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Edited by
Carlo Lozzia
Editorial

The meeting that was held in Volos, supported by the Benaki Institute, has ratified the 30 years of the working groups that today have become Integrated Protection and Production in Viticulture. It is my intention to remind and thank all those who, with their work, have contributed to the birth, the development and the extraordinary vitality of this group. When peoples' names are mentioned very often we forget someone. I would, in any case, like to thank Mario Baggio, the "founder fathers" M. Baillod, H. Milair, M. Martelli, W. Gartel, and P. Carceles and the co-ordinators who have preceded me: M. Baillod, S. Schmid and B. Dubos. A big thank you goes to all the researchers who have worked hard these years, many of whom are not with us any longer. Amongst all these I would like to remember my friend Jacques Stockel, who will probably follow the developments of the fight against moths from the other side of the world.

Returning to more current themes, viticulture is continuously moving towards high quality productions and the protection represents one fundamental aspect also in relation to the environment where the biodiversity and the healthiness are protected in favour of the quality of life of the farmer and of the consumer. All the works presented at this meeting go in this direction and the future prospectives are always to understand better the relationships between the environment and the diseases from the viewpoint of integrated production which will interest all the European countries, including those that have recently joined our Community. The great successes that this group is obtaining are also thanks to the co-ordinators of the sub-groups D. Thiéry, M. Maixner, M. Clerjeau, K.J. Schirra and J Sentenac. I thank them and hope they will continue in their researches and in co-ordinating the younger colleagues.

As far as I am concerned, I thank you for the renewed trust placed in me. I will continue to work hard in the next years for the development of viticulture and also so that young researchers, especially those, who do not have great economic means, can work and take part in our sessions. I hope that this confrontation between old and new will lead to greater integration among the sub-groups, which has always been and always will be highly profitable.

Hoping to see you all at the next meeting, which will probably be full of bubbles like Champagne, I wish you all a good work.

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The Working Group celebrates its 30th anniversary

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The evolution and historical chronology of the working group presented in the table illustrates that the information available on our past has different qualities. Whereas the year 1984 marks an important step in the working group’s records because the proceedings of the meeting held in 1983 at Toulouse were for the first time published as IOBC/wprs Bulletin, the examination of the early phase has to be based on unpublished internal reports and testimonies of eye witnesses that participated in the important decision making processes. Therefore I would like to focus my short historical review on that particular period ranging from 1973 to 1981.

The preparatory phase 1973

It is interesting to note that the first initiative for the establishment of an IOBC working unit dealing with viticulture was made by an entomologist that was already involved in the dynamic IOBC working group in orchards but covered in his professional activities also viticulture: Mario Baggiolini at the Swiss Federal Research Station at Changins/Nyon. Baggiolini’s activities and merits within IOBC as dynamic promoter of integrated plant protection and Integrated Production systems are well known and were instrumental for the organisation of the historic preparatory meeting at Changins in February 1973 and for the submission of a proposal to IOBC Council for the establishment of a Study Group. He was supported by a young acarologist in the same laboratory who became later the first convenor of the working group: Marc Baillod.

Since most of the IOBC activities at that time were almost exclusively in the hands of entomologist, the discussions and conclusions of the first preparatory reflection group (with 5 entomologists) are extraordinary.

Their agenda listed 4 points:

1. Situation of plant protection (in viticulture) in Europe;
2. Potentials of establishing an IOBC working unit for viticulture;
3. Inventory of major problems and research topics;
4. Conclusions and proposals.

In point 1 we find interesting statistics concerning the number of treatments applied the major pests and diseases in Switzerland, France and Germany. In all countries concerned the spray program was dominated by fungicide applications against mildews (Plasmopara and Oidium) and Botrytis and varied between 3 (dry regions) and 12 (humid areas). Second identified problem were the grape moths (2 - 6 applications) followed by spider mites (1-2 applications). Other problems (e.g. excoriose, Black rot, Sparganothis, leafhoppers (Cicadelles), noctuids, eriophyid mites) were considered to be of more limited and local importance. The problems were thought to be closely linked to suboptimal use of pesticides and fertilisers.
**Point 2:** Some basic principles of a future working unit were laid down:
- Interdisciplinary: the group should be as open as possible and hence not be limited to a single discipline (e.g. entomology)
- The activity should not replace or limit the work carried in each participating country
- The main objective would be an international podium for the exchange of information between members but also to act as catalyst in the relevant fields of research (i.e. to encourage and accelerate, but not impose rules).

It was concluded that at the beginning the group should not be too large but be flexible to grow according to needs. Best format to start and evolve was the Study Group with the following objectives:
- to act as liaison between countries, exchange of documents, harmonizing working methods.
- to act as liaison between researchers, coordination of research avenues even in a bilateral way where only 2 countries were interested
- regular meetings of scientists working in the same subject area.

**Interested countries**
Probably considering the IOBC rule that an IOBC working unit must have participants from at least 3 different countries it was concluded to start with France, Germany and Switzerland as core countries but it was also deemed highly desirable to identify the interest of the immediate neighbour countries, i.e. Austria, Italy and Spain. Other countries (e.g. eastern Europe) to be handled case by case.

**Structure of the group respecting the national research, viticultural and extension structures**

It was considered desirable to have representative of major institutions engaged in the future work:

- **France:** INRA, official plant protection services, ITV, ACTA
- **Germany:** The 2 national research centers BBA Bernkastel and research institute Geisenheim. At least 1 representative of the 8 regional research institutions. Weinbauinstitut Freiburg i.B.
- **Switzerland:** The 2 federal research stations at Nyon/Changins (French speaking Switzerland) and Wädenswil (German speaking part).

The research structures of Italy, Austria and Spain were not well known to the panel and it was decided to contact colleagues in these countries in due course.

**Disciplines concerned**
Having accepted in principle that the group should have a large scope it was deemed important to associate mycologists as well as generalists right from the beginning. For the start it was not considered to be of priority to associate virology, breeding and clone selection in order to avoid overburden of the vehicle. It was discussed whether 1-2 representatives working in cultural technologies were important at the beginning; they would be most welcome if the group was not too heavy.

**Point 3: Inventory of key problems and research fields**
It was admitted in the report that the panel could only produce an incomplete and biased list of items. The examination of the report confirms this entomological bias because the arthropod pests were discussed and ranked in detail whereas the fungal diseases were covered
Point 4. Proposals for further action
To be proposed to IOBC Council the establishment of a Study Group “IOBC-WPRS Study Group on Integrated Control in Viticulture”. To contact colleagues in other countries and to identify interest and participation. To organise a first meeting (2 working days) to take place in December 1973 at Nyon, Switzerland and with the following agenda:

- Structure of the group
- Discussion of some selected key problems and information about the respective national research activities
- Inventory of existing and lacking methodology in the respective areas. First proposals for the establishment of tolerance levels (pests).

IOBC Council gave green light for the establishment of the proposed Study Group in spring 1973.

The first meeting in 1974

was a full success with 35 participants from 7 European countries and the presence of the IOBC Secretary General Lukas Brader. Interesting to note that 10 participants were phytopathologists. This was a novum in IOBC since the phytopathologists were traditionally meeting in other international frames (such as EPPO).

The objectives of this first meeting were to

- analyse the possibilities to introduce Integrated Protection schemes in viticulture
- examine the key problems (pests, diseases, side-effects of pesticides)
- organise subgroups addressing the key issues and to transform the study group into an IOBC Working group in 1975.

Conclusions and decisions taken

- The establishment of subgroups was accepted and 7 units defined (see also table): Grape moths (Roehrich F), Mites (Baillod CH), Side-effects (Touzeau F), Sparganothis (Russ A), Autocidal control (Arroyo E; this subgroup was for the start integrated in the grape moth subgroup), Fungal diseases (Gärtel D) and “Representation of the group” Baillod (CH), Milaire (F), Gärtel (D), Martelli (I) and Carceles (E).
- The chairpersons of the subgroups are requested to proceed with the development of the unit and to seek the membership of scientists not present but interested in the activities.
- Each subgroup has to define by end 1974 its objectives and to prepare a working plan for 1975.

The priorities for each subgroup were also defined as follows

SG Grape moths: Relation Grape moths – Botrytis; damage thresholds; harmonising trapping systems; examination of antagonists; distribution maps of the 2 species; basic control technology and especially the use of Bacillus thuringiensis.
Autocidal control and use of pheromones: Research and development of a sex pheromone for *Eupoecilia ambiguella*; investigation on confusion technology; relation between trap catches and actual activity of the moths.

SG Mites: Sampling methods; tolerance levels; importance of antagonists especially typhlodromid predatory mites.

SG Fungal diseases: biology in view of integrated protection; influence of cultural practices; simultaneous control of *Plasmopora* and *Botrytis*; measures to increase plant resistance; improvement of forecasting and possibilities of warning systems.

SG Side-effects: Influence of pesticides on typhlodromid mites and the acarocenose in general; possibilities of foliar analyses to show and investigate trophic effects of pesticides; inventory of most evident trophic effects; side-effects of fungicides on other fungi.

Meeting frequency: It was decided that for the beginning meetings would take place in the framework of the subgroups in order to allow a rapid exchange of information between specialists interested in particular problems and to accelerate the development of working concepts.

**The subgroups start their work**

The records in the table show that in the period 1975 until 1980 the main activities were carried out at the subgroup level. As participant in most of these meetings I look back with a feeling of great satisfaction, innovation and visions since the amount of open questions and unsolved problems was gigantic. The main working tools applied were mutually developed project protocols for joint actions by participants especially interest in the respective topic. The working atmosphere was open, carried by mutual support and remained untouched by institutional barriers and secrecy. Also to note as a small side remark that it took more for the pathologists to find a mutual basis of straightforward collaboration that changed later with the organisation of plenary meetings where the subgroup of pathologists became a most viable and stimulation platform that prevailed up to the present.

**Plenary meetings become the standard format**

With the organisation of the second plenary meeting in 1979 the working group initiated its traditional biannual schedule of full meetings that has been continued up to the present. The meeting of 1981 at Gargnano / Italy can possibly considered a further milestone in the history of this working group where the subgroups were redefined and the working group has adopted the present structure. The number of participants in the plenary meetings fluctuated between some 30 persons and large audiences of some 160 persons participating in the Bordeaux meeting in 1993. The former project oriented approach was more and more replaced by a symposium type of presentations given in the subgroups that were meeting more and more in parallel. This development seems to reduce to a certain extent the initial vision of an interdisciplinary platform. This aspect was discussed in the meeting of 2001 taking place in Ponte do Lima where the question was raised if this IOBC working group should not consider to re-introduce in a modest way the notion of plenary sessions focussing on a few common topics while still providing ample space for the important discipline oriented discussions in the subgroup environments. The organisation and outcome of a plenary session and round table at the 3rd day of the present meeting 2003 in Volos will show whether this redesign of the working group’s activities is successful and desirable.
### Chronology of IOBC Working Group “Integrated Control in Viticulture”

<table>
<thead>
<tr>
<th>Year</th>
<th>Date and Location</th>
<th>Convenor</th>
<th>Type of Meeting</th>
<th>Bulletin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973</td>
<td>20 - 21 February Changins CH</td>
<td></td>
<td><strong>Preparatory meeting</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Baggioni CH, Baillod CH, Guignard CH, Milaire F, Schruft D</td>
<td>Report*</td>
</tr>
<tr>
<td>1973</td>
<td>May</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1974</td>
<td>27 - 28 February, Lausanne, CH</td>
<td>Baillod, CH</td>
<td><strong>First meeting of Study Group</strong></td>
<td>Report*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>with 35 participants from A (1), CH (9), D (4), E (4), F (9), I (6), Ru (1). Establishment of 5 subgroups – SG Grape moths, SIT (Roehrich, F) – SG Mites (Baillod, CH) – SG Side effects of pesticides (Touzeau, F) – SG Sparganothis (Russ, A) – SG Fungal Diseases (Gärtel, D)</td>
<td></td>
</tr>
<tr>
<td>1975</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1975</td>
<td>18 -19 February, Avignon, F</td>
<td>Roehrich, F</td>
<td>SG grape moths, 12 participants. CH, F, I</td>
<td>Report*</td>
</tr>
<tr>
<td>1976</td>
<td>12 -13 February, Changins, CH</td>
<td>Baillod CH (Schmid, CH)</td>
<td><strong>Plenary meeting</strong> with 32 participants</td>
<td>Report*</td>
</tr>
<tr>
<td>1976</td>
<td>8 – 9 December, Toulouse, F</td>
<td>Touzeau, F</td>
<td>SG Side-effects with restricted number of participants</td>
<td></td>
</tr>
<tr>
<td>1977</td>
<td>1 -2 March, Grau du Roi, F</td>
<td>Roehrich, F</td>
<td>SG grape moths</td>
<td>Report*</td>
</tr>
<tr>
<td>1977</td>
<td>8 – 10 March, Vienna, A</td>
<td>Russ, A</td>
<td>SG Sparganothis</td>
<td></td>
</tr>
<tr>
<td>1977</td>
<td>planned but no meeting</td>
<td>Gärtel, D</td>
<td>SG Fungal diseases</td>
<td></td>
</tr>
<tr>
<td>1978</td>
<td>22 – 24 February Zaragoza, E</td>
<td>Roehrich, F</td>
<td>SG Grape moths</td>
<td>Report*</td>
</tr>
<tr>
<td>1978</td>
<td>14 – 15 November Avignon, F</td>
<td>Baillod, CH</td>
<td>SG Mites</td>
<td>Report*</td>
</tr>
<tr>
<td>1979</td>
<td>21 – 23 February Beaune, F</td>
<td>Baillod, CH (Schmid, CH)</td>
<td><strong>Plenary meeting</strong> with 35 participants</td>
<td>Report*</td>
</tr>
<tr>
<td>1980</td>
<td>27 – 28 February Freiburg, D</td>
<td>Baillod, CH</td>
<td>SG Mites</td>
<td>Report*</td>
</tr>
<tr>
<td>1980</td>
<td>11 – 13 March, Keskemet, H</td>
<td>Roehrich, F</td>
<td>SG Grape moths</td>
<td>Report*</td>
</tr>
<tr>
<td>Year</td>
<td>Title</td>
<td>Participants</td>
<td>Location</td>
<td>Description</td>
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<tr>
<td>--------</td>
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<td>----------------------------------------------------------------------------</td>
</tr>
</tbody>
</table>
| 1981   | Plenary meeting                                                      | 51           | Gargnano, I | Schmid, CH | Subgroups redefined:  
- Grape moths and chewing insects (Roehrich, F)  
- Mites and sucking insects (Baillod, CH)  
- Fungal and bacterial diseases (Gärtel, D)  
- Side-effects of pesticides (Touzeau, F)  
- Practical application (Schmid CH) |
| 1983   | Plenary meeting                                                      | 81           | Toulouse, F | Schmid, CH | New SG on **Physiological disorders** |
| 1985   | Plenary meeting                                                      |              | Bernkastel, D | Schmid, CH |                                |
| 1987   | Plenary meeting                                                      |              | Logrono, E  | Schmid, CH |                                |
| 1988   | Joint symposium CCE/IOBC on Integrated Protection in Viticulture     |              | Lisbon, P   | Cavalloro, EU | Schmid, CH |
| 1989   | Plenary meeting                                                      | 77           | Sion, CH   | Schmid, CH | New SG on **soil management** |
| 1991   | Plenary meeting                                                      | 87           | Conegliano, I | Schmid, CH |                                |
| 1993   | Plenary meeting                                                      | 160          | Bordeaux, F | Dubos, F |                                |
| 1995   | Plenary meeting                                                      |              | Freiburg, D | Dubos, F |                                |
| 1997   | Plenary meeting                                                      | 70           | Gödöllő, H | Dubos, F |                                |
| 1999   | Plenary meeting                                                      | 122          | Firenze, I  | Lozzia, I |                                |
| 2001   | Plenary meeting                                                      | 138          | Ponte de Lima, P | Lozzia, I | Decision to reorganise meeting structure |
| 2003   | Plenary meeting                                                      |              | Volos, GR  | Lozzia, I |                                |

*) Unpublished internal reports distributed to IOBC secretariat and participants of the respective meeting
Growth-models, a tool to define spray intervals against downy mildew (Plasmopara viticola)

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Abstract: A model to control grapevine downy mildew was generated at the State Institute for viticulture, Freiburg. To improve the model, field trials were performed in order to determine the maximum leaf appearance respectively the leaf development between two applications under high and permanent infection pressure. The sprayings were carried out according to a growth-model simulating leaf appearance and leaf development. Under permanent and heavy infection pressure with P. viticola 2 to 3 leaves are allowed to emerge or 300 to 400 cm² unprotected leaf area can develop before the next treatment is necessary. A new aspect in our strategy is the consideration of the host, while a few years ago only the pathogen was regarded as important. The combination of a growth-model with a P. viticola- model is thought to be an important progress for the integrated control of downy mildew.

Key words: Vitis vinifera, grapevine, growth-model, modeling, Plasmopara viticola, downy mildew, integrated control, disease control

Introduction

A model for the controlled management of grapevine downy mildew was developed at the State Institute for viticulture, Freiburg (Bleyer & Huber 1995); an important question in the model was the effective period of fungicides. Several investigations showed that the duration of protective activity was more than 21 days on the old leaves, present at the time of spraying. On young leaves we found little and on leaves grown after application we found no effects (Huber et al. 2002). So it becomes obvious that the spray intervals are not limited by the degradation of the fungicide, but by the growth of the vine. To improve our strategy we tried to define the maximum leaf appearance respectively the leaf area, which can develop and is protected between two applications under high infection pressure (Bleyer et al. 2002). A useful tool is the growth-model developed by SCHULTZ for “Riesling” c.v. in 1992 (Schultz 1992). It simulates leaf appearance and leaf development of primary shoots and was validated for “Müller-Thurgau” c.v. and “Pinot noir” c.v. in 1999. During the seasons 2000, 2001 and 2002 leaves from 50 primary shoots were counted and compared with the simulation. A good correlation between the simulated and the measured data was found until the middle of July (Fig. 1).

In 2001, we performed a field trial in order to determine the maximum leaf number between two applications under high and permanent infection pressure. The vineyard was planted with the variety “Müller-Thurgau”, which is highly susceptible to downy mildew. In these experiment the protective compound “Metiram” was applied according to the growth-model. The results showed that 2 to 3 leaves are able to emerge under high and permanent infection pressure before the next treatment is necessary. We continued our studies with the growth-model in the year 2002. The objective of our field trial was to quantify the leaf area between two sprayings under permanent high infection pressure, because the leaf area is more important for the application than the leaf appearance.
Material and methods

The plots were inoculated with a sporangial suspension of \textit{P. viticola}. The first applications were done at the 90\% incubation progress (Table 1). The following applications were carried out according to the growth-model. In order to produce a high infection pressure the plots were irrigated regularly from the 2\textsuperscript{nd} June until 20\textsuperscript{th} June. All applications were done with the compound “Metiram”. On 3\textsuperscript{rd} July infestation with \textit{P. viticola} was assessed on every leaf on 40 marked shoots per variant.

Table 1. Plan of the trial, “Müller-Thurgau” c.v., Freiburg, 2002

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>0% Incubation progress</th>
<th>90% Incubation progress</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control inoculation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Leaf area 320cm\textsuperscript{2} inoculation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>Leaf area 400cm\textsuperscript{2} inoculation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>Leaf area 533cm\textsuperscript{2} inoculation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>Leaf area 800cm\textsuperscript{2} inoculation</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>Leaf area, 1600cm\textsuperscript{2} inoculation</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Results and discussion

\textit{Trial}

The propagation conditions of \textit{P. viticola} during the field trial were extremely favorable. Natural and artificial rainfall together amounted to 400mm. The disease level of the untreated
control was about 60%. Figure 2 shows the average disease severities of the 1st (oldest) to the 18th (youngest) leaf of 40 shoots per variant on 3rd July.

![Disease severity of each leaf on a primary shoot after different spray managements.](image_url)

**Fig. 2.** Disease severity of each leaf on a primary shoot after different spray managements. Arrows indicate first and last spraying, respectively, “Müller-Thurgau” c.v., Freiburg, 3rd July 2002

If the vines were sprayed after the development of 320cm² leaf area (var. 2), nearly no infestation was observed on the newly grown leaf area (leaf area between the first and last spraying). Also treatments after the development of 400cm² (var. 3) resulted only in a small increase of the disease. If applications were done after the development of 533cm² (var. 4), 800cm² (var. 5), and 1600cm² (var. 6), disease severity increased extremely, but mainly so on the newly grown leaf area. It has to be noted however that this result was obtained with the susceptible variety “Müller-Thurgau” under permanent high infection pressure.

**Improvement of the existing strategy**

The next step is the integration of the new results in our strategy to control downy mildew, and this idea is illustrated in Figure 3. Infection conditions are calculated on weather data. Infections can be caused by oospores (soil infections) or by zoospores (secondary infections). In contrast to our earlier strategy, when we considered only one single soil infection at the beginning of the season, we now are aware of soil infections occurring during the whole season (5, 6). In the case of a weak infection, meaning short duration of leaf wetness, we wait till 80% of the incubation period. Then a protective fungicide is sprayed. In the case of a strong infection, meaning long duration of leaf wetness or thunderstorms, a curative fungicide is applied as soon as possible. The estimation of the infection intensity plays a key role in the practical management of downy mildew. Up to now it is not possible, to calculate accurately the infection intensity. The only way to estimate it is with individual experience. After the
application, we wait until 300 to 400 cm² (data for “Müller-Thurgau” c.v.) of leaf area has developed, which can be calculated by the growth-model. If we work only with copper-fungicides we look at the weather forecasting. Before rain we spray again with copper. If we work with organic fungicides we consider with weather data the next infection conditions. Than the flow process starts again.

The used growth model is well suited for simulating leaf appearance and leaf area development of primary shoots for the varieties “Riesling”, “Pinot noir” and “Müller-Thurgau” (canopy system: espalier-type). But it has to be considered that the development of secondary shoots is not integrated in this model and that nutrition supply must be optimal. Still, the use of the described growth model will be a progress for the plant protection.

**Acknowledgements**

The support of H.R. Schultz FA Geisenheim is gratefully acknowledged.

**References**


Huber, B. et al. 2002: Studies on the effective period of protective fungicides against downy mildew (P. viticola); Proceedings of the 4th International Workshop on Powdery and Downy Mildew in Grapevine, Napa, California: 29-30.

A change in our conception of the life cycle of *Plasmopara viticola*: Oosporic infections versus asexual reproduction in epidemics

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³ Operative Unit of Plant Protection, Istituto Agrario di S. Michele all’Adige, S. Michele TN 38010, Italy,
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⁵ Stazione Federale di ricerche Agronomiche Changins, centro Cadenazzo, 6594 Contone, Switzerland.

Abstract: The prevalent conception of the life cycle of *Plasmopara viticola*, causal agent of grape downy mildew postulates that epidemic start from the overwintering oospores through splash dispersed macrosporangia and zoospores. Macrosporangia may germinate if sufficient wetting of the leaf debris occurs and average temperatures are above 10°C or a particular temperature sum is reached starting from a determined biofix (Gehmann). Once sporulating lesions from such primary infections are present the pathogen has the ability to infect through the secondary sporangia and zoospores. The potential reproduction rate of the secondary phase out weights massively the potential of oospores. Therefore it is assumed that once the first primary lesions are sporulating the relative contribution of oospores to the progress of the epidemic is irrelevant. In this paper we draw a different, more differentiated picture of the life cycle of *P. viticola* based on the results presented in this Bulletin and prior obtained through the genetical analysis of various pathogen populations collected at different times of the epidemics in vineyards across Europe.

Key words: population analysis, genetic dissection, down mildew, microsatellite

Introduction

The accepted biological concept of how an epidemic of *Plasmopara viticola* evolves can be resumed in: within a relatively short time lap, all oospores mature and with a rain event primary macrosporangia are dispersed on leaf material. The rule of the “three 10” (temperature above 10°C, more than 10 mm of rain in 24 h and shoots longer than 10 cm) usually adequately describes this event. In particular situation also sum of temperature above a threshold can indicate the point of maturity reached by the oospores. Simulations and oospore germination tests confirm this assumption. Once primary infection passed the latency period, *P. viticola* can produce asexual sporangia at night and high humidity conditions, which are then splash dispersed during a rain event and in the presence of a water film on leaves and berries they can infect within a few hours. This type of secondary cycle fuels the epidemic. Control and simulation programs are targeted on the secondary cycle once the primary event has taken place (Viret and Siegfried, 1998). In vine producing zone of southern Switzerland such secondary infection periods as registered by automatic weather stations and calculated by the program often are at regular intervals of 2-4 days reaching 20 or more separate periods during the months June to middle of August (Jermini et al., 2003 in this
Bulletin). Under such conditions only control strategies purely based on chemicals, oriented toward protecting continuously leaves and bunch can be successful. The relevant question discussed in this paper is: are the assumptions we currently accept on the life cycle and epidemics of *P. viticola* correct? Several independent research groups in the past decade have given more attention to the role of the oospores not at last also in the attempt to construct forecasting and risk models such as EPI (Strizyk, unpublished, Gomez and Amaro, 2001) which attributes a weather determined quantitative risks to the oosporic phase of the pathogen

**Oospore germination time:**

If the above mentioned epidemic life cycle is assumed to be correct, than the only relevant parameter is the exact date of the first possible germination of the oospores and infection conditions. Much work has been dedicated to determine this date (Gehmann). However several researchers revised the concept and extended their observations on oospore germinations introducing the dimension time (Hill, 1998, Loskill et al., 2001). Research concepts favour standardisation of the methodology, and clear and easy reproducible experimental conditions. This led to uniformity in treatment of the overwintering leaf debris for example under a layer of sand. Under such conditions oospores germination seams to be quite uniform in time, however if more natural overwintering conditions are chosen, oospores germinate over a much longer period up to two month (Jermini et al., 2003). To asses the true time laps in which oospores can germinate, the time of their formation in the prior year, the various possible overwintering conditions (dry-wet cycles, temperature, fully exposed, partially or always covered and so forth should be considered. Probably we will observe germination of oospores up into fall, therefore giving a potential of primary infections all year around climatic conditions permitting.

**Quantitative contribution of the primary infections to the epidemics:**

In past few years, molecular markers of various types have been develop which can be used to fingerprint and identify single genotypes. The first such DNA-based markers were the Random amplified polymorphic DNA (RAPD) using the PCR-technique. We used these markers to analyse a population of *P. viticola* (Kump et al., 1998) and by others to study the genetic variability of the pathogen (Seidel et al., 1998). However these marker were rather unsuited, as they needed pure *P. viticola* DNA, they are dominant (type presence/absence) and are often not reproducible. Only with the development of the microsatellite DNA-markers (SSR) (Gobbin et al., 2002) which are highly reproducible multiallelic (potentially two alleles per genotype and locus), variable and unambiguously identifiable also in DNA-probes with foreign (*Vitis*) DNA large scale genetic studies on *P. viticola* became feasible.

As presented elsewhere in this IOBC-wprs-Bulletin such studies showed first a high genetic heterogeneity in all populations sampled, second a extremely variable contribution of single genotypes to any of the epidemics analysed. Most genotypes were found only once, a few several times and rarely a genotype causing by itself a noticeable number of secondary lesions was identified. In most vineyards analysed a single or two genotypes reached more than a few percent of all lesions. This was noticed as well as early in the season, which we would expect, as also later in July and August which was rather unexpected.

**Discussion**

The preliminary results presented by Gobbin et al., 2003, Rumbou and Gessler, 2003, Pertot et al., 2003 (personal communication and in this Bulletin) point to a re-evaluation of the
quantitative contribution of the primary infections during the months June to August, e.g. the damaging phase of the epidemic. If the primary infections can contribute to the epidemic up to August to well over 50% and the dispersal of a single clone is rather limited and band to a single or few vines the potential availability of oospores should be relevant. Reducing the overwintering inoculum should at least theoretically also reduce linearly the number of primary infection and therefore proportionally to the relative importance of them the epidemic. However to complicate the picture enter the observation that only few to rare genotypes are able to reproduce to a relevant amount.

Several evolutionary questions arise therefore, first is this just by chance or have those most frequent genotypes genetically fixed advantages compared to the genotypes which appear once; second, if as we have to suppose they are truly more fit, why the alleles or allele combination giving this higher fitness did not get fixed in the population; third what would be the selection mechanisms responsible for maintaining this genetic diversity? Currently no response is available and a large scale effort over complete seasons (including fall) and several years will be necessary to gain sufficient data to attempt any explanation.

What are the consequences for the control measures? Again no clear answer can be given. However, it seems clear that under given climatic conditions a reduction of the overwintering inoculum or any reduction of the oospores formed in the prior seasons can lead to a much lower infection potential not only early (first possible oospore derived infections) but at least up to July, with the consequence that the epidemic curve will result less steep. This in turn would facilitate any control strategy which allows some lack in the protective cover such as proposed by Jermini et al., 2003.

It also seems reasonable that it is highly dangerous to propose a program based on the present lesions in a vineyard with early down mildew control. In such a case it will be impossible to know, nor even get a feeling for the potential quantity of oosporic inoculum present, and if climatic conditions for primary infections overlap with window in the protecting cover on the vines even in absence of sporulating lesions massive new infection could occur. Also the reverse may be plausible, even with sporulating lesions if the primary inoculum is rare leaving a cover window may not lead to dramatic increase of the epidemic. However if lesions are present we have already to suspect a high amount of oospore to be present and therefore a high potential for new primary infections at any time.

In most cases if hypothetically we would eliminate a particular number of spots at any time at random

Studies on particular cases such as unfavourable overwintering conditions with strong bottlenecks for *P. viticola* populations or accurate control also late in the season may help to determine the importance of sanitary measures oriented toward the reduction of the oospore potential similarly as today is recognized and applied for the control of apple scab (MacHardy et al., 2001).

First preliminary reports (Rumbou, personal communication) indicate that autumn epidemics may be initiated and driven by different populations than the spring epidemics at least under unfavourable summer conditions in Greece. What would be the significance of these findings if also valid in a more general way has to be discussed.

References


Spatial distribution of *Plasmopara viticola* secondary inoculum

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Abstract: *Plasmopara viticola* is the causal agent of downy mildew on grape. Several crucial biological questions, as temporal and spatial dispersal dynamic of airborne asexually produced microsporangia, are still unknown. Spatial population analysis of *P. viticola* was until now hampered by the inability to distinguish between the asexually and oosporic derived new infections. Population increases were usually attributed to the asexual phase of the pathogen, which biased the determination of the spread distance to such an extent that it often becomes impossible to establish a gradient from a source. With the use of the multiallelic co-dominant SSR markers (microsatellite) the asexual spread can be determined exactly.

The spatial-temporal dispersal of microsporangia generated by sporulating lesions was tracked by the genetic identification of a genotype through its microsatellite allele pattern. The natural situations reported in this paper show the pattern of sporangia distribution in vineyards and in single grapevines from the original primary infection to the target vines.

Key word: downy mildew, grapevine, oomycete, population genetics, SSR.

Introduction

Downy mildew, caused by the diploid Oomycete *Plasmopara viticola*, is one of the most important grape (*Vitis vinifera*) diseases world-wide. Secondary disease cycles can happen under suitable infection conditions, similar to those valid for oosporic-derived (primary) infections (Schruft & Kassemeyer 1999). Traditionally, responsibility for disease spread over both time and space was attributed to secondary sporangia (Agrios 1988), but no clear proof is yet available.

In this study, we combined synergistically all the genetic (Gobbin et al., 2003a) and statistical background, in order to quantify the spatial dispersion of particularly successful genotypes as a function of epidemic progression.

Materials and methods

Four vineyards were selected within four European-wine-producing countries (Table 1). Naturally occurring downy mildew epidemics were surveyed during years 2000-2002 within those plots. Oil spots were collected from 3 to 5 times per plot along the epidemic, and their
location was recorded. Samplings were performed as described in Gobbin et al., 2003 for a high throughput method (HTM). Due to the spatially-circumscribed epidemic development in Was (single vine infected), we additionally recorded the single lesion position according to a reference system defined by six sectors (top, medium, low and left, right vine sectors).

Table 1. Vineyard location (site abbreviation, site name in full and nation), number of rows (R), distance between rows (dr), number of vines per row (V), distance between vines in a row (dv), total vines (Nv), plot area (A), sampling dates (S date), sampling size (Nobs), number of different genotypes (Ngen), number of single genotypes (genotypes that occurred once all over the surveying period; Nsgen), name of the most frequent genotype (MFG name), occurrence of the most frequent genotype (Nobs MFG). Bold characters refer to the total number of lesions collected (tNobs), of genotypes identified (tNgen), of single genotypes identified (tNsgen) and of MFG identifications (tNobs MFG).

<table>
<thead>
<tr>
<th>Vineyard loc</th>
<th>R</th>
<th>V</th>
<th>Nv</th>
<th>S date</th>
<th>Nobs</th>
<th>Ngen</th>
<th>Nsgen</th>
<th>MFG name</th>
<th>Nobs MFG</th>
</tr>
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<tbody>
<tr>
<td>Bomübetosca</td>
<td>7</td>
<td>40-69</td>
<td>414</td>
<td>&lt;27.6.01</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>übetosca</td>
<td>112 (58.0%)</td>
</tr>
<tr>
<td>France</td>
<td>1.5 m</td>
<td>0.9 m</td>
<td>559 m²</td>
<td>27.6.01</td>
<td>63</td>
<td>3</td>
<td>1</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.7.01</td>
<td>18</td>
<td></td>
<td>9</td>
<td>2</td>
<td>4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.7.01</td>
<td>58</td>
<td></td>
<td>15</td>
<td>5</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>27.8.01</td>
<td>53</td>
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<td>27</td>
<td>20</td>
<td>22</td>
<td></td>
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<tr>
<td></td>
<td>total pop</td>
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<td></td>
<td>41</td>
<td>28</td>
<td></td>
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<tr>
<td>Geigenusra</td>
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<td>13</td>
<td>39</td>
<td>26.5.00</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>egignusra</td>
<td>3</td>
</tr>
<tr>
<td>Germany</td>
<td>1.8 m</td>
<td>1.2 m</td>
<td>84 m²</td>
<td>31.5.00</td>
<td>7</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>05.6.00</td>
<td>30</td>
<td></td>
<td>8</td>
<td>2</td>
<td>10</td>
<td></td>
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<td></td>
<td>19.6.00</td>
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<td>25</td>
<td>15</td>
<td>55</td>
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<tr>
<td></td>
<td>06.7.00</td>
<td>21</td>
<td></td>
<td>8</td>
<td>3</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>total pop</td>
<td>206</td>
<td></td>
<td>33</td>
<td>20</td>
<td>79 (38.2%)</td>
<td></td>
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<tr>
<td>Evizemca</td>
<td>16</td>
<td>18-23</td>
<td>328</td>
<td>23.6.00</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>1.5 m</td>
<td>0.8 m</td>
<td>394 m²</td>
<td>02.7.00</td>
<td>71</td>
<td>10</td>
<td>6</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.7.00</td>
<td>22</td>
<td></td>
<td>6</td>
<td>3</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>total pop</td>
<td>97</td>
<td></td>
<td>15</td>
<td>11</td>
<td>59 (60.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ezazerma</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>01.6.02</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>ezazerma</td>
<td>1</td>
</tr>
<tr>
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<td>1</td>
<td>0</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16.6.02</td>
<td>85</td>
<td></td>
<td>6</td>
<td>4</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>total pop</td>
<td>111</td>
<td></td>
<td>6</td>
<td>4</td>
<td>105 (94.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aNo sampling was performed prior to 27.06.01. An “open” date refers to the putative appearance of the very first primary infection caused by the MFG übetosca.*

For genotyping the collected samples, we used automated high throughput DNA extraction, PCR amplification of the four polymorphic P. viticola specific SSR loci BER, CES, GOB and ISA, sequencer based fragment analysis and automatic allele scoring. Every
step was performed exactly as described in Gobbin et al., (2003). For each of the four plots, we pooled all the individuals collected at the 3 to 5 sampling dates (16 independently sampled populations). The number of individuals ($tN_{obs}$), the total number of genotypes ($tN_{gen}$) and the number of single genotypes (genotypes identified once throughout the observation period, $tN_{sgen}$) were calculated for each of the 4 collection sites separately. For each total population (Table 1, bold numbers), the genotypes occurring with the highest frequency in the total population, were defined as most frequent genotypes (MFGs).

**Results**

**Downy mildew dispersion strategies**

*P. viticola* genotypes showed distinct spreading strategies after considering the epidemics studied. Essentially following four dispersion patterns were distinguished: 1) clonal multiplication on single, or few, vines; 2) clonal multiplication on single, or few, vines followed by vineyard-scaled dispersion; 3) multi-cluster vineyard-scaled dispersion without previous clonal multiplication on a few vines; 4) random vineyard-scaled dispersion without previous clonal multiplication on a few vines, and 5) irrelevant clonal multiplication and dispersal. The great majority of the genotypes were categorized into this class.

![Fig. 1. Spatio-temporal dispersion pattern of the most frequent genotype in Wädenswil (ezazerma). The single vine infected is represented by a bi-dimensional scaled rectangle, as if it was observed from a neighbouring vine on the same row. Dimensions on x-axis represent left-side and right-side vine extensions, while y-axis represents vine height (in metres). The grapevine was divided into six sectors (left and right, top, medium and low sectors). Dot size represents the number of MFG observations per infected leaf. The position of infected leaves was assigned randomly within the corresponding sector. Uninfected leaves and leaves infected by other genotypes are not shown. Histograms on x- and y-axes describe the disease distribution within six grapevine sectors, based on c$N_{obs} \cdot$MFG per sector. From left to right, plots describe the cumulative genotype spread as a function of time.](image-url)
**Strategy 1: clonal multiplication on a single vine**

In Wädenswil (Was) a heavy sporulating first oil spot (genotype *ezazerma*) was detected on 01.06.02 on a leaf located within the medium-right sector of a vine (Fig. 1 and Table 1). After an asexual disease cycle, on 10.06.02, we observed a spatially restricted infection focus generated by the same genotype in the immediate vicinity of the first infected leaf. A total of 25 new oil spots were detected on six adjacent newly infected leaves. On 16.06.02, presumably after a new secondary cycle, 85 lesions were detected within the same vine. Five additional genotypes were identified, suggesting the occurrence of new primary infections. *Ezazerma* was found 79 times, colonizing 23 new leaves. On the last sampling date, the left vine side was infected by 50 lesions (on 14 leaves), while the right vine side by 55 lesions (on 16 leaves), mostly (68%) distributed within the medium sectors. Secondary sporangia did not migrate more than one meter farther apart after two secondary cycles, never reaching nearby vines that were perfectly healthy.

Fig. 2a

Spatio-temporal dispersion patterns of the most frequent genotypes, *übetosca* (Fig. 2a), *evuzemca* (Fig. 2b) and *egigusra* (Fig. 2c) found in Bommes, Tesero and Geisenheim, respectively. The vineyard is represented by a scaled rectangle (distances are given by the dotted line on the right hand side). Every vertical x-axis thick describes the beginning and the end of a grapevine row. Each number on y- and x-axis represent grapevine coordinates in the plot (row and grapevine number, respectively). Dot size represents the cumulative number of MFG observations per vine. Histograms on x- and y-axes describe the disease distribution along rows and grapevines, respectively. ELL Gaussian bivariate confidence ellipses, centred on the sample means of x and y severities, were specified by a default probability value of 0.68. From left to right, plots describe the genotype spread as a function of time. In Bommes (Fig. 2a), the first plot describes the putative location of the very first infection that started the epidemic prior to 27.6.01. Row one was chemically treated and no sampling was done. The last 14 vines per row are not shown (12.6 m) because they were MFG-free. In Tesero (Fig. 2b), only the last seven rows (of 16) are shown because of disease hosting.
Among the 64 lesions collected in Bommes on 27.06.01, three distinct genotypes were identified. One, übetosca (MFG), was identified 61 times, while the other two, once and twice, respectively. At the time of collection, one oil spot out of the 61 lesions successively genotyped as übetosca resulted visibly wider in size and more necrotized than the remaining 63 lesions. Therefore, we speculated that this oil spot may have had an older origin (<27.06.01) than the other 60 ones, and that it may have been the very first lesion that was able to generate the whole progeny (60 lesions until 27.06 + 51 lesions from 28.06 until 27.08) by successive secondary cycles (Fig. 2a; Table 1).

On 27.06, 13 vines were unequally attacked by übetosca: 60% of the lesions were localised on the tenth vine located in the seventh row (vine 10/7), 13% on the vine where the above-mentioned partially necrotized lesion was found (11/7), while the remaining 16 lesions were found on 11 immediately surrounding vines. Admitting the vine 11/7 to be the location of the very first primary infection, secondary sporangia may have migrated, on average, 1.37 m (st. dev: 1.33 m). A maximal airborne dispersion of 6.5 m was found. During the next two weeks, one (or few) very mild secondary cycles occurred, which slightly increased the occurrence of the MFG (4 new lesions found). The next two samplings revealed that übetosca colonised intensively in the northern part of the vineyard, with 25 and 22 new lesions, respectively.

**Legend to Fig. 2 a-c:** see page left

**Strategy 2: clonal multiplication on few vines followed by vineyard-scaled dispersion**
Strategy 3: multi-cluster vineyard-scaled dispersion without previous clonal multiplication on a few vines

In Tesero, *evuzemca* is the genotype that contributed the most to the epidemic (MFG): it was identified on vine 1/14 on 23.06.00 and it was responsible for 60% of the disease severity on 02.07.00 and 68% on 14.07.00 (Fig. 2b). On 02.07.00, 14 vines were attacked, mostly vines 5/16, 7/15 and 9/15. The other 16 lesions were dispersed on 11 vines. Considering vine 1/14 as the secondary inoculum source, the average migration distance was 6.41 m (st. dev: 2.87 m; max: 16.88 m; min: 2.19 m). A weaker dispersion occurred in the period 02 to 14.07.00: the MFG was newly identified 15 times, mostly on the same vines that were already colonised (only vine 4/16 was newly colonized by a MFG clone). The 15 clones were identified on six vines, mostly on vine 11/15.

Strategy 4: random vineyard-scaled dispersion without previous clonal multiplication on a few vines

In Geisenheim on 26.05.00, the MFG *egigusra* was colonising two vines close-at-hand both located in the third row (Fig. 2c and Table 1). One oil spot was found on vine 7/3 and two lesions on vine 8/3. We speculated that a secondary cycle already occurred and that the very first primary infection was missed by the observers. On 31.05.00, *egigusra* was identified on four vines. Considering exclusively vine 8/3 as the secondary inoculum source, the average migration distance was 4.97 m with a st. dev. of 1.54 m (max: 7.00 m; min: 3.6 m). It took only 10 days after the first appearance date to colonise 10 vines (26%) dispersed throughout the plot. One disease focus was observed at the fourth sampling date on the two vines where the MFG was first observed. After 40 days from the first observation, on 06.07.00, 20 vines (51%) were attacked.

Discussion

This study is just a short report of a much wider research which surveyed 18 *P. viticola* populations all over Europe. In that wider study, we identified more than 2300 genotypes among 4685 samples collected. 71% of all genotypes were identified only once throughout the epidemic while seven genotypes showed a marked secondary multiplication and dispersal: four of them were described in this paper.

The finding of four main dispersion strategies was expected because of regional variation in climatic conditions, cultivation systems, as well as strain-specific aggressiveness. Pathogen aggressiveness was strikingly revealed in Was (*ezazerma*) and Tes (*evivurro*), where the genotypes colonised, intensively, a single vine without escaping from it. This initial spatially limited secondary inoculum accumulation (distance among clones < 1 m) seems to be the antecedent step that leads to genotype spreading at further distances (strategy 2); this was observed, for instance, in Bom (*übetosca*). Further dispersal patterns follow the examples of Tesero (*evuzemca*) and Geisenheim (*egigusra*), where secondary sporangia were dispersed at a vineyard scale, generating either a few clusters or a random pattern, respectively.

One of the most salient consequences of this study consists in the deviation from the generally accepted view that an epidemic starts from a few simultaneously appearing primary infections, followed by a massive asexual reproduction. Instead, we observed that there was a continuous input of genotypes to the epidemic, which, overwhelmingly, contributed with much restriction (one or a few lesions each) to the total disease severity. The same trend occurred in every epidemic surveyed, less markedly only in Gei and in Was. About ¼ of the genotypes sampled were constituted by single genotypes that never gave rise to an asexual
progeny or gave rise to progeny that were undetected by the observers. Massive clonal reproduction seems therefore to represent the exception than the rule.

References


Response of the grapevine growth and yield quantity to the application of a minimal fungicide strategy for the control of the downy mildew (Plasmopara viticola)

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Abstract
The minimal fungicide strategy (MFS) tested in an experimental vineyard allowed a relevant reduction in the number of treatments without significant influences on yield quantity and quality. We assumed that the plant compensated the leaf damage during ripening period with the mobilisation of the reserves stored in the roots. The application of the MFS during the period 1999-2002, always in the same plot, permitted to evaluate the potential negative effects of this reaction during the following seasons until the beginning of a new epidemic. The comparison was made with a plot treated according to the normal schedule applied in the vineyard. On plant growth, the observed effects were a significantly reduction of the shoot length only after 3 stress years and the tendency to reduce the main and lateral leaf surface after 2 stress years. The most important influence was a significantly decrease in the potential yield quantity, evaluated in the late July, from the 2nd stress years. Therefore, the productivity in MFS plot was still 0.500 kg/m² higher than the production limit of 1 kg/m² valid in our country. These results indicated a minimal impact of the downy mildew stress caused by the incomplete control of P. viticola with the MFS on the plant growth and productivity. The risk due to the application of the MFS over several years is minimal and we assume that a year with a low disease impact is enough to reconstitute the reserve pool and to eliminate the stress situation.

Key words: Downy mildew, Plasmopara viticola, epidemic growth, leaf area, yield

Introduction
Grapevine is a perennial woody plant that has evolved survival mechanisms of reproduction, vegetative maturation and acclimation to environmental stresses. Such a strategy requires that vine effectively assigns available annual resources to mature both reproductive and vegetative tissues (Howell et al., 1994). Consequently, the amount of reserves contents in the woody parts of the plant is an important element to secure the early shoot growth in the spring.

The minimal fungicide strategy proposed and tested in an experimental vineyard allowed a relevant reduction in the number of treatments and resulted in almost identical yield as well as quantitatively as qualitatively (Jermini et al., 2003). Prior studies of the interactions between downy mildew (Plasmopara viticola) and grapevine have showed that the application of the minimal fungicide strategy induced a limited carbohydrate mobilisation from the roots (Jermini et al., 2001). The influence of this reserve mobilisation on the following season was also limited and no significant differences had been observed in plant growth and productivity (Jermini et al., 2000 and 2001). If the influence of single year application of the minimal fungicide strategy on the following season is limited, it is possible to suppose a negative effect if the stress is repeated on the same plant for more than a single
season. As a consequence, the minimal fungicide strategy has been applied during the period 1999-2002 in the same plot with the aim to observe from 2000 the cumulated effect of the stresses on the following seasons from bud burst until the beginning of a new epidemic on plant growth and yield production.

Material and methods

Plant material and experimental design
The experiment was conducted in South part of Switzerland in the plot prior described (Jermini et al., 2003)

The experimental design and the fungicides application in the MFS and SS plot are described in Jermini et al. (2003).

Phenology, plant growth and leaf area assessment
From 2000, the first stress year, until 2002 third stress year, 20 plants per treatment distributed between the sub-plots were selected at begin of April and the phenology evolution of the buds and shoots were followed weekly.

Between 13 and 16 representative shoots (1 shoot every 2 plants) per each sub-plot were selected at the phenological stadium E. Shoot growth (shoot length from basis until apex), number of main leaves, lateral shoots, leaves on lateral shoots and leaf area was assessed with non-destructive methods.

The area of main and laterals leaves was estimated on 10 shoot per treatment with the method described by Carbonneau (1976).

Generally these controls were carried out until begin of the new epidemic (apparition of the first sporulation).

Yield parameters
Number of shoots per plant, including the spurs, has been regulated before bloom. Shoot fertility (number of clusters per shoot calculated on 10 plant in each sub-plot) and the expected yield quantity have been estimated at end of July without to consider the eventual downy mildew damage on clusters. The expected yield/m² is calculated on the basis of the number of berries per cluster (determined from the average of the number of berries of 10 representative clusters per plot), the number of clusters/plant (determined as average of the number of clusters/plant of the sub-plots) and a berry weight of 1.7 g, that represent the average of the berry weight at harvest of the 10 years controls, and the plant density of 2.4 plants/m².

Statistical analysis
The statistical comparison between the 2 plots was performed with a paired t-test.

Results and discussion

Influence of cumulated stress years on plant phenology, plant growth and leaf area
The cumulated stress; 2000 (1 stress year), 2001 (2 stress years) and 2002 (3 stress years), led to no differences on bud burst date and on bud and shoot phenology.

On plant growth the most important effects has been measured on the shoots length (tab. 1) where a significantly difference appeared only at the 3rd stress year with a difference of 3.72 cm, at the control of May 21, and 7.09 cm at the May 30. These results confirm the observations made comparing the effect of a high stress in a untreated canopy plot with a normally treated plot during the following season (Jermini et al., 2000).

No difference was found on leaf number/shoot (main and lateral leaves), on number of lateral shoots/main shoot and on number of shoots/plant.
Table 1. Shoot growth, expressed in cm, measured in the MFS (minimal fungicide strategy) and SS (standard schedule) plots during the 3 stress years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Stress Year</th>
<th>May 19</th>
<th>June 2</th>
<th>May 21</th>
<th>June 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average (cm)</td>
<td>s</td>
<td>Average (cm)</td>
<td>s</td>
<td>Average (cm)</td>
<td>s</td>
</tr>
<tr>
<td>2000</td>
<td>1st stress year</td>
<td>40.33</td>
<td>4.85</td>
<td>76.19</td>
<td>6.58</td>
</tr>
<tr>
<td></td>
<td>MFS</td>
<td>40.55</td>
<td>3.40</td>
<td>76.24</td>
<td>6.63</td>
</tr>
<tr>
<td>2001</td>
<td>2nd stress year</td>
<td>39.96</td>
<td>3.95</td>
<td>68.44</td>
<td>4.76</td>
</tr>
<tr>
<td></td>
<td>MFS</td>
<td>40.45</td>
<td>2.91</td>
<td>67.79</td>
<td>6.63</td>
</tr>
<tr>
<td>2002</td>
<td>3rd stress year</td>
<td>47.24</td>
<td>1.31</td>
<td>80.23</td>
<td>2.59</td>
</tr>
<tr>
<td></td>
<td>MFS</td>
<td>50.96</td>
<td>1.69</td>
<td>87.32</td>
<td>4.76</td>
</tr>
</tbody>
</table>

The differences were statistically significant for the 2000 and 2001 stress years, with 1 P = 0.022 and 2 P = 0.031.

A second effect was observed from the 2nd stress year on with a decrease of leaf area measured in June on the main and lateral leaves (tab. 2). However these differences were not statistically significant.

Table 2. Leaf area expressed as cm² of the main and lateral leaves in the MFS (minimal fungicide strategy) and SS (standard schedule) plots during the 3 stress years. Measurements made at June 5 for 2000, June 15 and 27 for 2001 and respectively 2002. In the MFS plot were chosen plants without downy mildew symptoms.

<table>
<thead>
<tr>
<th>Year</th>
<th>Main leaf area (cm²)</th>
<th>Lateral leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>MFS 118.84 16.02</td>
<td>19.82 7.65</td>
</tr>
<tr>
<td></td>
<td>SS 114.53 16.58</td>
<td>17.73 3.29</td>
</tr>
<tr>
<td>2001</td>
<td>MFS 126.12 15.86</td>
<td>22.19 5.70</td>
</tr>
<tr>
<td></td>
<td>SS 138.19 28.12</td>
<td>28.88 6.60</td>
</tr>
<tr>
<td>2002</td>
<td>MFS 155.57 25.92</td>
<td>39.34 3.69</td>
</tr>
<tr>
<td></td>
<td>SS 170.51 27.19</td>
<td>41.97 7.21</td>
</tr>
</tbody>
</table>

Influence of cumulated stress years on yield quantity
The plant productivity was defined in late July as estimation of the potential yield, because before onset of ripening the crop load was thinned to a potential of 1 kg/m² in all plots according to the production limit of our country. Differences have been observed on plant fertility, expressed as number of cluster/shoot, only in 2001. The estimated potential yield shows a significantly decrease from the 2nd stress years by 0.290 kg/m² and by 0.392 kg/m² after 3rd stress years. Despite these differences the potential productivity in the MFS plot was still 0.500 kg/m² higher than the production limit of 1 kg/m².
Table 3. Shoot fertility expressed as number of clusters per shoot and potential yield quantity estimated at the end of July in the MFS (minimal fungicide strategy) and SS (standard schedule) plots for the stress years 2000-2002.

<table>
<thead>
<tr>
<th></th>
<th>Fertility (clusters/shoot)</th>
<th>Yield (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average s</td>
<td>Average S</td>
</tr>
<tr>
<td>2000</td>
<td>MFS 1.64 0.094</td>
<td>1.572 0.149</td>
</tr>
<tr>
<td></td>
<td>SS 1.60 0.126</td>
<td>1.610 0.152</td>
</tr>
<tr>
<td>2001</td>
<td>MFS 1.461 0.264</td>
<td>1.5422 0.289</td>
</tr>
<tr>
<td></td>
<td>SS 1.571 0.158</td>
<td>1.8322 0.188</td>
</tr>
<tr>
<td>2002</td>
<td>MFS 1.79 0.079</td>
<td>1.5103 0.039</td>
</tr>
<tr>
<td></td>
<td>SS 1.73 0.110</td>
<td>1.9023 0.218</td>
</tr>
</tbody>
</table>

P 1 = 0.043
P 2 = 0.003
P 3 = 0.001

Therefore, the influence on yield quantity can be considered the main impact resulting from the stress caused by downy mildew infections of the prior years in the MFS plot.

This effect, with the differences observed in the shoot growth (tab. 1), could considered the consequence of repeated series of years with important downy mildew epidemics, which have induced a “cumulated” stress and a reduction of carbohydrate reserves of the plants, which was not sufficient in the following year to cover the requirements of the plant for its maximal productivity during the pre-bloom period until fruit set period (Koblet et al., 1993; Jermini et al., 2000).

Nevertheless, we should consider that each growing season is independently from the previous one, because the reconstitution or depletion of the reserve pool depends of the plant stress during ripening period (e.g. years with a high downy mildew severity versus years without relevant down mildew). Our previous results (data not showed) are in according with experiments made from the Koblet et al. (1993), in which these authors show that a season without stress is enough to turn back the carbohydrates reserves to a normal level.

However these results indicate that the impact of the downy mildew stress caused by the incomplete control of P. viticola with the MFS on the plant growth and productivity is small enough to still allow sufficient photosynthesis to maintain productivity above the limit of 1 kg/m². Therefore the impact during the three years on reserves was insufficient to impede mobilisation of reserves to compensate for foliage damage as it did not result at harvest in any loss and therefore the risk due to the application of the MFS over several years is minimal.

Acknowledgements

The authors would like to thanks S. Rigamonti, K. Besomi and A Sassella for help in the data collection.

References


Jermini, M., Blaise, Ph. & Gessler, C. 2001: Quantification of the influence of *Plasmopara viticola* on *Vitis vinifera* as a basis for the optimisation of the control. – IOBC/wprs Bulletin 24(7): 37-44.

Application of the Minimal Fungicide Strategy for the control of the downy mildew (*Plasmopara viticola*): effect on epidemics and yield quantity and quality

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Abstract

Prior studies of the interactions between downy mildew (*Plasmopara viticola*) and the grapevine has permitted to propose a minimal fungicide strategy (MFS), which consists in treatments during the early epidemic phase to delay epidemic under a proposed economic injury level of 5% severity at the beginning of veraison. During the period 1999-2002 the impact of the MFS on epidemic, yield quantity and quality was studied in comparison with a standard schedule (SS) and an untreated plot. The MFS application has permitted to reduce between 43% and 67% the number of fungicide applications in comparison with the SS under downy mildew conducive conditions. The disease severity measured on the leaf canopy was, at the middle of August, limited between 13.1% and 2.0% indicated a delay of the epidemic phase. In the untreated plots the severity was at the same period greater than 30% with a total yield damage. At harvest, the yield in the MFS plot did not differ statistically, with the exception of 1999 and 2001, from that of the normally treated plot. The must soluble solids contents were lower of 0.96°Brix for 1999, 0.5°Brix for 2000 and 2001 and 0.1°Brix for 2002. The difficulty in the fungicide application timing in the MFS was determined from the lack of knowledge on the role of primary and secondary infection in the epidemic development. Their quantification and implementation in a quantitative forecasting model, that integrates the yield formation and their interactions with the disease, is the basis for optimally application of the MFS.

Key words: Downy mildew, *Plasmopara viticola*, epidemic, yield, fungicide reduction

Introduction

The downy mildew (*Plasmopara viticola*) is the major disease in the vineyards of the South part of Switzerland. The recurrent regular leaf damages make the downy mildew the key pathogen in the spray schedule of the vine-growers, which, for its control, apply a preventive strategy consisting in 7-9 fungicide application from May until middle of August.

During the period 1994-1998 we have quantified the interactions between downy mildew and the grapevine analysing the damage and the compensation mechanisms (Jermini *et al.*, 2001). This approach has permitted to propose a minimal fungicide strategy based on a first treatment at the appearance of the first symptoms, to avoid yield quantity losses. At this first treatment follows one or two additional fungicide applications during the early epidemic phase with the aim to delay epidemic under a economic injury level of 5% severity at the veraison to avoid plant stress during the first ripening phase and consequently yield quality damages (Jermini *et al.*, 2001).

Until now the MFS has been applied only on a little plot with a significant reduction of the fungicide application without negative influences on yield quantity and quality (Jermini et
The validation of these results necessitates the application of this strategy for some years on the same plot to evaluate its possible negative influences over the years on the plant, the epidemic and the yield.

Therefore, the aim of this work is to verify the effect of the minimal fungicide strategy, applied on the same plot for the period 1999-2002, on the downy mildew epidemics and its impact on yield quantity and quality.

**Material and methods**

**Plant material and experimental design**

The experiment was conducted in South part of Switzerland in a 10 years old Merlot grapevines plot grafted on 3309 rootstock, trained to a double Guyot and with a vine spacing of 2.00 x 1.20 m between and respectively within the rows.

The experimental design consisted in the comparison of two treatments: minimal fungicide application strategy (MFS) and standard schedule (SS), which corresponds to the normal preventive downy mildew control applied in the vineyard. An untreated control has served as verification of the damage potential of the downy mildew. Each treatment consisted in a plot of 4 rows with 61 plant/row for an amount of 244 plants divided in 8 sub-plots. This experimental design has maintained for the period 1999-2002.

**Application of the Minimal Fungicide Strategy**

The fungicides application in the MFS was defined without the support of a model. The spraying decisions were based on:

- first fungicide application; after the discovery of the first downy mildew sporulation in the plot and considering the weather forecast;
- following treatments: 1) epidemic progress, defined through regularly visual controls of the disease incidence in the plot, considering the appearance of new symptoms and the increase of infected leaves in the plot areas where the first sporulation were found; 2) the time passed from the last fungicide application and the disease incidence increase observed during that period 3) weather (past and forecast);
- the possible last fungicide application is middle of August (last term in Switzerland admitted for the application of organic fungicides),

To avoid bias by effects of other pathogens (powdery mildew and black rot) the minimal fungicide strategy plot was treated during the seasons with ISS fungicides.

**Disease assessment**

Between 13 and 16 representative shoots (1 shoot every 2 plants) per each sub-plot was selected at the phenological stadium E before the beginning of the downy mildew epidemic. Disease severity was assessed on these shoots, with non-destructive methods at the finding of the first sporulation, after the following treatments and at the veraison. Disease severity was estimated with an extended Horsfall scale (Horsfall and Cowling, 1978), in which the first class, 0-3% damaged area, was divided in two new classes from 0-1% and 1-3% of damaged area to avoid an overestimation of the low diseased levels. The disease severity on clusters was estimated during the second half of July before grape load adjustment.

**Yield parameters**

Number of shoots per plant, including the spurs, has been regulated before bloom. The expected yield quantity has been estimated at the end of July and grape load adjusted to a theoretical production of 1 kg/m² for each sub-plot, corresponding at the production limit admitted in our country.

At vintage each plot was harvested individually and yield components analysed.
Statistical analysis
The statistical comparison between the 2 plots was performed with a paired t-test.

Results and discussion

Impact of the minimal fungicide strategy on epidemics
In the 1999, the first downy mildew symptoms were found on June 2. The first fungicide application in the MFS was made only on clusters at June 4 with a disease severity on leaf from 0.0013% (tab. 1). Unfortunately, an important rainfall period during June (17 days with 321.4 mm) has induced a rapidly increase of the epidemic and the second treatment has been applied at June 30 with a severity of 1.48%. A third and fourth treatment, made at July 29 and respectively at August 17, have not sufficiently to keep the epidemic under a EIL of 5% at the beginning of August in correspondence with the veraison (tab 1) and to avoid a damage of 34.7% severity on clusters (tab 2). This first application year of the MFS demonstrates the necessity to avoid, after the finding of the first sporulation, new primary infections, and consequently a early disease severity increment, with a risk to select rapidly fit genotype which are responsible of the disease spread in the plot (Gobbin et al., 2003). In the SS plot 7 treatments was applied during the season.

In the 2000, the first downy mildew symptoms were found on May 24 and the climatic conditions from this date until middle of August has been favourable to the downy mildew epidemic with 23 potentially infection periods (determinate from a Lufft warning system), corresponding at 1 infection every 4 days. Three fungicides applications (May 26, July 5 and August 11) have been made in the MFS plot against the 9 of the SS plot with a reduction of 67% of the treatments. Contrary to 1999, the first treatment has been applied immediately after the finding of the first sporulation and has permitted to delay of the epidemic, which was at the veraison under the EIL (tab 1). The cluster damage severity was only of 0.6% (tab. 2). Contrary, in the untreated plot the damage was of 37.7% on leaf at August 18 and of 30.2% on June 30 on clusters.

Table 1. Severity of the downy mildew epidemics expressed as % of damaged leaf area/shoot for the years 1999-2002 evaluated at the first treatments, made at the apparition of the first sporulation, until veraison.

<table>
<thead>
<tr>
<th>Date</th>
<th>Severity (%)</th>
<th>Date</th>
<th>Severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td></td>
<td>2001</td>
<td></td>
</tr>
<tr>
<td>June 9</td>
<td>0.0013</td>
<td>May 30</td>
<td>0.00040</td>
</tr>
<tr>
<td>July 1</td>
<td>1.48</td>
<td>June 27</td>
<td>0.00031</td>
</tr>
<tr>
<td>July 30</td>
<td>5.07</td>
<td>August 13</td>
<td>13.03</td>
</tr>
<tr>
<td>August 13</td>
<td>13.07</td>
<td></td>
<td>4.76</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>2002</td>
<td></td>
</tr>
<tr>
<td>June 2</td>
<td>0.038</td>
<td>June 3</td>
<td>7.46E-8</td>
</tr>
<tr>
<td>July 17</td>
<td>0.057</td>
<td>June 26</td>
<td>0.00093</td>
</tr>
<tr>
<td>August 10</td>
<td>2.04</td>
<td>July 9</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>1.222</td>
<td>September 9²</td>
<td>13.62</td>
</tr>
</tbody>
</table>

² Control made at the end of the first ripening period

During the 2001, 42 potentially infection periods, corresponding at 1 infection every 2, have been detected from the apparition of the first sporulation at the May 22 until August 18.
Four fungicide applications were made in the MFS (June 7 and 26, July 30 and August 14) and 8 in the SS plot. The epidemic was maintained under control until end of June and no damages were found on clusters (tab. 2). From end of July the disease increased rapidly and at the middle of August the severity was 13% (tab. 1). Severity was clearly lower than in the untreated plot, where at 14 August the severity was 86.06%. The genetic analysis made during this year in the MSF plot has indicated that probably this epidemic behaviour is due to an important migration of fit genotypes from the untreated plots (Gobbin et al., 2003).

In the 2002, like 2001, from the appearance of the first symptoms, June 3 until August 18, 42 potentially infections periods were registered, corresponding at 1 infection every 2 days. In the MFS 4 treatments have been applied (June 7, July 4 and 17 and August 2) and 7 in the SS plot. The MFS has permitted an important delay of the epidemic (severity of 35.7% in the untreated plot at August 14), which has increased only during the ripening period (tab. 1) with a low damage on clusters (tab. 2).

For each experimental year, the disease severity measured on the leaf canopy was, at the middle of August, limited between 13.1% and 2.0%, which indicates a delay of the epidemic phase. In the untreated plots the severity was at the same period greater than 30% and the SS plot showed only sporadic lesions. An exception is the 1999, where the choice to begin the leaf protection at a severity of 1.48% has demonstrated the impossibility to manage the epidemic. The difficult for the fungicide application decision in the MFS is due to the important and rapidly increases of the epidemic during August and particularly on lateral shoots.

The selection in the MFS of fit genotypes and the migration of these from untreated plots (Gobbin et al., 2003) in immediate proximity is probably at the origin of this epidemic increase. Consequently, the application of a fungicide in August was practically obligatory to preserve the leaf area of the lateral shoots, which is responsible to the carbohydrate production for the berry ripening (Candolfi-Vasconcelos, 1990).

Table 2. Severity of the downy mildew damage on clusters expressed as % of damaged cluster of the SS (standard schedule), MFS (minimal fungicide strategy) and untreated plot. The results reported for the untreated plots corresponds to a single plot for 1999 and at the average of 4 replications for the others years. The controls have been made at the August 2 for 1999, June 30, July 11 and July 15 for 2000 and respectively 2001 and 2002.

<table>
<thead>
<tr>
<th>Date of control</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.2% ± 0.27</td>
</tr>
<tr>
<td>MFS</td>
<td>34.7% ± 4.201</td>
<td>0.6% ± 0.47</td>
<td>0.0%</td>
<td>17.5% ± 8.03</td>
</tr>
<tr>
<td>Untreated plot</td>
<td>94.7%</td>
<td>30.2% ± 8.25</td>
<td>48.5% ± 19.40</td>
<td>59.1% ± 19.10</td>
</tr>
</tbody>
</table>

1 Standard deviation

In 1999, despite the difficult in the downy mildew epidemic management, the disease severity of 34.7% on cluster was evaluated acceptable, because the estimated yield quantity production, considering the yield due to downy mildew infection, was of 1.424 kg/m², 0.424 kg/m² higher than the production limit of 1 kg/m² standing in our country. For the same reason the 17.5% damage of 2002 did not influenced directly the yield quantity because the potential yield production was 0.245 kg/m² higher than the production limit. In all experimental years we had reduced the cluster number to attaint 1 kg/m².
At harvest, the yield quantity in the MFS plot differs significantly from that of the normally treated plot for the years 1999 and 2001 with a difference of 37.9% and 14.4% for 1999 and respectively 2001 (tab. 3). Generally, with the exception of 1999, the yield quantity differences were due to the difficulty to reduce yield to a maximum of 1 kg/m² by eliminating the appropriate the number of cluster/plant. In both treatments equal reduction was made, which in the case of MFS lead to too severe reduction. During the experimental years 2000 until 2002 no yield was harvested in the untreated plots.

Table 3. Results of the most important yield component at harvest (date of harvest: September 29 for 1999, September 22, September 26 and October 2 for 2000, 2001 and respectively 2002) from minimal strategy (MFS) and standard schedule (SS) plots.

<table>
<thead>
<tr>
<th></th>
<th>Plot</th>
<th>Yield (kg/m²)</th>
<th>Must soluble contents (°Brix)</th>
<th>Total acidity (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
<td>Average s</td>
<td>Average</td>
</tr>
<tr>
<td>1999</td>
<td>MFS</td>
<td>0.820</td>
<td>0.077</td>
<td>18.08</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>1.320</td>
<td>0.067</td>
<td>19.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = &lt; 0.001</td>
<td>P = 0.03</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>2000</td>
<td>MFS</td>
<td>1.160</td>
<td>0.198</td>
<td>19.29</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>1.112</td>
<td>0.262</td>
<td>19.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.44</td>
<td>P = &lt; 0.001</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>2001</td>
<td>MFS</td>
<td>0.793</td>
<td>0.212</td>
<td>19.44</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>0.926</td>
<td>0.257</td>
<td>19.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.03</td>
<td>P = &lt; 0.001</td>
<td>P = 0.39</td>
</tr>
<tr>
<td>2002</td>
<td>MFS</td>
<td>0.865</td>
<td>0.101</td>
<td>19.70</td>
</tr>
<tr>
<td></td>
<td>SS</td>
<td>1.016</td>
<td>0.196</td>
<td>19.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P = 0.067</td>
<td>P = 0.155</td>
<td>P = 0.04</td>
</tr>
</tbody>
</table>

The must soluble solids contents was significantly lower for 1999, 2000 and 2001 in comparison with the SS plot with a difference of 0.96°Brix for 1999, 0.5°Brix for 2000 and 2001 (tab. 3) corresponding to a reduction of 5% and respectively 2.5%. The damages observed in 1999 was amplified by late topping in August of the plants with a consequently elimination of an important parts of healthy leaf area of the lateral shoots. For the others years no topping was applied in the MFS plot after the initiation of the veraison. The total acidity was significantly higher in the MFS plot with exception of 2001 (tab. 3).

These results indicate that under critically epidemic conditions, the application of the MFS permits a reduction between 43% and 67% of the number of fungicide application without important negative influences on yield quantity and quality. The optimally application of MFS necessities the quantification of the role of primary and secondary infection in the epidemic development and their implementation in a quantitative forecasting model that integrates, besides the epidemic development, the yield formation and their interactions with the disease. MFS is a good basis for the full application of the IP concept. However the reduction of yield to the imposed maximal quantity still appears as a difficult task under the MF strategy, as is difficult if not impossible to account for the reduction caused by downy mildew in these plots.
Acknowledgements

The authors would like to thank S. Rigamonti, K. Besomi and A Sassella for the help in the data collection and the laboratory of the RAC Changins for the yield quality analysis.

References


Jermini, M., Blaise, Ph. & Gessler C. 2001. Quantification of the influence of Plasmopara viticola on Vitis vinifera as a basis for the optimisation of the control. IOBC/wprs Bulletin 24(7): 37-44.
Influence of the overwintering methods on the germination dynamic of downy mildew (*Plasmopara viticola*) oospores

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Abstract

During the years 2001 and 2002, the oospore germination was followed with the aim to quantify the macrosporangia production in time. Leaf disks were cut from oospores colonized zones of leaves collected the previous year in vineyard and arranged in Agryl bags. Samples of 2000 were covered with 1-2 cm sand. The 2001 samples were exposed to the natural atmospheric conditions on ground. From bud burst of grapevine on, samples were weekly brought into the lab and left under ideally climatic conditions to stimulate the oospore germination and controlled daily. The macrosporangia observed were counted and destroyed. In 2001, sampling started April 10 and the macrosporangia emission produced the 81.8% of the total of counted at the end of the season. The other samples gave the 9.5% and the 6.2% respectively of the total macrosporangia production. These results were apparently in contrast with the genetic population studies of downy mildew, which showed an important primary infection during the whole season. In 2002, contrary to previous year, the germination dynamic was in accordance with the data from the genetic population analysis. The difference between 2001 and 2002 consisted in the overlaying with sand resp. letting the leaves disk overwintering more naturally. We supposed that the concept of a homogeneous ripening of the oospores during winter could be a methodological artifact and we proposed the necessity to develop and standardize a methodology better reflecting the field conditions in which the oospores wintering. The results indicated that the oospore field population was not homogenous but composed from different age-physiological cohorts, which correspond at different ripening levels modulated by the climatic conditions and possibly by leaf effects.

Key words: *Plasmopara viticola*, oospore, germination, macrosporangia production

Introduction

The biological cycle of downy mildew (*Plasmopara viticola*) comprises an asexual multiplication phase during the vegetative period and a sexual phase that ensures the survival of the pathogen over winter. The sexual stage has been considered, until now, solely and only responsible for the initiation of the disease in the spring with little quantitative relevance. The asexual phase is considered the multiplication phase and responsible for the dispersion of the disease in time and space. Recent studies on the genetic populations of downy mildew (Gobbin et al., 2001, 2003; Pertot et al., 2003) showed that, contrary to what has been assumed until now, infections due to oospores are not only important for the initiating the disease, but they play a significant role in the epidemic development.

Little is known on the temperature and humidity requirements of formation, and maturation of oospores. Minimal temperature sums until first germination has been indicated by Gehman (1987), however starting from an arbitrary chosen biofixe. Most research was
dedicated to the time lap necessary for a final maturation of oospores at controlled temperature starting from a précis date with the scope to predict the time still necessary until the first germination in nature could occur (Hill et al., 1994 and Tran Manh Sung, et al. 1990). No indication is available on the influence of the time of formation or on the particular microclimatic conditions of overwintering on the maturation and therefore on the potential germination moment. This lack of knowledge impedes any quantitative prediction of this event neither in time and in its extension. The lack of precise quantitative knowledge of the oospore stage represents currently the main obstacle to an accurate forecast of downy mildew epidemic. The aim of this study is therefore that to have a first approach to a quantification of the macrosporangia production in the time from bud burst of the grapevine in field with the obvious shortcoming that variability of the time of formation and true overwintering conditions are not considered.

Material and methods

Sample preparation and overwintering method
Leaves with downy mildew sporulation and necrosis were collected in vineyard during the first weeks of October on Merlot grapevine. The colonized leaf areas were controlled with a binocular for the presence of oospores. On these areas leaf disks from 1 cm diameter were cut and arranged in Agryl bags. Each bag contained 40 leaf disks. Samples of 2000 were covered with 1-2 cm sand and that of 2001 were only left outside on ground.

Oospore incubation and method of control
From bud burst of grapevine on, each week a sample consisting 10-12 leaf disks was brought into the lab. Leaf disk were disposed on wetted blotting paper in a Petri-dish and kept at 18-20°C for the oospore germination. Samples were controlled daily. The macrosporangia observed were counted and destroyed with a needle. Observation of each sample set was continued until no more germination occurred for at least two days.

Results and discussion

In 2001, the first samples were brought into the lab April 10 and on this samples the macrosporangia emission began after a day of incubation and finished after 22 days, producing the 81.8% of the total of macrosporangia counted at the end of the season and with, in average, a production of 176.4 macrosporangia per leaf disk (tab. 1). The samples of April 23 and May 2 gave the 9.5% and the 6.2% respectively of the total macrosporangia produced in the season with an average production of macrosporangia per leaf that was 10 times lower than that observed for the first simple of April 10 (tab. 1). Later samples only showed sporadic germination and no germination was found from June 27 onwards.

In 2002 the first macrosporangia were found in the sample of April 23. However, also in the later samples up to July germination was observed. The sample of April 23 produced the 15.5% of the total macrosporangia production, followed with the 18.2%, 23.4%, 20.2% and 21.8% of the sample fro May 16, 22 28 and June 6 (tab. 2).

The average of macrosporangia counted per leaf disk in these five periods varied between the 26 of the May 22 and the 11 of the April 23 series (tab. 2). Like for 2001, the samples of April produced for the longest time period macrosporangia.

The results of 2001 are apparently in contrast with the genetic population studies (Gobbin et al., 2001) of downy mildew and particularly with the analysis made in our minimal fungicide strategy plot (Gobbin et al., 2003), which shows that important primary infections occur during the whole season.
Table 1. Macrosporangia production in leaf-disk collected in 2000 and overwintered outdoors under 1-2 cm of sand. The data represents the mean of 10 leaf-disk.

<table>
<thead>
<tr>
<th>Date of sample analysis</th>
<th>Number of days until first sporangia was observed / Duration macrosporangia production (days)</th>
<th>Macrosporangia produced per leaf disk</th>
<th>% of the total macrosporangia produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 10</td>
<td>1 / 22</td>
<td>176.4</td>
<td>81.79</td>
</tr>
<tr>
<td>April 23</td>
<td>14</td>
<td>18.3</td>
<td>9.53</td>
</tr>
<tr>
<td>May 2</td>
<td>11</td>
<td>12.3</td>
<td>6.24</td>
</tr>
<tr>
<td>May 7</td>
<td>13</td>
<td>3.7</td>
<td>1.73</td>
</tr>
<tr>
<td>May 14</td>
<td>7</td>
<td>0.8</td>
<td>0.42</td>
</tr>
<tr>
<td>May 28</td>
<td>9</td>
<td>0.3</td>
<td>0.17</td>
</tr>
<tr>
<td>June 6</td>
<td>1</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>June 11</td>
<td>1</td>
<td>0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Contrary, the results of 2002 are in accordance with the data from the genetic population analysis. The difference between 2001 and 2002 consisted in the overlaying with sand resp. letting the leaves disk overwintering more naturally. Therefore, we can suppose that the concept of a homogeneous ripening of the oospores during winter could be a methodological artifact. To avoid this problem, it is necessary to develop and standardize a methodology better reflecting the field conditions in which the oospores wintering.

Table 2. Macrosporangia production in leaf-disk collected in 2001 and overwintered outdoor overlaying with letting. The data represents the mean of 12 leaf-disk.

<table>
<thead>
<tr>
<th>Date of sample analysis</th>
<th>Number of days until first sporangia was observed / Duration macrosporangia production (days)</th>
<th>Macrosporangia produced per leaf disk</th>
<th>% of the total macrosporangia produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 23</td>
<td>21</td>
<td>12.1</td>
<td>15.49</td>
</tr>
<tr>
<td>May 7</td>
<td>1</td>
<td>0.20</td>
<td>0.26</td>
</tr>
<tr>
<td>May 16</td>
<td>9</td>
<td>14.2</td>
<td>18.80</td>
</tr>
<tr>
<td>May 22</td>
<td>7</td>
<td>18.3</td>
<td>23.43</td>
</tr>
<tr>
<td>May 28</td>
<td>7</td>
<td>15.8</td>
<td>20.23</td>
</tr>
<tr>
<td>June 6</td>
<td>7</td>
<td>17.0</td>
<td>21.77</td>
</tr>
<tr>
<td>June 11</td>
<td>4</td>
<td>0.30</td>
<td>0.38</td>
</tr>
<tr>
<td>June 18</td>
<td>1</td>
<td>0.10</td>
<td>0.13</td>
</tr>
<tr>
<td>June 25</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>July 2</td>
<td>1</td>
<td>0.10</td>
<td>0.13</td>
</tr>
</tbody>
</table>
The germination dynamic of the two years, and particularly for 2002, show that after mid-June the germination level is very low and from mid-July it does not occur anymore. These results confirm previous observations (Cortesi and Zerbetto, 1994, Hill et al., 1997).

Gehmann (1987) has showed that oospore formation can be distributed all over the season, which then leads to a very inhomogeneous population of oospores entering in winter. Our data shows that the oospores present in a single leaf-disk and between leaf-disk need quite different time laps until the germinate even under optimal conditions with the greatest variance in the early samples (from one up to 22 days) Also a high variance is present in the number of macroporangia produced per leaf disk during the sporulation period inside and between the different series. The variability inside a particular series may be attributed to a different number of oospores present per leaf-disk, the decrease noted between series in correspondence to the progressing of the season may be attributed to progressing sporulation occurring also on the storage site and possibly to the progressive decay. These results indicate clearly that this inhomogeneous population of oospores entering in winter is also maintained during the sporulation period. Therefore we can suppose that the oospores field population is composed from oospores distributed in different age-physiological cohorts, which correspond at different ripening levels and modulated by the climatic conditions and possibly by leaf effects. The quantification of oospores maturation and germination and their relation to the effective primary infection is therefore a need for the realization of a quantitative forecasting model and the optimally application of the minimal fungicide strategy. Our experimental set up in 2002 corresponded better to the natural conditions, however they are still far from the real variability to which leaf debris may subjected so that it can be assumed that oospore germination variability in time goes well beyond the two to three month registered in the 2002 trial.

Acknowledgements

The authors would like to thank S. Rigamonti and K. Besomi for the help in the laboratory controls.

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Studies on *Plasmopara viticola* oospore germination in Trentino, Italy

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**Abstract:** Oospores are the driving inoculum for *Plasmopara viticola* primary infections on grapevine, but their correlation with the severity of primary infection is still debated. The oospores germination was studied since 1998 in an experimental untreated vineyard located in Adige Valley, Trentino, Italy, with a sensitive floating disk test, following the method proposed by Hill. The method was developed to detect the production of very low number of zoospore deriving from oospore germination and it is based on the use of leaf disks floating on crushed infected leaves material, which was maintained in natural condition in the vineyard. Meteorological data (precipitation, relative humidity and temperature) and leaf wetness periods were recorded and the appearing of the oil spots in the vineyard was daily checked. In the four year study high germination rate was recorded during the period in which the first infections usually take places in Trentino and which corresponds to high grapevine susceptibility to the disease. The germination rate stayed high for a long period, generally till mid summer, permitting primary infections to be possible for long time, as it was stated by genetic studies on *P. viticola*. At the beginning of the season, oospores germination is not simultaneous and they were able to germinate for some days, but all oospores germinated in less then 24 hours during the periods when primary infections usually took place in Trentino. No prediction of infection occurrence and severity could be made from the germination test, as previously stated in Germany.

**Key words:** *Plasmopara viticola*, oospore germination, floating disk test.

**Introduction**

Oospores are the driving inoculum for *Plasmopara viticola* primary infections on grapevine, but their correlation with the severity of primary infection is still debated. The oospores germination was studied since 1998 in an experimental untreated vineyard located in Adige Valley – Trentino (north-eastern Italy), with a sensitive floating disk test, following the method proposed by Hill (Hill, 2000). The method was developed to detect the production of very low number of zoospore deriving from oospore germination and it is based on the use of leaf disks floating on crushed infected leaves material, which was maintained in natural condition in the vineyard.

**Materials and methods**

*P. viticola* infected leaves were collected in an untreated vineyard at the beginning of September and crushed in a blender. Resulting mud was put on the surface of small holed boxes (used in cheese industry) filled for 2/3 with sand. Boxes were buried in vineyard soil, in such a way as the treated leaf material was at the same level of the ground. Starting from March a fixed amount of leaf material was collected and placed into boxes with floating leaf disks of healthy grapevine. Leaf disks were daily changed and left in humidity chambers for 4-5 day for evaluation of possible infections occurrence. Meteorological data (precipitation,
relative humidity and temperature) and leaf wetness periods were recorded and the appearing of the oil spots in the vineyard was daily checked.

Results and discussion

In the four year study, a high germination rate (fig. 1) was recorded during the period in which the first infections usually take places in Trentino and which corresponds to a high grapevine susceptibility to the disease. The germination rate stayed high for a long period, generally till mid summer, allowing primary infections to be possible for long time, as it was stated by genetic studies on \textit{P. viticola} (Gessler et al., 2002). At the beginning of the season, oospores germination is not simultaneous and they were able to germinate for some days (fig. 2, upper graph and fig. 3), but all oospores germinated in less than 24 hours during the periods when primary infections usually took place in Trentino Region (fig. 2, central graph and fig. 3). No prediction of infection occurrence and severity could be made from the germination test.

Fig. 1. Time for oospore germination in the four years study
Fig. 2. Oospore germination during 2002, period with no disease susceptibility (up), period in which usually primary infections take place in Trentino (centre) and last part of the primary infection period (low).
Fig. 3. Days required for oospore germination in year 2002.

Acknowledgements

The authors kindly acknowledge G. Hill for the precious information on the floating leaf disk method and A. Vecchione, F. De Luca, M. Delaiti for helping in performing the tests.

This work was funded by the Fund for Research of the Autonomous Province of Trento, Italy, Research project AGRIBIO.

References


Occurrence of *Plasmopara viticola* primary and secondary infections in the early stage of the season in Northern Italy (Trentino)

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² Phytopathology Group, Institute of Plant Sciences, Swiss Federal Institute of Technology, 8092 Zurich, Switzerland

Abstract: In areas where the climate is warm and wet during the growing season, downy mildew, caused by *Plasmopara viticola*, represents the most destructive grapevine disease. Although it is one of the most studied grape pathogens, many epidemiological aspects are still unknown, in particular the quantitative contribution of oosporic-derived primary infections vs. secondary (asexual) infections. In this study the early stage epidemic in an Italian vineyard (Navicello, Trentino, Italy) was analyzed. This study contradicts current accepted theory about downy mildew disease dynamics. It was clearly shown that infections occurring during the early epidemic stages were oosporic-derived and, that single genotypes don’t multiply significantly enough to cause an important damage at the end of summer.

Key words: *Plasmopara viticola*, grapevine, epidemiology.

Introduction

In areas where the climate is warm and wet during the growing season, downy mildew, caused by *Plasmopara viticola*, represents the most destructive grapevine disease. Although it is one of the most studied grape pathogens, many epidemiological aspects are still unknown, in particular the quantitative contribution of oosporic-derived primary infections vs. secondary (asexual) infections. In this study the quantitative contribution of the primary infections derived from oospores in relation to the contribution of the secondary asexual infections to the early stage of the epidemic in an Italian vineyard was analyzed. Distinction between primary and secondary infections could be based on the assumption that *P. viticola* strains having different SSR allele pattern derive from different sexually produced oospores, whereas strains presenting identical SSR allele pattern derive from the same oospore (asexual reproduced clones).

Materials and methods

The study was performed in the early stage epidemic in an Italian vineyard (Navicello, Trentino, Italy).

In 2000 a plot (4 rows x 33 vines) was maintained untreated within an experimental vineyard weekly treated from F stage (Baggiolini) against downy mildew. As soon as symptoms appeared, a half of each visible oil spot was collected for genetic analyses (samplings: 15th May, 22th May and 30th May). Two additional sampling were performed on 21th June and 6th July but only 1-2 oil spots per vine were collected. Exact locations of lesions in the vineyard were recorded, as well as meteorological data (precipitation, relative humidity and temperature) and leaf wetness.
Oosporic germination potential (number of days necessary for germination) was evaluated using the floating disc test proposed by Hill (2000).

SSR analyses were perform according to the method proposed by Gobbin et al. (2003).

Fig. 1. Oospore germination rate: days that are necessary for germination with the floating disk test.

Fig. 2. Average temperature (Tm), rain, leaf wetness (LW) symptom appearing, estimated infections sampling date and sporulation, in the first stage of the epidemic in the studied vineyard.
Results and discussion

Floating disk test revealed that primary infections were possible until 14th June (fig. 1).

The first 6 lesions that were recognized by the observers appeared on 13th of May. They were all genetically different, therefore we assume that they have been generated by six primary infections. Less then 10 mm of rain were enough to start afore mentioned primary infections. Only a single primary lesion gave rise to a clonal progeny detected by successive sampling, while the other 5 were not re-sampled, suggesting their extinction. Similar disease dynamic was revealed by the next two pathogen collections: primary infections were significantly more numerous than secondary infections. Clones of the genotypes, individually considered, showed both aggregation (clones within same plant) and wider dispersion (>10 plants distance).

The primary infection contribution during the first epidemic stages was very high (63-77 %) (fig. 3). In the last two samplings, we detected only few genotypes that were previously identified, that indicates either reduced clonal multiplication or genotype extinction. Less then 10 mm of rain were enough to start a primary infection (fig. 2).

Table 1. Primary and secondary infections in the first stage of the epidemic in the studied vineyard. In the IV and V only a sample of the oil spot present on the plants were collected (respectively 0.5 and 1 for each plant).

<table>
<thead>
<tr>
<th>sampling</th>
<th>date</th>
<th>spots</th>
<th>collected spots</th>
<th>analysed spots</th>
<th>genetically different</th>
<th>primary (%)</th>
<th>genetically identical (clones)</th>
<th>secondary (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.05.00</td>
<td>all</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>100.00</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>II</td>
<td>22.05.00</td>
<td>all</td>
<td>79</td>
<td>68</td>
<td>43</td>
<td>63.24</td>
<td>25</td>
<td>36.76</td>
</tr>
<tr>
<td>III</td>
<td>30.05.00</td>
<td>all</td>
<td>455</td>
<td>347</td>
<td>268</td>
<td>77.23</td>
<td>79</td>
<td>22.77</td>
</tr>
<tr>
<td>IV</td>
<td>21.06.00</td>
<td>0.5 x p</td>
<td>71</td>
<td>71</td>
<td>52</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>06.07.00</td>
<td>1 x p</td>
<td>103</td>
<td>103</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This study contradicts current accepted theory about downy mildew disease dynamics. It was clearly shown that infections occurring during the early epidemic stages were oosporic-derived and, that single genotypes don’t multiply significantly enough to cause an important damage at the end of summer.

Acknowledgements

This work was co-funded by the Fund for Research of the Autonomous Province of Trento, Italy, Research project “AGRIBIO”.

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Expression of hypersensitive reaction to *Plasmopara viticola* infection on a grapevine segregating population

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² DBADP, University of Udine, Udine, Italy

Abstract: All varieties of *Vitis vinifera* are highly susceptible to downy mildew (*Plasmopara viticola*), while resistance/tolerance is shown in many American *Vitis* species. In some American *Vitis* species a defence mechanism, that leads to the quick death of cells near the pathogen penetration site, occurs. This hypersensitive reaction is therefore associated with disease resistance and it is generally visible as a distinct necrosis correlated with the accumulation of autofluorescent compounds, particularly phenolics, and other irreversible cell damages.

To detect Quantitative Trait Loci (QTLs) for resistance to downy mildew and fruit quality, a mapping population derived from an interspecific cross between *V. vinifera* cv. Moscato bianco (susceptible) and an accession of *Vitis riparia* (resistant) was developed. *Vitis riparia* accession shows hypersensitive reaction against *P. viticola*. Classes of phenotypic resistance/susceptibility were identified on the basis of proportion of leaf tissue with sporulation or chlorosis and of extension and severity of hypersensitive response against downy mildew in grape leaves, both after artificial infections done in controlled condition and after field natural infections. These data were applied for QTLs detection by using an interval mapping method.

A small percentage of progeny with hypersensitive flecking were identified, but several phenotypic pattern of hypersensitive reaction were present after natural infections in field. Ultrastructural alterations of these different tissue reactions were evaluated by means of transmission electron microscopy (TEM). Leaves with different symptoms of disease and different phenotypic reactions were collected and prepared for TEM according standard methods. TEM observations showed different ultrastructural alterations according to the phenotypic class of leaf symptoms.

Key words: hypersensitive reaction, TEM, *Plasmopara viticola*, *Vitis* spp.

Introduction

*Plasmopara viticola* (B. et C.) Berl. and de Toni, the grapevine downy mildew pathogen, causes economically a very important disease (Langcake P. and Lovell P.A. 1980).

All varieties of *Vitis vinifera* are highly susceptible to downy mildew (*P. viticola*), while resistance/tolerance is shown in some American *Vitis* species. In some American *Vitis* species a defence mechanism named Hypersensitive Reaction (HR), that leads to the quick death of cells near the pathogen penetration site, occurs.

In plants the classification of this defence mechanism is based mainly on morphological criteria of the resultant cell-death lesions as well as the functional suppression of pathogen growth (Lam *et al.*, 2001).

In several virus, fungus and bacteria–host plant system showing the HR, associated with structural changes in the cells have been observed in the close areas surrounding leaf tissue showing necrotic local lesions (Goodman 1972; Ishihara *et al.*, 2002; Meyer *et al.*, 1988).

The aim of this work is to compare symptoms, corresponding to different levels of susceptibility to *P. viticola*, observed in a mapping population derived from an interspecific
cross between *V. vinifera* cv. Moscato bianco (susceptible to *P. viticola*) and an accession of *Vitis riparia* (resistant to *P. viticola*) naturally infected, to ultrastructural alterations in the cells of the infected host.

**Materials and methods**

This study was conducted in an experimental vineyard located in S. Michele all’Adige (Trento), Italy. Vines were planted in 1996. The adopted training system was simple Guyot. During the summer of 2002 the natural symptoms of downy mildew on the infected leaves were evaluated. Observations were made on the first susceptible six leaves for three shoots in each genotype. Classes of phenotypic resistance/susceptibility were identified on the basis of proportion of leaf tissue with sporulation or chlorosis and of extension and presence of hypersensitive reaction against downy mildew in grape leaves.

Leaf samples of several genotypes infected with *P. viticola* showing HR were analysed at ultrastructural level by means of electron transmission microscopy (Musetti et al., 2000). Leaf samples with typical oil spots with sporulation were compared.

**Results and discussion**

The average of the infected leaf area on the first four leaves in greenhouse and field conditions produced QTL information on linkage groups of the paternal parent (Marino et al., 2002), but on the other side, HR symptoms did not allowed the mapping of the trait, when evaluated as presence/absence on the whole segregating population.

Genotypes showing HR in field can be divided in three groups according ultrastructural alterations.

Some common features in macroscopic symptoms can be found in the three groups:

1. At ultrastructural level, tissue presents an alteration of chloroplasts and thylakoids. Vacuoles are filled with dark phenolic material (Fig. 1B, arrows). *P. viticola* is observed in the substomatal zone and in the intercellular space of spongy parenchyma. Hyphae appear vacuolated (Fig. 1C), but austoria are few and not easy detected. Yellowish, dark or necrotic oil spots with presence of HR on leaves (Fig. 1A).

2. Leaf tissue appears highly damaged. Cells walls are thin and distorted (Fig. 2C), chloroplasts contain starch and lipidic drops, plasmolysis and abnormal vacuolisation. Palisade cells are filled with a dark material (2D, arrows). Small necrotic spots and mosaic spots with HR on leaves. Small necrotic lesions have a concentric development (Fig. 2A and Fig. 2B).

3. TEM observation reveals that the palisade cells have a big phenolic accumulations in the vacuoles (Fig. 3B) and plasmolysis in the spongy parenchymal cells. *P. viticola* has abnormal vacuolisation and necrotic austria surrounded by a big amount of callose (Fig. 3C). Necrotic spots with HR on leaves. This group is constituted by necrotic spot in healthy or in chlorotic tissue with absence of sporulation (Fig. 3A).

(Susceptible) – In this group tissue appears highly damaged, cells are filled with dark amorphous material (Fig. 4B, arrows). Fungus also presents alteration: some hypae and austria have dark and necrotic content (Fig. 4C). Yellowish, dark or necrotic oil spots with sporulation and absence of HR on leaves. (Fig. 4A).

HR evaluation, based only on presence/absence criteria, did not allowed the trait mapping, probably because more than one pathway is involved.
TEM observations confirm that some differences are present after infections at cytological level. Further studies are necessary to better understand differences among different reactions in the whole segregating population.

Fig. 1a. Yellowish oil spots with presence of HR.

Fig. 1b. Ultrastructurally alteration of leaf tissue.

Fig. 1c. Hyphae of *P. viticola*
Fig. 2a. Necrotic punctiform spots with HR
Fig. 2b. Mosaic spots with HR
Fig. 2c. Necrotic punctiform spots with HR
Fig. 2d. Mosaic spots with HR

Acknowledgements

This work was supported by the project “Advanced Biology” funded by the Fondazione delle Casse di Risparmio di Trento e Rovereto, Trento-Italy.
Fig. 3a. Necrotic spots with HR

Fig. 3b. Palizade cells with phenolic accumulations

Fig. 3c. Austoría of *P. viticola* surrounded by callose

References

Fig 4a. Yellowish oil spots with presence of sporulation

Fig 4b. Ultrastructurally alteration of leaf tissue

Fig 4c. Transversal hyphae of *P. viticola*


Early evaluation of grape berry susceptibility to *Botrytis cinerea*

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**Abstract:** Two indicators of susceptibility of berries to *Botrytis cinerea* were identify. They are the acid oses (or soluble pectic compounds in water: SPW) and the phenolic compounds (PC) linked to the parietal structures of the cells in the pellicular skin complex. High quantities of SPW and low quantities of PC correspond to high levels of grey mold in the vineyard. These indicators are relevant very early, as soon as bunch closure. They indicate a general tendency on a great climatic area scale, and they can be taken into account in the development of a rule of decision to control grey mold.

**Key words:** grapevine, grey mold, *Botrytis cinerea*, susceptibility, risk indicators.

**Introduction**

The follow-up of the epidemic development of the grey mold of grapes within the framework of the Epidemiology National Network of Study of this disease reinforced the idea, that the evolution of the skin structure of grape berries plays a key role in the expression of the rot (Prudet, 1994). Three successive theses were carried out on this topic. The study of two model cultivar of vines, sensitive Sauvignon, and Arriloba (crossing of Sauvignon and rafiat of Moncade) tolerant, made it possible to identify two markers of susceptibility of berries to *Botrytis cinerea*. They are the acid oses or soluble pectic compounds in water (SPW) easily available and favorable by the enzymes of *B. cinerea*, and the phenolic compounds (PC) linked to the parietal structures of the cells of the pellicular complex, compounds known for their property inhibiting enzymatic activities. Thus if the quantity of SPW is high and the quantity of PC is weak, potential susceptibility of berries to *Botrytis cinerea* is raised, and conversely. The interest of these two indicators is that they can be given very early (at closure of bunch stage).

**Material and methods**

The method was developed by Chenet (1997). The berries are taken randomly in a reference plot of Sauvignon at the closure of bunch stage. After freezing, they are peeled: 100g of skin are necessary. After various operations in alcohol and crushing, the Alcohol Insoluble Material (A.I.M.) is collected. From the A.I.M., we obtained:

- Soluble pectic compounds in water (SPW) by fractionation with water. The proportioning of the SPW is carried out by colorimetry with a spectrophotometer at 520 nm optical density by using the metaphenylphenol method. The results are given in mg of galacturonic acid per g of A.I.M.
- Phenolic compounds by extraction with hot NaOH. They are estimated by colorimetry with a spectrophotometer at 725 nm optical density. The results are given in mg of guaïacol acid per g of A.I.M.
Results

For example we show the results obtained in 1994, year marked by strong grey mold rates vintaging in the untreated plots (70 % on Sauvignon) and in 1995, where surprisingly, there was no damage of grey mold regarding the climatic conditions very favorable to the development of the disease.

Evolution of soluble pectins in water (SPW)

Quantity of SPW easily available and favorable by the enzymes of *B. cinerea* is linked to the intensity of the damage at harvest. Indeed, to large quantities of SPW correspond high grey mold rates and vice versa. The quantitative differences observed between the years are already visible as soon as bunch closure.

![Fig. 1. Evolution of SPW of the skin of healthy berries of Sauvignon during their development in 1994 and 1995.](image)

Evolution of the phenolic compounds (PC)

Content of phenolic compounds which present antifongic activities and take part in parietal cohesion is weaker in 1994 than in 1995. As previously, the same remark can be made on the precocity of the quantitative differences observed.

Validity of the indicators

The value of the indicators of the PSB (Potential of susceptibility of berries to *B. cinerea*) is established starting from a plot test of Sauvignon of average vigor in which no prophylactic action is carried out. It is estimated that the results obtained under these conditions indicate a general tendency for the whole of a great climatic area. Until now, these indicators were
validated since 1994 until 2002 (fig. 3). They make it possible to envisage very early (at bunch closure) and probably before, as soon as berry setting, the risk of development of grey mold at harvest.

![Graph showing WSP and PC contents in relation to grey mold in the test plot at harvest from 1994 to 2002. Grey mold damage are visually assessed one week after technological maturity in order to better observe differences.](image)

**Fig. 3.** WSP and PC contents in relation to grey mold in the test plot at harvest from 1994 to 2002. Grey mold damage are visually assessed one week after technological maturity in order to better observe differences.

**Factors of variation of the potential of susceptibility of berries to *B. cinerea* (PSB)**

As it has just been mentioned, indicators of the PSB seem to have general value for the whole of a great area. This report suggests a climatic determinism of the value of the PSB. Nevertheless, it is well known that the vine susceptibility to *B. cinerea* is also strongly related to environmental biotic and abiotic (Bulit & Dubos, 1982). Thus the influence of the nitrogenous fertilization was evaluated.

**Nitrogenous fertilization influence**

We evaluated the quantities of soluble pectic compounds in water (SPW) and the phenolic compounds (PC) linked to the parietal structures of the cells in the pellicular complex of berries taken in a Sauvignon plot in which a part had received a nitrogenous fertilization and the other part was left in the state (fig. 4)

![Graph showing influence of nitrogenous fertilization on potential of susceptibility of berries to *B. cinerea* (PSB).](image)

**Fig. 4.** Influence of nitrogenous fertilization on potential of susceptibility of berries to *B. cinerea* (PSB)
The pellicular complex of berries taken from the nitrogenous fertilized plot has a high susceptibility potential characterized by high SPW and weak PC quantities, and a ratio PSW/PC twice higher.

**Discussion and conclusion**

It is now shown that, from the first stages of the development of berry, the pellicular complex plays a key role in the susceptibility of berry to *B cinerea*. The "biochemical state" of the berry skin allows to account for the years with strong potentialities of grey mold severity. Two parameters relatively simple to quantify make possible to evaluate the state of the skin. However, these results constitute only one first step before having a system to forecast the risks with general value. It would be necessary to have a forecasting model of the PSB, (this would be only to exempt long and tiresome task to peel berries) and to validate it in other wine areas. The PSB is possibly determined by climatic factors and we currently have a sufficient data base (9 years) to approach the development of a forecasting model. It would also be advisable to identify and quantify the factors of fluctuations of the PSB, such as impact of prophylactic actions, the side effect of fungicides and others environmental factors...

**But what are practical repercussions of this work at present?**

Let us take the case of 2002 and vineyard of Bordeaux: The Conseil interprofessionnel des Vins de Bordeaux (CIVB), at bunch closure stage presented on its web site the information of the risk of an explosive development of grey mold at vintage if the climatic conditions were favorable. It is clear that an evaluation of the PSB at flowering stage will be helpful to decide to carry out an additional treatment. Two situations can be identified:

- High added value vineyards where 2 treatments are carried out at end of flowering and veraison stages, a third and late treatment could be considered.
- Low added value vineyards where no anti-rot treatment is applied, a treatment around veraison is economically very profitable.

Lastly the PSB is going to be taken into account in the development of a "decision rule to control grey mold" within the framework of an experimentation aiming at validating decision rules treatments in the vineyards (Program INRA 2001-2003 Integrated Pest Management).

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Precursory climatic indices of Botrytis rot development in mature grapes

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Abstract: In 2002, grey mould progressed quickly in many French vineyards, due to conducive climatic conditions. Near Bordeaux, the symptom development was studied on Merlot in the absence of specific fungicide to control Botrytis cinerea. The disease incidence increased linearly from the beginning of August (5% of rotted clusters) until the end of September (100% of rotted clusters). The disease severity (percentage of rotted berries in the diseased clusters) increased up to 20% during the first 3 weeks of September, from ca. 2% in August. Plurifactorial statistical analyses, using a 1993-2001 database, allowed us to select standard climatic variables highly correlated to population dynamics parameters at the end of the season. From this, climatic indices were calculated in order to evaluate the potential development of symptoms within one week. For each cultivar tested (Merlot, Cabernet Sauvignon and Cabernet franc), 2 indices were specially developed: one for the incidence and the other for the severity. They can be used to simulate the disease progression curves (incidence and severity, independently) and to forecast the epidemic risk from 5 to 15 days in advance. The potential of these precursory indices for use by growers is discussed in the context of integrated control in viticulture.

Key words: Botrytis cinerea, epidemiology, grey mould, integrated control, risk factors, Vitis vinifera

Introduction

The final quality of the vintage is conditioned, to some extent, by the grape evolution after veraison, the stage when grape berries beginning to ripen lose their green colour. The evolution of grape maturity is generally monitored regularly in the vineyard in order to help growers to make a choice with regard to the date of grape harvest. In the event of a rainy climate, as in 2002 in France, the grey mould progression becomes also an essential parameter to be considered. However, no indicator is available in order to appreciate and anticipate the progression potential of the disease, due to Botrytis cinerea. For this purpose, in Bordeaux vineyards, we have investigated the risk factors at the end of the season and the different subpopulations of B. cinerea (Martinez et al., 2003). Various environmental factors (canopy management, soil effect ...) can affect the disease development in time and space. The climatic conditions getting near to harvest (frequent precipitations, high relative humidity, mild temperatures ...) are considered, rightly, as key elements which rule the epidemics.

Material and methods

Experimental vine plot
The experiment was conducted in an INRA experimental vineyard near Bordeaux. The Merlot vines, planted in 1991 on a gravelly soil (5347 vines per ha), were vertical-trellised and cane-pruned. The natural epidemic development occurred in the absence of damage by grape berry moth larvae (Lobesia botrana, Lepidoptera). No specific fungicide was applied to control
B. cinerea. Standard climatic data came from an automatic agrometeorological station being next to the plot.

**Summer climatic conditions in 2002**
The climatic conditions contributed to the development of grey mould (Fig. 1). Precipitations were regular enhancing the relative humidity of the air. From July to September, i.e. for 92 days, 28 rainy days were recorded, the daily relative humidity was mostly > 70%. The temperatures were rather cool: only 10 days showed daily maximal temperatures exceeding 30°C.

**Fig. 1. Climate features in 2002 (INRA, Bordeaux)**

**Development of the climatic risk indicators**
The climatic indices of risk of grey mould result from multidimensional statistical analyses. The analyzed data originated from a survey in various French wine producing areas from 1993 to 2001. We selected standard climatic variables relevant to the epidemic because they were highly correlated with disease progression data. The periods when these variables were highly correlated were also determined. In this way, the indices, calculated on the basis of climatic data only, enabled us to evaluate the potential development of the disease within one week at the end of the season. Two indices have been specially developed, one for the frequency of rotted bunches, the other for symptom severity in rotted bunches. Specific indices are calculated for each of the 2 black cultivars we studied: Cabernet sauvignon and Merlot. They can be used to forecast the epidemic risk from 5 to 15 days in advance (according to the cultivar).

**Results and discussion**

**Epidemic development on Merlot in 2002**
The symptom evolution was monitored during the season in order to assess the percentage of rotted bunches (incidence) and the percentage of rotted berries within the diseased bunches (severity). The latter parameter was measured in the laboratory by picking off grapes from the bunch. As shown in Fig. 2 (solid line), the disease incidence increased linearly from the beginning of August (5% on the 7th) until the end of September (100% on the 30th).
increase rate was fast: 12% of bunches newly diseased per week. The disease severity evolved in 3 stages (Fig. 3 solid line). At first, until the end of August, the severity remained low and stable (2-3%). Then, it increased up to 20% during the first 3 weeks of September. Lastly, a plateau was reached at ca. 20% corresponding to a stopping of the symptom development.

**Fig. 2.** Percentage of rotted bunches in 2002 on Merlot

**Fig. 3.** Grey mold sensitivity, i.e. percentage of rotted berries per rotted bunch in 2002 on Merlot

**Fig. 4.** Variation of the climatic indices for Merlot in 2002

**Simulations of disease progression**

In 2002 on Merlot, the variations of the calculated climatic indices are presented in Figure 4. The variations of both indices revealed four periods corresponding to an increased risk of symptom outbreaks, i.e. at the middle and at the end of August and September. The disease development was simulated using these calculations and an assessment in the vineyard, when the first grey mould symptoms appeared in the bunches (Figs. 2 and 3, dotted lines). The simulated disease progression matched satisfactorily the observed data. The disease incidence was correctly simulated until mid-September corresponding to the main part of the kinetics of
disease progression (Fig. 2). On the other hand, calculations of severity over-estimated the risk (Fig. 3). This shift in severity between real development and simulation came from a reduced colonization of bunches by *B. cinerea* due to 2 features of 2002:

1) Grapes late in maturing (ratio "sugars/total acidity" of *circa* 38, the 1/10);  
2) A low bunch compactness due to flower abortion (almost 100 berries per bunch).

**Conclusions**

A better understanding of development potential of grey mould near harvest should enable risk of epidemics to be forecasted in the end. This step is an essential one in the context of Integrated Pest Management (IPM), i.e. for integrated protection of the vineyard.

The indices of grey mould risk, calculated on the basis of climatic data only, should allow vine growers to evaluate the epidemic tendency in real time and with complete objectivity. This corresponds to a "modelling of tendency" because, at a local plot level, the final severity of grey mould depends on the climate, obviously, but also on other environmental factors, such as vigour of the vines or wounds caused by insects (Fermaud, 1998). Moreover, the reliability of climatic data must be optimal. The greatest attention must be paid to the site and the maintenance of automatic meteorological stations.

Lastly, it has to be stressed that an anticipation of the epidemic risk is possible in the short-term, up to 15 days. Use of these precursory indices by vine growers should result in an improvement of strategies to control the disease at the end of the season. Thus, the use of specific fungicides, at or after veraison, should depend on the risk of grey mould development as indicated by the indices. An other important prospect is to use the indices as helpful tools in decision-making in order to optimize the date of grape harvest.

**References**

The ecology of *Botrytis cinerea* on grape in the Western Cape province, South Africa

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**Abstract:** The occurrence of *Botrytis cinerea* in air currents in table and wine grape vineyards, and subsequent disease expression at different positions on leaves and bunches of grape, was determined from 1996 to 2002. *B. cinerea* in vineyards was mainly carried as conidia in air currents, and less commonly in rain drops. The number of conidia in air currents was high from bloom to bunch closure, and then drastically decreased. Estimations of viable *B. cinerea* residing in leaves and bunches showed that their amounts reflected levels occurring in air currents. The pathogen also occurred in a fixed pattern in bunches and it mostly occupied the bases of the berry and the pedicel. The next prominent positions colonised were the rachises and laterals. It was found infrequently on the berry cheek, and the stylar end of the berries was virtually free of the pathogen. Disease expression displayed the pattern showed by the inoculum ecology. The findings indicate that conidia dispersed in early season in bunches, and residing superficially at the berry-pedicel attachment zone, are a major factor in causing *B. cinerea* bunch rot.

**Key words:** grey mould, bunch rot, natural infection.

**Introduction**

In the Western Cape province of South Africa, grapevine is cultivated for wine and table grape production and *Botrytis cinerea*, or grey mould, poses a menace to the producer. The pathogen follows a similar trend in local vineyards as in other parts of the world, namely to go unnoticed early in the season, and then to cause grey mould late in the season, or during storage. This elusive characteristic of the fungus has perplexed grape producers and plant pathologists when making recommendations towards effective disease control. A handful of reasons exist why plant doctors find it difficult to combat this pathogen on grape. Firstly, *Botrytis cinerea* infects different organs of the grapevine, namely leaves, buds, canes and bunches (Nair & Hill, 1992). However, studies on timing of fungicide application, biological control, host resistance and disease prediction models usually comprise investigations on berries. The rationale for this is that the most prominent phase of the disease is found on berries. So, for plant doctors grey mould is usually associated with symptom expression on the berries. Secondly, in most studies plant doctors predominantly make use of mature berries (Coertze & Holz, 1999; Coertze, Holz & Sadie, 2001). The reason for this is that we usually assess the outcome of our experiments according to symptom expression. Thirdly, the pathogen can invade berries by different infection pathways, namely through stigmata, pedicels, natural openings, wounds, or by direct penetration of the cuticle (Holz, Gütschow, Coertze & Calitz, 2003). Furthermore, inoculation of immature berries is often followed by a latent period. Lastly, berries are considered resistant to symptom expression when immature, and susceptible when mature (Coertze & Holz, 1999; Coertze, Holz & Sadie, 2001). Plant doctors therefore prefer to use mature grapes in their experiments, and to use conidial suspensions containing millions of conidia. The mature berries are atomized with, dipped in, or injected with the conidial suspensions, or suspension droplets are placed onto the berry cheek. However, it should be kept in mind that these methods allow for the deposition and
growth of clusters of conidia on one site, and may differ from primary natural inoculation in the vineyard, where single conidia may be deposited intermittent and develop at several sites on the berry surface (Coertze & Holz, 1999; Coertze, Holz & Sadie, 2001).

It should be evident from this discussion that difficulties encountered in combating Botrytis bunch rot can partially be ascribed to a poor understanding of the ecology of the pathogen’s inocula in vineyards. The behaviour of the pathogen is regulated by different sets of cultivation practices, environmental and climatic conditions in each growing region. A broad programme "The ecology of Botrytis cinerea in grape bunches and the implementation of disease control programmes" was thus started in 1995 with the aim to study the behaviour of the pathogen in vineyards of the Western Cape, South Africa. In this paper highlights of our findings on the ecology and behaviour of the pathogen in grape bunches are discussed.

The aim of the first part of the study was to investigate germination and establishment of infection on grape berries by dry, single airborne conidia of the pathogen. Natural infection on grapes at different phenological stages was simulated in the laboratory by dusting them in a settling tower with dry conidia (Coertze & Holz, 1999; Coertze, Holz & Sadie, 2001). The berries were incubated for various periods at high relative humidity, or were covered with a film of water. Germination of the solitary conidia, appressorium formation, stilbene and suberin induction by germlings, and germling viability were examined after each incubation period by fluorescence microscopy. Isolation and freezing studies were conducted to determine surface colonisation and penetration. The material was used untreated to detect the pathogen on the surface, or were surface-sterilised to detect mycelia in the tissue. The studies with dry, airborne conidia showed that disease expression by Botrytis cinerea on berries is not dependent upon inoculum density. We found that the skins of berries at different phenological stages provide an effective barrier to penetration. The airborne conidia did not survive for extended periods on the surface of moist or wet berries. Consequently few infections in grape berry skins were established by airborne conidia. The solitary conidia were furthermore unable to induce disease symptoms on berries at different phenological stages. Collectively, these findings indicated that this mode of infection should not contribute to a gradual build-up of secondary inoculum in the vineyard, nor to B. cinerea epiphytotics. Our findings furthermore suggested that symptom expression on berries might be a very late event in the disease cycle, and that the primary events may occur at other positions in the bunch. We therefore decided to investigated those primary events at other positions in the bunch.

Whereas natural infection was simulated in the first part of the investigation, only naturally infected grapevine material was used the second part. Our aims were (i) to estimate the amount of viable conidia occurring in air currents in vineyards, (ii) to estimate the amount of viable Botrytis occurring on leaves and bunches, and (iii) to determine the relationship between the occurrence of the pathogen and subsequent disease expression at different positions on leaves and bunches.

Different techniques were used to detect viable conidia in air currents in table (cultivars Dauphine and Waltham Cross) and wine grape (cultivars Chardonnay, Sauvignon Blanc and Merlot) vineyards in various growing regions, and on plant material obtained from these vineyards (Holz, Gütschow, Coertze & Calitz, 2003). For four consecutive days during prebloom, bloom, pea-size, bunch closure, véraison and harvest, sets of Petri dishes with freshly prepared Kerssies’ B. cinerea selective medium (Kerssies, 1990) were left overnight in the bunch zone of vines. The dishes were collected at approximately 11:00 each morning. Plant material was collected from the vines on the fourth day. Isolations were made from berry skins on the selective medium or on water agar medium supplemented with paraquat (Grindrat & Pezet, 1994). Leaves and parts of bunches bearing three to seven berries on a short rachis section were used untreated, treated with paraquat, or frozen for 1 h at –12°C.
Paraquat and freezing were used to terminate host resistance and to promote the development of the pathogen from the tissues. The material was used untreated to detect the pathogen on the surface, or were surface-sterilised to detect mycelia in the tissue. The number of colonies occurring on dishes, and the incidences of tissues yielding the pathogen were used to quantify the amount of *B. cinerea* occurring in air currents, and superficially or in the tissue at the various positions on leaves and bunches.

**Results**

The spore trap data indicated that *B. cinerea* in vineyards were mainly carried as conidia in air currents, and less commonly in rain droplets. Conidia were not carried in groups, but as single spores, and were deposited individually on the vine. The number of spores in air currents, from pre-bloom to late pea-size stage, was initially high, and then drastically decreased. Estimates of fungus numbers on healthy shoots indicated that their numbers were the highest early in the season. On leaves, the fungus mainly developed from the blade, which latently carries the fungus. The patterns of pathogen occurrence in bunches reflected its levels in air currents and on shoots. The fungus occurred in a consistent pattern in leaves and bunches in all vineyards. Based on the combined data for tissues exposed and unexposed to paraquat, *B. cinerea* occurred predominantly in bunches and was mostly associated with the bases of the berry and the pedicel. Overall, approximately 30% of the berries yielded *B. cinerea* at these positions. The next prominent positions occupied were leaf blades, rachises and laterals, of which approximately 20% yielded *B. cinerea*. The pathogen occupied the petioles less often (10%), and infrequently (5%) the berry cheek. The stylar end of the berries, on the other hand, was virtually free (0.02%) of the pathogen. Disease expression in bunches displayed the pattern showed by the inoculum ecology, and symptoms consistently developed first at the berry-pedicel attachment zone. The isolation studies showed that the pathogen seldom occurred on the surface or in the skin tissue near the base, cheek or stylar end of berries. Latent infections in the berry base were also few at véraison and harvest. The data showed that for the structures of the inner bunch, no clear relationship existed between the incidence of *B. cinerea* occupation and subsequent symptom expression at pea size and bunch closure. Disease expression only developed when host resistance was terminated by applying paraquat or freezing as stress factors. Furthermore, due to the spreading lifestyle of the pathogen, a single berry that became symptomatic at the berry base or pedicel area, caused extensive berry decay and finally rot of the entire bunch. This property therefore gave a distorted relationship between conidial density and disease expression in the bunch.

**Discussion**

Collectively, these findings showed that grey mould in these vineyards was unlikely to be caused by the very low amounts of *B. cinerea* occurring on the skin surface, or in the skin tissue. It was also unlikely for berry rot to be caused by colonisation of the pistil, and subsequent latency in the stylar end. Instead, berry rot developed primarily from the berry-pedicel attachment zone. These findings indicated that conidia dispersed in the early season in bunches, and residing superficially at the berry-pedicel attachment zone, are a major factor in bunch rot. The *B. cinerea* occupation pattern thus explains why grey mould develops mostly from the inner bunch and why disease management strategies should concentrate on the pre-bunch closure stage and on inhibiting *B. cinerea* development in the inner bunch during the early part of the season.

The data also showed that cultivars may differ in resistance of their bunch parts to natural *B. cinerea* infection. For example, for Merlot, a strong positive linear relationship between *B.
**cinerea** incidence data of paraquat treated and untreated material was found at harvest for both the pedicel and the berry base. On Dauphine the incidence of *B. cinerea* declined at the berry base and on the pedicel, but on Merlot the incidence remained high throughout the season at these positions. *B. cinerea* conidia and germlings may therefore have different survival periods on tissues of the various positions, as is indicated by the low incidence at which the pathogen was detected at the cheek, and the high incidence of occurrence on the rachis, lateral and pedicel. In this context it was previously shown that single conidia of the pathogen did not survive for extended periods on berry surfaces. Passive defence and active defence mechanisms may therefore play a differential role in the resistance of the different tissues to infection by *B. cinerea*, and in the survival of conidia, germlings and latent mycelia of the pathogen.

**Take home message**

Throughout this study it was noted that sound, healthy bunches developed grey mould only after being subjected to stress. Furthermore, in all our studies where bunches were subjected to paraquat and freezing treatment, other fungi (*Penicillium*, *Aspergillus*, *Alternaria*, *Mucor* and *Rhizopus* spp.) commonly associated with bunch rot developed in a similar pattern as *B. cinerea* at the various positions in bunches. From this it can be concluded that (i) the importance of these fungi residing at the berry-pedicel attachment zone is underestimated in their epidemiology, and the development of bunch rot epiphytotics in grapevine; (ii) *Botrytis cinerea*, and its companions, requires assistance to enable the infection cycle to run its full course, and to generate a symptom; (iii) stress factors operative on vines, for example those that cause rupture at the berry base, and insects, should play an important role in disease expression by the different fungi in the vineyard. Plant pathologists, viticulturists and entomologists should thus unite in their effort to understand the ecology of these fungi in grape bunches and collectively investigate the so-called "helping hand" needed by the fungi to generate a symptom.

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Influence of inoculation time after wounding on the action of Isabella volatiles against *Botrytis cinerea*

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Abstract: The aim of the research was to study the influence of the inoculation time after wounding on the action of the volatile substances produced by grapes of the Isabella variety (*Vitis labrusca*), with a view to determining whether these substances could be used as biocontrol agents for the postharvest control of the fungus *Botrytis cinerea*. To perform a quantitative analysis of the biological action of these volatile substances on the growth of *B. cinerea*, use was made, as a bioassay method, of the closed Mariotte system. In the *in vivo* experiment performed to study the effect of the time of inoculation after wounding on the action of the volatile substances on *B. cinerea*, use was made of the following interactive model: volatiles from grapes of the Isabella variety – *B. cinerea* – kiwifruit of the Hayward variety (*Actinidia deliciosa*). Three different times of inoculation after wounding were studied. The results confirmed the antifungal action of the volatile substances from the Isabella grapes as they limited the incidence of infection, reducing considerably both the amount of the inoculum and the activity of the pathogen. The time of inoculation after wounding did not play an important role in the inhibitory action of the Isabella volatiles.

Key words: gray mold, biological control

Introduction

*Botrytis cinerea* Pers. Fr. is the most important cause of postharvest rots on kiwi berry fruit (Opgenorth, 1983; Pennycook, 1985; Eckert and Ogawa, 1988; Niklis *et al*., 1995. Michailides and Elmer, 2000). Kulakiotu (2001) found that natural volatile substances emitted by grapes of the Isabella variety, of the *Vitis labrusca* species, have antifungal properties against *B. cinerea*. Sporulation and sclerotia formation of the fungus were strongly inhibited by Isabella volatiles in *in vitro* bioassays (Kulakiotu *et al*., 2002a), while in *in vivo* bioassays the results showed strong antimicrobial action of the Isabella volatiles against *B. cinerea*, leading to a reduction in the inoculum and in the activity of the pathogen (Kulakiotu *et al*., 2002b; Kulakiotu *et al*., 2003).

The aim of the research was to study the influence of the inoculation time after wounding on the action of the volatile substances produced by grapes of the Isabella variety (*V. labrusca*) against the *in vivo* growth of *B. cinerea* on kiwi berry fruit (*Actinidia deliciosa*), with a view to determining whether these substances could be used as biocontrol agents for the postharvest control of the fungus.

Material and methods

The modified closed Mariotte system was used as the bioassay method (Sfakiotakis, 1972).

In the *in vivo* experiment performed to study the effect of the time of inoculation after wounding on the action of the volatile substances from the Isabella grapes against *B. cinerea*,...
Fig. 1. Number of *B. cinerea* conidia on kiwi berry fruit, in the presence of grapes of the Isabella variety (*V. labrusca*), in the presence of grapes of the Roditis variety (*V. vinifera*) and in the absence of any grapes (Control), inoculated with the fungus immediately (I), 24 hours (II) and 48 hours (III) after the wounding, 7, 10 and 11 days, respectively, after insertion in the closed Mariotte system, at 21° C (LSD5%; 338.09).

Fig. 2. Depth of rot of kiwifruit - in the presence of grapes of the Isabella variety (*V. labrusca*), in the presence of grapes of the Roditis variety (*V. vinifera*) and in the absence of any grapes (Control) - inoculated with the fungus *B. cinerea* immediately (I), 24 hours (II) and 48 hours (III) after the wounding, 7, 10 and 11 days, respectively, after insertion in the closed Mariotte system, at 21° C (LSD5%; 3.37).
use was made of the following models: (i) volatile substances emitted by grapes of the resistant variety Isabella (*V. labrusca*) – *B. cinerea* – kiwifruit of the Hayward variety (*A. deliciosa*), (ii) volatile substances emitted by grapes of the susceptible variety Roditis (*V. vinifera*) – *B. cinerea* – kiwifruit of the Hayward variety, and (iii) no volatile substances at all – *B. cinerea* – kiwifruit of the Hayward variety. Three different times of inoculation were studied: immediately (I), 24 hours (II) and 48 hours (III) after the wounding.

The analyses of data variance in the experiment were performed with the MSTAT (Michigan State University, Version 4.00/EM) program. When the F values were significant, the mean comparisons were performed with the least significant difference value, at the significant level of *P*=0.05.

**Results**

The results confirmed the antifungal action of the volatile substances from the Isabella grapes as they limited the incidence of infection, reducing considerably both the amount of the inoculum (Fig. 1) and the activity of the pathogen (Fig. 2). The time of inoculation after wounding did not play an important role in the inhibitory action of the Isabella volatiles.

**Discussion**

This research presents experimental data which demonstrate the strong antifungal action of the volatiles produced by grapes of the Isabella variety against *B. cinerea* on kiwifruit. The volatiles inhibit the fungus’ capacity to sporulate and grow via plant tissue, leading to a decrease in the inoculum and in the activity of the pathogen.

In conclusion, it appears that the natural volatile substances produced by the Isabella grapes of the *V. labrusca* species have potential as biocontrol agents for the postharvest control of *B. cinerea* on fruit of the *A. deliciosa* species.

**References**


Influence of volatiles of Isabella grapes at different developmental stages on *Botrytis cinerea*

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Abstract: The aim of the research was to study the effects of the stages of development of grapes of the Isabella variety (*Vitis labrusca*), from Siviri in Halkidiki, on the action of the volatile substances produced by these grapes against the growth of the fungus *Botrytis cinerea*. The closed Mariotte system was used as a bioassay method. In the *in vitro* experiment performed to study the effect of different developmental stages on the action of the volatile substances against *B. cinerea*, use was made of: (i) volatiles excreted by grapes of the Isabella variety, and (ii) volatiles excreted by grapes of the Roditis variety (*V. vinifera*). In the *in vivo* experiment use was made of the following interactive model: Isabella volatiles – *B. cinerea* – kiwi berry fruit of the Hayward variety (*Actinidia deliciosa*). Three different developmental stages of the Isabella variety of grape were used: immature, semi-mature and mature. The results confirmed the antibiotic action of the volatile substances from the Isabella variety of grape on the sporulation of the fungus and formation of sclerotia. The Isabella volatiles also limited the incidence of infection by *B. cinerea* on *A. deliciosa* fruit, reducing considerably both the amount of the inoculum and the activity of the pathogen. The inhibitory action of the volatiles from the mature Isabella grapes against *B. cinerea* on the kiwifruit was more intense than that of the volatiles from the immature and semi-mature grapes.

Key words: biological control, postharvest pathology

Introduction

*Botrytis cinerea* Pers.:Fr. is the most serious cause of postharvest rots on grapes and kiwifruit (Eckert and Ogawa, 1988; Snowdon, 1990; Niklis *et al*., 1995; Michailides and Elmer, 2000).

Kulakiotu (2001) found that volatiles produced by grapes of the Isabella variety, of the *Vitis labrusca* species, which is resistant to powdery mildew, downy mildew, and winter cold (Galet & Morton, 1988), have an antimicrobial action on *B. cinerea*. In the *in vitro* bioassays that were performed, volatiles produced by grapes of the Isabella variety inhibited sporulation of *B. cinerea*, while volatiles emitted by grapes of the Roditis variety, of the *V. vinifera* species, had a stimulating action on sporulation of the fungus (Kulakiotu *et al*., 2002).

The aim of the research was to study the influence of the developmental stages of grapes of the Isabella variety (*V. labrusca*), from Siviri in Halkidiki, on the action of the volatile substances emitted by these grapes against the growth of the fungus *B. cinerea*.

Material and methods

The antifungal action against *B. cinerea* of the volatile substances produced at different stages of development of the Isabella grapes was studied in *in vitro* and *in vivo* experiments. The modified closed Mariotte system was used as a bioassay method (Sfakiotakis, 1972). In the *in vitro* experiment use was made of: (i) volatiles emitted by grapes of the Isabella variety and *B.
cinerea on PDA, and (ii) volatiles emitted by grapes of the Roditis variety (V. vinifera) and B. cinerea on PDA. In the in vivo experiment use was made of the following interactive model: Isabella volatiles - B. cinerea - kiwi berry fruit of the Hayward variety (Actinidia deliciosa). Three different developmental stages of the Isabella grapes were used: immature, semi-mature and mature.

The analyses of data variance in the experiments were performed with the MSTAT (Michigan State University, Version 4.00/EM) program. When the F values were significant, the mean comparisons were performed with the least significant difference value, at the significant level of $P=0.05$.

**Results**

A study was made of the influence of the volatile substances produced by Isabella grapes at different developmental stages on the in vitro and in vivo growth of the fungus B. cinerea on kiwifruit.

The results confirmed the antifungal action of the volatile substances produced by grapes of the Isabella variety on the sporulation (Fig. 1) of the fungus and formation of sclerotia (Fig. 2). The Isabella volatiles limited the incidence of infection by B. cinerea on A. deliciosa fruit, reducing considerably both the amount of the inoculum (Fig. 3) and the activity of the pathogen (Fig. 4). The inhibitory action of the volatiles from the mature Isabella grapes against B. cinerea on the kiwifruit was greater than that of the volatiles from the immature and semi-mature grapes (Figs 3 and 4).

Fig. 1. Influence of volatile substances produced by Isabella (V. labrusca) grapes at different developmental stages (I: immature, II: semi-mature and III: mature) and of volatiles produced by Roditis (V. vinifera) grapes on the in vitro production of conidia by B. cinerea at 21°C, 10 days after insertion in the closed Mariotte system.
Fig. 2. Influence of volatile substances produced by Isabella (V. labrusca) grapes at different developmental stages (I: immature, II: semi-mature and III: mature) and of volatiles produced by Roditis (V. vinifera) grapes on the in vitro formation of sclerotia by B. cinerea at 21°C, 10 days after insertion in the closed Mariotte system.

Fig. 3. Sporulation rate of B. cinerea on kiwifruit inoculated with the fungus, in the presence of immature (I), semi-mature (II) and mature (III) grapes of the Isabella variety (V. labrusca), and in the presence of mature grapes of the Roditis variety (V. vinifera), 5 days after inoculation and 6 days after insertion in the closed Mariotte system, at 21°C (LSD0.05: 27.36).
Fig. 4. Rot depth of kiwifruit inoculated with *B. cinerea*, in the presence of immature (I), semi-mature (II) and mature (III) grapes of the Isabella variety (*V. labrusca*), and in the presence of mature grapes of the Roditis variety (*V. vinifera*), 5 days after inoculation and 6 days after insertion in the closed Mariotte system, at 21°C (LSD$_{0.05}$:1.46).

**Discussion**

This research presents experimental data which demonstrate the intense antimicrobial action of the volatiles of mature Isabella grapes against *B. cinerea*.

The inhibitory action of the volatiles of the mature grapes was stronger, as they completely inhibited the sporulation of the fungus on the kiwifruit and considerably reduced its growth via plant tissue, leading to a sharper decrease in the inoculum and in the activity of the fungus than those caused by volatiles from grapes at the other stages of development. The above corroborates the hypothesis that the concentration and quality of the synthesis of the volatiles from Isabella grapes is superior in the mature grapes of this variety.

**References**


Antifungal action of Isabella volatiles against Botrytis cinerea

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Abstract: The aim of the research was to study the volatile substances produced by grapes of the Isabella variety (Vitis labrusca) as possible biocontrol agents for the postharvest control of the fungus Botrytis cinerea on fruit of the Vitis vinifera and Actinidia deliciosa species. To perform a quantitative analysis of the biological action of these substances on the growth of B. cinerea, use was made, as a bioassay method, of the closed Mariotte system. In the in vivo experiments performed to study the action of the volatile substances on B. cinerea, use was made of the following interactive models: (i) volatile substances from grapes of the Isabella variety (V. labrusca) – B. cinerea – grape berries of the Roditis variety (V. vinifera) and (ii) volatile substances from grapes of the Isabella variety – B. cinerea – kiwi berry fruit of the Hayward variety (A. deliciosa). The results confirmed the antifungal action of the volatile substances from the Isabella grapes as they limited the incidence of infection, reducing considerably both the amount of inoculum and the activity of the pathogen. In this study, for the first time, an in vivo examination has been made of the action of the volatile substances produced by Isabella grapes on the growth of B. cinerea on kiwi berry fruit with encouraging results for the postharvest control of the fungus on these fruit.

Key words: Botrytis cinerea, gray mold, biological control, postharvest pathology

Introduction

Botrytis spp., and especially B. cinerea, are important pathogens of stored and transported fruits, vegetables, ornamental crops and nursery stocks (Jarvis, 1977). Kulakiotu (2001) found that volatile substances emitted by grapes of the Isabella variety, of the Vitis labrusca species, were highly inhibitory to the sporulation and sclerotia formation of the fungus B. cinerea in in vitro experiments. Volatiles produced by grapes of the Isabella variety inhibited the in vitro production of conidia by B. cinerea, while volatiles produced by grapes of the Roditis variety (V. vinifera) stimulated it (Kulakiotu et al., 2002).

The aim of this research was to study the volatiles produced by Isabella grapes (V. labrusca) as possible biocontrol agents in the postharvest control of the fungus Botrytis cinerea on grape and kiwi berry fruit.

Material and methods

To perform a quantitative analysis of the antifungal action of volatiles on the growth of B. cinerea, use was made, as a bioassay method, of the modified closed Mariotte system (Sfakiotakis, 1972).

In the in vivo experiments performed to study the action of the volatile substances on B. cinerea, use was made of the following interactive models: (i) volatile substances from grapes of the resistant variety Isabella (V. labrusca) – B. cinerea – grape berries of the susceptible
variety Roditis (*V. vinifera*), and (ii) volatile substances from grapes of the Isabella variety – *B. cinerea* – kiwi berry fruit of the Hayward variety (*A. deliciosa*). The antifungal action of the volatile substances was studied on grape berries, under different temperatures, and on kiwi berry fruit, under different grape weights.

The analyses of data variance in the experiments were performed with the MSTAT (Michigan State University, Version 4.00/EM) program. When the F values were significant, the mean comparisons were performed with the least significant difference value, at the significant level of \( P=0.05 \).

**Results**

The results confirmed the antifungal action of the volatile substances from the Isabella grapes as they limited the incidence of infection, reducing considerably both the amount of inoculum (Figs 1 and 3) and the activity of the pathogen (Figs 2 and 4).

At 21\(^\circ\) C the density of inoculum on grapes of the Roditis variety in the presence of Isabella grapes was considerably less than that on grapes of the Roditis variety when no Isabella volatiles were present (Fig. 1). The contribution of volatiles from Isabella grapes to the decrease in the activity of the pathogen was smaller but still significant even at 0\(^\circ\) C (Fig. 2). On kiwi berry fruit, the number of conidia and the depth of rot were reduced in proportion to the increase in weight of the Isabella grapes (Figs 3 and 4).

![Fig. 1. Influence of temperature (21\(^\circ\) C, 10\(^\circ\) C and 0\(^\circ\) C) on the number of *Botrytis cinerea* conidia on grape berries of the Isabella (*Vitis labrusca*) and Roditis (*V. vinifera*) varieties and on Roditis in the presence of Isabella 7, 15 and 75 days after the inoculation and their insertion in the closed Mariotte system (LSD\(_{5\%}\): 61.9).](image-url)
Fig. 2. Influence of temperature on the rotten tissue rate of the infected grape berries (LSD$_{5\%}$: 4.34).

Fig. 3. Number of *B. cinerea* conidia on kiwifruit in the absence of grapes and in the presence of 300 g, 400 g and 500 g of Isabella grapes, 7 days after the inoculation and the insertion in the closed Mariotte system, at 21ºC (LSD$_{0.05}$: 209.31).
Fig. 4. Extent of *B. cinerea* on kiwifruit in the absence of grapes and in the presence of 300 g, 400 g and 500 g of Isabella grapes, 7 days after the inoculation and the insertion in the closed Mariotte system, at 21°C (LSD$_{0.05}$: 0.63).

**Discussion**

This research presents evidence which demonstrates the inhibitory effects of the Isabella volatiles upon the growth of *B. cinerea* on grape and kiwi berry fruit.

The antifungal action of the Isabella volatiles on *B. cinerea* on grapes was evident at 0, 10 and 21°C, though more pronounced at 21°C, while on kiwifruit it was more intense in proportion to an increase in the weight of the grapes. Its effects consisted of a reduction in the population density of the inoculum and a decrease in the activity of the pathogen.

The above justifies the conclusion that natural volatile compounds produced by grapes of the *V. labrusca* species, could be used as biocontrol agents for the postharvest control of *B. cinerea* on fruit of the *V. vinifera* and the *A. deliciosa* species (Baker, 1987). It also accords with Fries’ (1973) conclusion that ‘volatile substances must be included among the environmental factors which determine the distribution, the rate of growth and the mode of development of fungi in nature’ and that of French (1985) that “an understanding of the role of these compounds may help to explain some of the biological mysteries in germination and infectivity, and contribute new techniques in the development of pest control”.

![Graph showing depth of rot (mm) for different grape weights and kiwifruit species.](image-url)
References


Results of spray schedules using knowledge about ontogenetic resistance of grapes against powdery mildew

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Berries of *Vitis vinifera* are reportedly susceptible to infection by *Uncinula necator* until the begin of ripening and established colonies sporulate even longer. However, severe infections occur during a limited period from BBCH61 (beginning of flowering) until BBCH73 (berries like pellets) and berries become substantively resistant after BBCH73 (Gadoury et al. 1988, Kast and Stark-Urnau, 2000). Extensive colonisation of rachis occurred from two weeks before bloom (BBCH56) until 80 % of caps are dropped (BBCH68). Severe infections on berries were found during bloom and three weeks post bloom (BBCH73). Only small levels of powdery mildew developed, if berries were infected later than BBCH73. Brown necrotic spots were observed, when berries were infected after this developmental stage. The fungus had unsuccessfullly tried to penetrate the cuticle and the formation of conidiophores was inhibited. This is true not only for resistant varieties but also for extremely susceptible varieties.

It is obvious that severe fruit infection may mostly be a result of infection during the period from prebloom to the time when berries reach the size of pellets. The aim of this study was to proof the relevance of these findings using practical spraying schedules.

Material and methods

Field experiments were performed in a vineyard at Weinsberg (Schemelsberg) planted with Silvaner grape vines, a moderately susceptible variety. The experiment was carried out using 4 replications in a randomised block design with 22 vine/plot in a single line. Each experimental plot was placed between two untreated rows. The untreated lines of the year before were used for the experiment. Therefore, a lot of flagshoots occurred and disease pressure was extremely high, much higher than in conventionally treated vineyards.

Treatments:

01 untreated
02 7 treatments in intervals of 12 - 14 days
03 3 sprays at BBCH58, 68 and 73

All treatments were carried out in the recommended doses using a one row tunnel sprayer. 300 - 1.000 l/ha water dependent on the development stage of the vines were used.
Fungicides used:

<table>
<thead>
<tr>
<th>Year</th>
<th>Fungicide name</th>
<th>Active substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Discus</td>
<td>Kresoxim-methyl</td>
</tr>
<tr>
<td>2000</td>
<td>Vento</td>
<td>Quinoxifen + Fenarimol</td>
</tr>
<tr>
<td>2001</td>
<td>Flint</td>
<td>Trifloxistrobin</td>
</tr>
<tr>
<td>2002</td>
<td>Vento</td>
<td>Quinoxifen + Fenarimol</td>
</tr>
</tbody>
</table>

Remarks for 2002: The spraying equipment was out of order during the bloom period and the spraying interval was 16 days for all treatments due to an accident.

Disease incidence on grapes was evaluated on 100 grapes/plot at development stage BBCH85 (veraison).

Results and discussion

In 1999 and 2000 disease incidence of 3 treatments was significantly higher than for 2 sprays (Fig. 1 + 2). But in relation to the untreated control and regarding to the extreme disease pressure (flagshoots present) the 3 sprays covering the critical period during bloom were very effective. In 2001 and 2002 the differences between two and three treatments were not significant (Fig. 3 + 4). Furthermore, in 2002 the effect of both 3 and 7 sprays was also insufficient (Fig. 4). It should be mentioned that accidentally during the most critical period during bloom the interval was too long. Infections may have taken place during this bug and the following sprays were not able to stop these infections: The results clearly show, that sprays in the critical period from BBCH87 - 73 are most effective. Mistakes during this period are irreparable. In this period the most effective fungicides should be applied in short intervals. Other sprays are of minor importance.

Fig. 1. Disease incidence 1999 after 3 sprays using Discus during the critical period compared to 7 spray in intervals of 12 - 14 days
Fig. 2. Disease incidence 2000 after 3 sprays using Vento during the critical period compared to 7 spray in intervals of 12 - 14 days

Fig. 3. Disease incidence 2001 after 3 sprays using Flint during the critical period compared to 7 spray in intervals of 12 - 14 days

Fig. 4. Disease incidence 2002 after 3 sprays using Discus during the critical period compared to 7 spray in intervals of 12 - 14 days (accidentally longer interval in all treatments during bloom)
References


Evaluation of grapevine rootstocks for *Armillaria mellea* root rot resistance

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**Abstract:** Root rot caused by *Armillaria mellea* s.s. is an increasing problem in some grapevine growing areas in Trentino, Italy. Disease control is based only on preventive methods since chemical treatments are ineffective against the pathogen. Although *A. mellea* resistance has not been identified in genus *Vitis*, some degree of tolerance could be found in currently used rootstocks.

The aim of the research was to evaluate differences in susceptibility to the disease of the most common rootstocks used for Teroldego Rotaliano variety.

Seven grape rootstocks namely Schwarzmann (*V. Riparia* X *V. Rupestris*), 3309 (*V. Riparia* X *V. Rupestris*), 101-14 (*V. Riparia* X *V. Rupestris*), Teleki 5C (*V. Berlandieri* X *V. Riparia*), S.O. 4 (*V. Berlandieri* X *V. Riparia*), Kober 5bb (*V. Berlandieri* X *V. Riparia*), 41B (*V. Vinifera* X *V. Riparia*), were evaluated for resistance to *A. mellea* root rot. Teroldego was the grafted variety. Two years old potted grapevine were inoculated with two different methods. The inocula were obtained from naturally infected roots and *in vitro* cultivated rhyzomorphs. The plants died after two years from the inoculation. In order to evaluate *A. mellea* incidence, the roots of dead vines were examined. The method, in which the inoculum was based on infected root pieces, gave a higher percentage of infections than the technique in which rhyzomorphs were used.

The root rot incidence was not significantly different among the tested rootstocks.

**Key words:** *Armillaria mellea*, inoculum, rootstock resistance.

**Introduction**

*Armillaria mellea* is a basidiomycete that causes root rot on grapevine and on several others crops and it is a severe problem both in forests and in orchards. In the first stage of the infection *A. mellea* kills the cambium, which leads to the plant death, and in the late stage it causes the decay of the whole root tissues. Its mycelium decomposes cellulose, hemicellulose, and lignin and it can survive for several years as saprophyte in the soil, without the presence of the host plant. This causes the infection of plants when the new vineyard is set up after an old infected one. In fact *A. mellea* moves from decayed wood to healthy plant roots by the rhyzomorphs (which are black, root like structures made of fungal hyphae).

Diagnosis based on symptoms on the aerial part of the plant is not easy: disease symptoms are not specific and they appear only when the infection is on a late stage. The plant often dies even after several years from the pathogen infection. In summer diseased grapevines present stunted shoots and foliage, dwarfed bunches and drying of berries. In autumn infected plants show an early change in leaf colour. This reddening (if a red variety) or yellowing (if a yellow variety) usually appears two weeks before the physiological change of colour on healthy plants. Leaves are not turgid and veins are yellow.

At root level the symptom based diagnosis is easier. Indeed the roots are rotten and dark and removing the plant from the soil is easy. Moreover typical white mycelial fans and rhyzomorphs are often present on the rotted roots. Root gives a characteristic smell of fresh
mushroom. Fruit bodies occasionally appear on grapevine infected plants and usually are single big fruiting body and not numerous as commonly happens on other plant species.

In Trentino Region, in the North East of Italy, in a valley called “Piana Rotaliana”, A. mellea root rot is a severe and increasing problem on grapevine. Teroldego Rotaliano is the prevalent variety, which is a local, well paid, red variety.

The incidence of root rot in Piana Rotaliana is high (Chini, 1983). On a total area of 140.37 hectares, evaluated in a previous study, the 13 %, which corresponds to 18.25 hectares, resulted infected by A. mellea (Harrington, 1995). The level of infection on plants vary from 1 to more than 10 % of the plants (Sannicolò et al., 2002).

A. mellea root rot can not be satisfactory controlled by pesticides. In such kind of disease the control should based on agronomical methods (cultural rotation, inter cropping with non host species, etc.) or on the use of resistant rootstocks.

The aim of the present research was the evaluation of artificial inoculum techniques to test for pathogen resistance on grapevine and to test grapevine rootstock currently used on Teroldego Rotaliano, and to possibly find source of resistance.

Materials and methods

The tested rootstocks were the ones that give the highest quality in Teroldego wine. They were: Schwarzmann (V. Riparia X V. Rupestris), 3309 (V. Riparia X V. Rupestris), 101-14 (V. Riparia X V. Rupestris), Teleki 5C (V. Berlandieri X V. Riparia), S.O. 4 (V. Berlandieri X V. Riparia), Kober 5bb (V. Berlandieri X V. Riparia), 41B (V. Vinifera X V. Riparia).

The used variety was Teroldego Rotaliano and the plants were two-year potted plants. For each rootstock were used 54 plants. Untreated plants were maintained as untreated control.

Two A. mellea artificial inoculation methods were evaluated. In the first method A. mellea naturally infected roots pieces were maintained for three months at high humidity in peat (1:1) and this substrate was used in pots for planting the plants.

In the second method A. mellea rhizomorphs were inserted below the rind of the roots (plant were three times inoculated: twice during first year, once during the second). The result evaluation was done two years after inoculation.

Fig. 1. A. mellea incidence on plants treated with two inoculation methods. Values with different letters were significantly different at P=0.05 (Duncan test)
Results and discussion

The inoculation method based on infected substrate, lead to higher infections on plants (it causes the infection on 46.4% of the dead plants), whereas the other method, based on the use of rhizomorphs was inefficacy, in fact it shows no significant difference with the untreated control (fig. 1).

Furthermore no statistically significant differences were present among the evaluated rootstocks (fig. 2).

The best *A. mellea* inoculum technique on grapevine is method which is based on the use of infected substrate. No resistant rootstocks were found among tested ones and also tolerance was not evidenced among the tested rootstocks.

We can conclude that at the moment disease control must be based only on infection prevention with agronomical methods and rootstocks can not be an instrument for controlling disease in infected sites.

Acknowledgements

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References


Optimisation of pruning wound protection for the control of Eutypa dieback of grapevine in France

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Key words: Eutypa dieback, control, pruning wound.

Introduction

Eutypa dieback, due to the ascomycete Eutypa lata (Pers.: Fr.) Tul. & C. Tul., causes significant declines in the temperate and humid areas devoted to grapevine production in Europe. This fungus is also involved in the development of wedge-shaped and whitish necroses considered characteristic of esca (Larignon and Dubos, 1997).

In France, the present and main control measures include, in this order of importance, replacing dead or too severely affected vines with young vines (replanting), restoring vines, applying prophylactic methods and painting pruning wounds with a specific fungicide compound (Escudo) or a paste (Phytopast). Since sodium arsenite, which was the only curative compound used to control esca for the last century, was forbidden in 2001, there is now a renewal of interest for preventative control measures either chemical or biological.

In order to improve our epidemiological knowledge of this disease and to optimize the protection of wounds, an applied research programme started in 1998 at Bordeaux. Preliminary and summarized results are presented here.

Material and methods

All experiments, carried out for epidemiological or control purpose in winter (mostly in January or in early February), were based on artificial inoculations wounds (25 to 30 per treatment) after the pruning of labeled 1-yr-old canes of Cabernet Sauvignon. In order to check the E. lata infections, two assessment methods were used and will be later compared: re-isolation of the fungal pathogen was done after an incubation period of either two weeks or one year.

Results and discussion

Pruning wound susceptibility
Preliminary studies, regarding the effect of inoculum dose, revealed that a quantity of ±100 ascospores inoculated per wound (1500 spores/wound were used previously) was adequate in providing a reproducible and highly sufficient level of infection. On the basis of this result, the susceptibility of pruning wounds was re-examined: inoculations confirmed that fresh wounds are very susceptible but that susceptibility seemed to decrease more rapidly than previously shown. This suggested that the first week after pruning should be considered the
most critical period for wound protection. Complete epidemiological results will be presented and discussed elsewhere.

Pruning wound protection

Ongoing projects involve the assessment of efficacy of fungicides spray-applied with a recycling system. First experiments were done by spraying (dose: 1%) an experimental and commercial chemical mixture, ALERT S from Dupont de Nemours (presently allowed and used for the control of fungal diseases on various annual crops: rape, cereals,...) with fluzilazole (125g/L) and carbendazime (250 g/L) : ratio 1/2 similar to those of Escudo already registered for painting. Results obtained in 2001 and 2002 (Table 1) showed that this kind of application, more rapid and more convenient than previous techniques of treatment (painting, individual sprayers or manual spraying pruning shears) may reduce significantly the *E. lata* infections of fresh pruning wounds in winter. Nevertheless, further work will be obviously necessary to assess the duration of this kind of control and its effectiveness under natural infection conditions in particular when vines will be pruned early in the dormant season.

Table 1. Efficacy results obtained in 2001 and 2002 with a commercial chemical mixture (fluzilazole: 125g/L) and carbendazime: 250 g/L): applied with a spray-recycling material for the preventative protection of grapevine pruning wounds.

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th></th>
<th>2002</th>
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<td></td>
<td>2 weeks</td>
<td>1 year</td>
<td>2 weeks</td>
<td>1 year</td>
</tr>
<tr>
<td>Non inoculated control</td>
<td>0</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>Inoculated control</td>
<td>12</td>
<td>-</td>
<td>34</td>
<td>-</td>
</tr>
<tr>
<td>Treated</td>
<td>4</td>
<td>66</td>
<td>8</td>
<td>76</td>
</tr>
</tbody>
</table>

Inf.: % of infection (based on the number of infected canes/number of inoculated canes); Eff.: Relative efficacy (with regard to inoculated control); NT: Not tested

Acknowledgements

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Reference

Fungi associated with esca and grapevine declines in North Ribatejo, Portugal

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Abstract: A survey of 179 vineyards in eight municipalities of North Ribatejo, Portugal, namely Abrantes, Almeirim, Alpiarça, Chamusca, Golegã, Santarém, Tomar, and Torres Novas was conducted in 2002 to identify pathogenic wood fungi. Grapevines showing esca and decline symptoms were examined for the presence of pathogenic fungi. Slightly less than half of the vineyards (46.4%) showed symptoms of grapevine decline.

Eighteen phytopathogenic fungi were isolated from the wood of the grapevines with decline symptoms. The isolates were identified according to their morphological characteristics on PDA. The fungi isolated and the percentage of the vineyards affected were: Phaeoacremonium spp. (22.9%), Phaeomoniella chlamydospora (20.5%), Phomopsis viticola (18.1%), Phoma sp. (14.5%), Penicillium sp. (10.8%), Sphaeropsis sp. (8.4%), Seimatosporium sp. (7.2%), Alternaria sp. (6.0%), Acremonium sp. (4.8%), Conyothyrium sp. (4.8%), Truncatella sp. (4.8%), Cladosporium sp. (3.6%), Aureobasidium sp. (2.4%), Fusarium sp. (2.4%), Arthrinium sp. (1.2%), Fomitiporia punctata (1.2%), Pestalotiopsis sp. (1.2%), and Phytophthora sp. (1.2%).

The spatial distribution of the fungi in North Ribatejo is presented and discussed.

Introduction

The Petri disease and other grapevine declines have been causing a considerable destruction of grapevines in Portugal. The species of Phaeomoniella and Phaeoacremonium associated with these declines were first detected in central and southern Portugal by Rego et al. (2000) and in the Vinho Verde region (northwest Portugal) by Chicau et al. (2000). Research on the aetiology of Esca suggests that different pathogens are probably involved. Recently, it has further been suggested that different fungi, causing at least two different diseases (Esca and Petri disease), can cause similar symptoms on grapevine (Surico & Mugnai, 2001).

The fungi associated with grapevine decline in northwest Ribatejo, along the Tagus river valley, and the spatial distribution of the fungi associated are presented in this work.

Materials and methods

The grapevine decline was surveyed in 179 vineyards of different ages and 197 trunks were collected from symptomatic vines. Fungi were isolated by transferring small pieces of wood tissue from different types of necrosis to plates of PDA (Merck).

Cultures were incubated at 25°C in the dark for about 7 days. Isolates were transferred to PDA, incubated at room temperature and identified according to their morphological characteristics. Identifications were made from squash mounts of fruiting structures mounted in lactophenol.
Results and discussion

The results of the percentage of fungi detected in trunks associated with grapevine decline symptoms are shown in Figure 1. The main results are the following:

- *Phaeoacremonium* spp. was the most frequently isolated fungi (22.9%), being isolated nine times as the only fungi present, six times associated with *Phaeomoniella chlamydospora*, other six with *Phomopsis viticola*, and one with *Acremonium* sp.
- *Phaeomoniella chlamydospora* was the second most isolated fungi (20.5%), being seven times isolated alone, six associated with *Phaeoacremonium* spp., and five with *Phomopsis viticola*.
- *Phomopsis viticola* was the third most isolated fungi (18.1%), being six times isolated alone, other six associated with *Phaeoacremonium* spp., and five times with *Phaeomoniella chlamydospora*.
- *Acremonium* sp. was isolated four times (4.8%), three alone and one associated with *Phaeoacremonium* sp.
- *Fomitiporia punctata* was isolated only once (1.2%) and it was not possible to detect other fungi associated.

![Pie Chart](image)

Fig. 1. Percentage of fungi detected in grapevine trunks with decline symptoms in Ribatejo Vineyards.

The spatial distribution of the fungi detected in grapevine trunks with decline symptoms can be seen in Figure 2. The results show that:

- *Phaeoacremonium* spp., *Phaeomoniella chlamydospora* and *Phomopsis viticola* are the fungi with highest incidence and have a more regular spatial distribution in the areas surveyed;
The *Phaeoacremonium* spp. + *Phomopsis viticola* association appears only in the left bank of Tagus River, probably because that area is frequently flooded;

*Phaeomoniella chlamydospora* and *Phaeoacremonium* spp. are commonly found alone and in association with other fungi, mainly together or with *Phomopsis viticola*;

*Acremonium* sp. was only found alone in the north of the surveyed area.

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**Fig. 2.** Distribution of the isolated fungi in the Northwest Ribatejo region.

**References**

Rego, C., Oliveira, H., Carvalho, A. & Philips, A. 2000: Involvement of *Phaeoacremonium* spp. and *Cylindrocarpon destructans* with grapevine decline in Portugal. – Phytopathologia Mediterranea 39: 76-79.


The genetic underpinning of the minimal fungicide strategy

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Abstract: Minimal fungicide strategy (MFS) is a downy mildew management strategy developed in Southern Switzerland by M. Jermini (see Jermini et al. 2003).

In 2001, three samplings of P. viticola oil spots were performed both in the MFS plot and in two untreated plots. The MFS plot was chemically treated 4 times along the vegetative season, therefore resulted much less infected by P. viticola than the C1 and C2 plots, completely destroyed by the disease at the end of July. The P. viticola populations causing the epidemics in C1, C2 and MFS plots were genotyped according to Gobbin et al., 2003. Mainly two very aggressive genotypes were responsible for about 50% and 17% of the total attack in the C1 plot and in the MFS plot, respectively. They first originated presumably within the C1 plot and then migrated to the MFS plot. Despite observational evidence of the efficacy of the MFS, no clear proof of fungicide efficacy on genotype reduction is provided by molecular genetic techniques.

Key words: population genetics, downy mildew, SSR

Introduction

Minimal fungicide strategy (MFS) is a downy mildew management strategy developed in Southern Switzerland regularly applied since 1999 in Cugnasco (CH) (see Jermini et al. 2003). With this research, we investigated the effect of fungicide treatments on a downy mildew epidemic occurring in a plot where MFS was applied (MFS plot). We compared it to an undisturbed epidemic occurring in an untreated adjacent plot.

Materials and methods

In 2001, the P. viticola population causing the downy mildew epidemic was genotyped. Three samplings (7.6 – 25.6 – 23.7) of P. viticola oil spots were performed both in the MFS plot (4 rows x 71 vines) and in an untreated (C1) plot (1 row x 6 vines). On 25 June, a second control (C2) plot (1 row x 6 vines) was sampled. In total 609 lesions could be used for genetic analysis. The MFS plot was chemically treated 4 times along the vegetative season (on 7.6, 26.6, 30.7 and 14.8), therefore resulted much less infected by P. viticola than the C1 and C2 plots, completely destroyed by the disease at the end of July.

DNA was extracted from the oil spots and P. viticola specific microsatellite markers amplified as prior described (Gobbin et al 2003). The allelic pattern was used to identify the genotypes.
Fig. 1. Downy mildew epidemic progress surveyed in the C1 (grey) and MFS (black) plots from 7.6.01 to 23.7.01 in Cugnasco (CH). Numbers at the beginning of each arrow refer to the number of single genotypes identified at the time of sampling. Numbers preceded by “Σ” refer to clonal genotypes (genotypes that at the time of sampling were already present in more copies). Percentages of total disease severity (tDS) are reported to the right of the arrows, only if relevant. Lines or arrows spanning from one date to the next, indicate that genotypes were identified at both sampling dates. Names of particularly aggressive genotypes are reported near the corresponding arrow. The most important migration event of *ibetosna* and *alovispa* is indicated by the dotted grey arrow. Vertical arrows refer to fungicide applications within the MFG plot.

Fig. 2. Migration events of *alovispa* (black) and *ibetosna* (grey) from the C1 plot to the MFG plot. The putative locations of the very first primary infections of both genotypes are reported in the left figure. Vineyard dimensions are expressed in meters.
Results

Within the 609 genotyped lesions, 222 different genotypes were detected all over the plots during the observation lap: 56 in the C1 plot, 20 in the C2 plot and 164 in the MFS plot. In the C1 plot on 7.6, a single genotype unable to produce progeny, was identified. On 25 June, 13 single genotypes (13 lesions) were identified; only three of them were re-identified at the last sampling (14 lesions), while the other disappeared from the population. Additionally, 4 genotypes already present in clones were recognized. Two of them, *alovispa* and *ibetosna*, were colonising a few vines (10 and 14 lesions, respectively) and were responsible for 57% of the total disease severity (*tDS*). We speculated that the primary infection occurred after 7.6 and that at least one asexual cycle may have occurred (Fig. 2). Even the C2 plot was colonised by both genotypes. On the next sampling date, the control plot C1 was massively invaded by those genotypes (C1: 34 new clones of *alovispa* + 21 new clones of *ibetosna*, 41% of *tDS*). 29% of *tDS* was generated by 39 new single genotypes (Fig. 1).

On 7 June, in the MFS plot nine genotypes were identified, causing 17 lesions (Fig. 1). One dominant genotype (*ebebesra*, 6 lesions) was attacking a single vine. Chemical treatment (and/or natural selection?) eliminated 8/9 of the genotypes present with exception of *ebebesra*, which continued undergoing asexual cycles. On 25 June, it caused 49% of *tDS*, (20 new lesions), while other 20 newly appeared genotypes were identified (21 lesions). The second treatment finally stopped the potentially aggressive genotype *ebebesra*, protected from a new massive attack and eliminated (in combination with natural selection?) 18/20 genotypes. Between 25.6 and 23.7, 84 single genotypes (84 lesions, 25% *tDS*) and 53 clonal genotypes, comprehending *alovispa* and *ibetosna* (causing 17% of *tDS*), invaded the MFS plot (Fig. 2).

Migration events, as observed for *alovispa* and *ibetosna*, were not the only ones occurring throughout the epidemic. The spreading pattern revealed an almost unidirectional movement of microsporangia from the C1 and/or C2 plots to the MFS plot (12 genotypes firstly found on the C1 or C2 plots moved successively to the MFS plot; 3 genotypes were sampled simultaneously on C1 and C2 migrated to the MFS plot, 10 genotypes were sampled simultaneously on C1 and MFS plot and 2 genotypes originated in the MFS plot attacked successively the C1 plot). The majority of genotypes (195) didn’t migrate to any of the plots.

Discussion

At the time of sampling, visual disease assessment clearly indicated that fungicide sprays strongly reduced disease severity within the MFS plot in comparison to both control plots. Nevertheless, the employment of genetics did not furnish clear evidence of their efficacy. We can not distinguish yet if genotypes were killed by fungicide or by natural selection. We express this concern, because a recent study which surveyed 18 European downy mildew populations (4685 samples), revealed that about 70% of all genotypes normally fade out without undergoing asexual cycles (genotypes identified once throughout the epidemic). The high mortality of genotypes, or restricted asexual reproduction, seems therefore to be a intrinsic natural characteristic of *P. viticola*.

We just can speculate that the second fungicide application was the most strategic, because it protected the vines from a massive genotype appearance (observed in C1) and it killed one potentially dangerous genotype, which showed similar behavior to the two migrating genotypes.

An even more impressive proof of efficacy of the MFS would have appeared, if no control plots were used to monitor the real development of the disease. Disease severity
would have been reduced by 17% on 23 July in the MFS plot, only by impeding *alovispa* and *ibetosna* to multiply and to migrate, but we would not have had any proof of it!

**References**


Physiological disorders affecting vine plants longevity: the FD disease in North-West of Italy

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Abstracts: The early senescence of vines is a serious problem in many regions. Predisposing factors for plant decay are certainly many and, in the last ten – years period, FD disease has been affecting many vineyards in Liguria, Lombardy and Piedmont. In order to understand the vine decay, several observations were made on: vineyard growers proficiency and vineyard technique, vine cultivars, soil characteristics and local climatic conditions, reputation and wine economy of the area. From the results it appears that several factors may combine together to give rise the physiological disorder where phytoplasms are in cause. FD symptoms have been found closely related to plant stress where overcrop, bad soil structure, trunk wounds more involved. Difficult climatic conditions may increase the phenomena and among grape vine cultivars there are clear differences in showing symptoms but this mainly proves a vine – site relationship and shows that the older traditional cultivars are more tolerant. Vine growing techniques may increase plant stresses and vascular disorders; in order to reduce problems, improving cultivation factors (yield equilibrium, plant wounds, soil capacity) are suggested; any FD symptoms have been found in deliberately abandoned vineyards. More attention should be made as well before the introduction of new cultivars into ancient growing areas.

Key words: vine, plant decay, FD, vineyard technique.

Introduction

The last few decades have seen the development of great changes in viticulture. Specific examples are: the use of not so suitable land, the great increase of mechanisation, the cultivation, in a wide sense, of few grape varieties everywhere.

The early senescence of vines is a serious problem with heavy economic impact in many regions. Formerly in Piedmont the economic life of a vineyard was normally more than 50 years; nowadays many vineyards are replaced at 25-30 years and old vineyards are becoming rare. Predisposing factors for plant decay are certainly many but in some ways we may simplify into few main reasons: soil and plant management, unsuitable grape varieties. Than climatic conditions may affect the phenomena spreading.

Intensive viticulture and high productivity goals may be responsible to endanger soil health; conditions in which the mechanisation is used, may give rise to problems such as erosion and soil compaction. Relating to plant management, we are seeing decay in the vine growers profession, definitely more skilled in machine use, but less skilled in pruning. The complexity of the subject is great and the aim of this paper is just to consider what call FD symptoms affecting descendent sap flow in phloem tissue. This problem in the last decade has become a serious problem in some wine zones of North Italy and for some vine growers whilst in others almost negligible.

The aim of this paper is not to investigate the pathogens themselves but to try to understand inside the complexity of grape growing system.
Materials and methods

A survey on FD symptoms was carried out starting 1989 in different vine growing areas of North West of Italy and particularly in Piedmont, Lombardy and Liguria. FD symptoms can be confused with similar symptoms due to girdling, wounds of the trunk, incompatibility phenomena on grafted vines, physiological disorders. For this reason, the diagnosis was not restricted to a precise disease description in the actual place, but combined with factors which in some way may contribute to the disease occurrence and evolution. Some basic rules were followed before any observation on the decay of a specific grape vine variety: vineyard technique (yields over the years, plant and soil management) vineyard grower’s proficiency (professional grape grower, part time or income augmentation), vine cultivars, soil characteristics, local climatic conditions and altitude. Observations were first made in East Liguria, than starting 1996 in Lombardy (Oltrepo) and Piedmont, Tortona and northern Monferrato areas. A few years later observations started in Asti and Langa Regions, which at that time had only few incidences. Others experiences were carried out in Piedmont and Liguria by leaving vineyards deliberately uncultivated for years in order to verify affected plants by FD.

Results

Red sandstone and clay sticky soils have been showing firstly the symptoms; in the case of marn calcareous soils almost unaffected plants were observed if proper growing practices were used. In the same vineyard affected by FD symptoms, the frequency of diseased plants was definitely higher or almost only in the border of clay sticky soil and not in the marn calcareous soil. Higher frequency of affected plants on soil with bad management leading to erosion and compaction (aggressive technique). Green cover soil proved better performances but only if not competitive with vine plants.

<table>
<thead>
<tr>
<th>Site</th>
<th>FD-frequency</th>
<th>Total CaCO3 %</th>
<th>Total CaCO3 active %</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>pH</th>
<th>E.S.C meq/100 g</th>
<th>O.M (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costigliole</td>
<td>low</td>
<td>20</td>
<td>6,2</td>
<td>18</td>
<td>41</td>
<td>41</td>
<td>8,1</td>
<td>18,1</td>
<td>1,27</td>
</tr>
<tr>
<td>Vigliano</td>
<td>medium</td>
<td>5</td>
<td>1,4</td>
<td>23</td>
<td>63</td>
<td>14</td>
<td>7,9</td>
<td>6</td>
<td>0,63</td>
</tr>
<tr>
<td>Dogliani</td>
<td>medium</td>
<td>–</td>
<td>–</td>
<td>30</td>
<td>45</td>
<td>25</td>
<td>5,3</td>
<td>14,3</td>
<td>0,31</td>
</tr>
<tr>
<td>Cortiglione</td>
<td>fairly-high</td>
<td>1</td>
<td>0,8</td>
<td>20</td>
<td>40</td>
<td>40</td>
<td>7,9</td>
<td>25</td>
<td>0,29</td>
</tr>
<tr>
<td>Moncalvo</td>
<td>medium-high</td>
<td>54</td>
<td>7</td>
<td>30</td>
<td>32</td>
<td>38</td>
<td>8,3</td>
<td>21,4</td>
<td>1,24</td>
</tr>
<tr>
<td>Canelli</td>
<td>very-low</td>
<td>25</td>
<td>10,7</td>
<td>17</td>
<td>44</td>
<td>39</td>
<td>8,3</td>
<td>11,8</td>
<td>0,58</td>
</tr>
<tr>
<td>Rosignano M.to</td>
<td>very-high</td>
<td>27</td>
<td>11</td>
<td>16</td>
<td>41</td>
<td>43</td>
<td>7,7</td>
<td>12,2</td>
<td>0,88</td>
</tr>
</tbody>
</table>
During the survey was evidence that ability in pruning has reduced and receiving an attention largely below its importance. As vine is characterised by its inability to heal wounds, FD symptoms were mainly recorded on vine plants with large diameter wounds opposite each other. Or wounds on the trunk by mechanical strokes. Vineyards with bad pruning technique have been seriously damaged while nearby others with proper management were almost safe. Plant decay was first observed on vineyards with high / very high yields and on high cordon training system. Some pruning experiences on FD affected vineyards showed that most of plants were successfully recovered with the shoots from the bottom of the trunk (table 2).

Among vine cultivars, the behaviour for FD symptoms was found rather different: Chardonnay and Rheinriesling with high sensitivity, White Muscat for the best tolerance (table 3).

The effect of altitude on Pinot noir clonal selection showed increasing problems from upper hills to lower altitude cultivation sites (table 4).

Table 2. FD’ symptoms (%) 1999-2001 and recovery in year 2002 on Barbera cultivar.

<table>
<thead>
<tr>
<th>Localities</th>
<th>Vine grower</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002 recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agliano</td>
<td>Pavese Mario¹</td>
<td>&gt; 38 %</td>
<td>&gt; 42%</td>
<td>&lt; 5 %</td>
<td>84 %</td>
</tr>
<tr>
<td>Costigliole</td>
<td>DurettoFiorenzo²</td>
<td>&gt; 42 %</td>
<td>&gt; 46%</td>
<td>&lt; 7 %</td>
<td>89 %</td>
</tr>
<tr>
<td>Costigliole</td>
<td>Stella Achille³</td>
<td>&lt; 8 %</td>
<td>&gt; 25%</td>
<td>&gt; 41 %</td>
<td>82 %</td>
</tr>
</tbody>
</table>

¹ retired, not professional, bad soil management, very high yields;
² professional vine grower, very high yields, large wounds by trunk shortening;
³ professional vine grower, very high yields, aggressive on soil, rather bad pruning

Table 3. Behaviour of some varieties in Piemonte and Lombardia * for FD symptoms.

<table>
<thead>
<tr>
<th>Grape variety</th>
<th>Localities</th>
<th>FD sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbera</td>
<td>Asti, Alessandria, Oltreo</td>
<td>medium / high</td>
</tr>
<tr>
<td>Croatina</td>
<td>Oltreo</td>
<td>Low</td>
</tr>
<tr>
<td>Grignolino</td>
<td>Asti, Monferrato</td>
<td>Low</td>
</tr>
<tr>
<td>Moscato Bianco.</td>
<td>Asti region</td>
<td>very low</td>
</tr>
<tr>
<td>Nebbiolo</td>
<td>Barolo area</td>
<td>Low</td>
</tr>
<tr>
<td>Wälschriesling</td>
<td>Oltreo</td>
<td>Low</td>
</tr>
<tr>
<td>Rheinriesling</td>
<td>Oltreo</td>
<td>very high</td>
</tr>
<tr>
<td>Uva Rara</td>
<td>Oltreo</td>
<td>Low</td>
</tr>
</tbody>
</table>

* Observations are not from a field of comparative variety trials, but from repeated survey vineyards in different situations where, frequently, at least two varieties were closely cultivated

Table 4. Effect of altitude on Pinot noir in Oltrepo and Piedmont.

<table>
<thead>
<tr>
<th>Localities</th>
<th>&lt; 150 m ASL</th>
<th>&gt; 280 m ASL</th>
<th>&gt; 360 m ASL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oltrepo</td>
<td>very affected</td>
<td>Some problems</td>
<td>not affected</td>
</tr>
<tr>
<td>Monferrato</td>
<td>Affected</td>
<td>Few problems</td>
<td>not affected</td>
</tr>
<tr>
<td>Langa</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In all of the total abandoned vineyards (any cultural technique was applied) were never recorded FD affected plants and after years, then vineyards were recovered by convenient pruning and now a days they look as normal.

Tab. 5. Experiences on deliberately abandoned vineyards.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Surface ha</th>
<th>Vine cultivars</th>
<th>Start abandon</th>
<th>Recovery</th>
<th>FD symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cicagna (Ge)</td>
<td>0.50</td>
<td>Scimiscià</td>
<td>1995</td>
<td>1999</td>
<td>any</td>
</tr>
<tr>
<td>Costigliole (At)</td>
<td>0.60</td>
<td>Barbera</td>
<td>1998</td>
<td>2002</td>
<td>any</td>
</tr>
<tr>
<td>Costigliole (At)</td>
<td>0.29</td>
<td>Barbera</td>
<td>1999</td>
<td>2003</td>
<td>any</td>
</tr>
<tr>
<td>Isola D’Asti (At)</td>
<td>0.78</td>
<td>Barbera</td>
<td>1998</td>
<td>2003</td>
<td>any</td>
</tr>
<tr>
<td>Isola D’Asti (At)</td>
<td>0.27</td>
<td>Sauvignon</td>
<td>1998</td>
<td>2003</td>
<td>any</td>
</tr>
<tr>
<td>Mongardino (At)</td>
<td>2.20</td>
<td>Barbera</td>
<td>1999</td>
<td>2001</td>
<td>any</td>
</tr>
</tbody>
</table>

Concerning climatic condition it appears that, in the last 15 years, the rainfall has been greatly lower if compared with a long-term period of 130 years. Moreover in the period many precipitations were rather intense and flood happened in 1994 and 1999. Air temperature has been increasing in the late 90’s and Winkler index is showing clear evidence of increase of temperatures starting 1997 up to year 2001 to extremes never recorded before.

![Fig. 1. Canelli (AT) - Rainfall in the period 1987 - 2002 in comparison with an average of 130 years.](image)

**Discussion**

FD disease has been damaging, with different intensity, many vine growing areas in Liguria, Lombardy, Piedmont. From our results it appears that several factors may combine together to give rise the physiological disorder where phytoplasms are in cause and we may come to some conclusions. FD symptoms are closely related to plant stress where overcrop, bad soil structure, trunk wounds are most involved. Difficult climatic conditions may have increased the phenomena; there is some coincidence indeed between exceptional drought and hight temperatures evolution in the recent years and FD symptoms spreading, where first affected the places with greatest limiting plant survival factors. The cultivar Barbera was found rather affected by FD but this mainly in condition of over crop and unsuitable growing technique.
Fig. 2. Winkler index

Fig. 3. Monthly rainfall in the years 1997 and 2002

(cluster grower). In convenient and more balanced situation (wine growers) the problem was irrelevant. The vine cultivar Welchriesling that proved fairly tolerant toward FD, is largely cultivated in Central and East Europe whilst Rheinriesling is successfully grown in some German terroir. This mainly proved a vine – site relationship and shows that older traditional cultivars are more tolerant. Some modern growing techniques are aggressive; plant are accumulating stress which gradually are weakening the tissues and increasing vascular disorders. In order to reduce the above mentioned problems we need as a priory, to improve cultivation factors, to obtain better equilibrium in the yields, make proper wounds, improve soil capacity and create more professionalism in vine growing. The recovery of affected plant by a new shoot from the bottom of the trunk proved to be a proper technique. More careful thought should be made before the introduction of new cultivars into ancient growing areas.

As in abandoned vineyards were never found FD affected plants this demonstrates that uncultivated fields are not responsible for FD spreading.
Unfortunately, the FD problems are faced with an increasing use of insecticides with secondary effects on entomofauna, *pronuba* and responsible of reappearance of Tetranychidae mites. We are observing the coming back of vineyards problems that, with great efforts, have been almost solved in the past.

**References**


First Intern.Workshop on Grapevine Trunk Diseases, Siena, Italy 1-2 October 1999.


Preliminary investigations on the interaction between spiders (Araneae) and grapevine moth (Lobesia botrana (Denis et Schiffermüller)) populations in Apulian vineyards

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Abstract: The relations between spider population and grapevine moth (Lobesia botrana (Denis et Schiffermüller)) infestation were studied in 2000-2001 on an experimental vineyard of the Agricultural Faculty, sited in Valenzano (Bari). The spider species composition and their seasonal fluctuations were also investigated.

Starting from vine blooming, samples of 50 clusters were weekly collected from each of the two vine cultivars, Italia and Sangiovese, until vintage. Spiders, grapevine moth larvae and infested berries of each cluster were counted. Spiders were identified up to family, genus or species level according to their developmental stage. Since vintage till the end of 2001 further spider samples were collected by beating 50 vine-shoots per cultivar on an entomological umbrella. Grapevine moth flights were also monitored.

About 1400 specimens of spiders were collected altogether. The most represented spider families were, in a decreasing order, Clubionidae, Theridiidae, Thomisidae (including Philodromidae), Salticidae, Linyphiidae and Gnaphosidae. These families accounted for over 90% of all collected spiders. Hunting spiders reached about 70% of the whole spider population, thus prevailing on the orb web weavers.

The most frequent species were Cheiracanthium mildei L. Koch, Theridion melanurum Hahn, Philodromus rufus (Walckenaer), Icius hamatus (C.L. Koch) and Meioneta rurestris (C.L. Koch).

Significant relations were established comparing the population trend of single spider families and grapevine moth flight diagram, indicating that L. botrana was one of the main preys making up the spider diet. Clubionidae, Theridiidae and Thomisidae, which were the most represented families, showed the best fitting to grapevine moth adult population. On the contrary, the spider population did not show any close relation with grapevine moth larval infestation. The population trend of spiders was very similar in the two cultivars, thus revealing the absence of any preference related to cluster colour and compactness. In 2001 almost the same number of spiders was collected on Italia and Sangiovese cultivars (about 500 spiders/cultivar).

Some differences emerged in the total number of spiders collected on cv Sangiovese in 2000 (about 300 spiders through the whole year) and 2001 (about 500 spiders); however, the general population trend during the year, on the same cultivar, was basically the same in both years.

Introduction

Spiders (Araneae) are among the most important generic predators in ecosystems. They represent an important mortality factor for insects, as they affect their population dynamics (Nyffeler & Benz, 1987; Nyffeler et al., 1990; Wise, 1993; Nyffeler et al., 1994). Ecological studies on spiders regard different agro-ecosystems such as citrus and peach orchards, cotton fields, etc. (Nyffeler & Benz, 1987; Benfatto et al., 1995; Thaler & Zapparoli, 1993). Costello

1 The present research was funded by ex 60%.

2 The Authors equally contributed to the carrying out and drafting of the present work.

On the basis of the previous considerations, a study on spiders’ composition and their seasonal fluctuations in an Apulian vineyard was carried out in 2000-2001. Adult flight diagrams and larval infestation induced by *Lobesia botrana* (Denis & Schiffermüller) were also determined in the same period and for the same vineyard. A possible relationship between spider and *L. botrana* populations was then observed.

**Materials and methods**

The research was carried out in 2000-2001 on an experimental “tendone” vineyard of the Agricultural Faculty, sited in Valenzano (Bari).

In the vineyard, 5 plots of white table grape, cv Italia, and 5 plots of black vine grape, cv Sangiovese, were randomly selected. Each plot was made up of 10 vines.

Starting from vine blooming, samples of 50 clusters were weekly collected from each of the two vine cultivars until vintage. Spiders, alive grapevine moth larvae and total infested berries of each collected cluster were counted. Spiders were identified up to family, genus or species level according to their developmental stage. Since vintage till the end of 2001 further spider samples were collected by beating 50 vine-shoots per cultivar on an entomological umbrella. Grapevine moth flights were monitored by displacing two sexual pheromone traps in the vineyard and counting the males caught once a week.

Temperature, rain and relative humidity were daily recorded through the whole experimental period.

The normal cultivation techniques were adopted in the vineyard. No insecticide treatment was applied in the experimental plots except one on cv Italia on 2nd August 2000. Owing to the insecticide treatment, samplings on cv Italia stopped at the end of July 2000.

**Results and discussion**

In 2000-2001, about 1400 specimens of spiders were collected altogether (Tab. 1). The most represented spider families were, in a decreasing order, Clubionidae, Theridiidae, Thomisidae (including Philodromidae), Salticidae, Linyphiidae and Gnaphosidae. These families accounted for over 90% of all collected spiders. Hunting spiders reached about 70% of the whole spider population, thus prevailing on the orb web weavers.

The most frequent species were *Cheiracanthium mildei* L. Koch, *Theridion melanurum* Hahn, *Philodromus rufus* (Walckenaer), *Icius hamatus* (C.L. Koch) and *Meioneta rurestris* (C.L. Koch) (Tab. 1).

No close relation was found between the spider population and grapevine moth larval infestation, either considering the total spider population or the population of single families. There were little possibilities that larvae could represent an important part in the spider diet because grapevine moth larvae penetrated into the berries soon after hatching, thus leading to an endophytic life.

Comparing the total spider population with the adult *L. botrana*, some general prey-predator relation could be appreciated: the second moth flight, peaking on June 20th, was
associated with an increment in spider population with a peak at the beginning of July; after the occurrence of the third and fourth moth flights, new increments in spider population were observed.

Table 1. Total spider number of each genus or species collected in 2000-2001.

<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Species</th>
<th>Author</th>
<th>Male</th>
<th>Female</th>
<th>Spiderlings</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anyphaenidae</td>
<td>Anyphaena</td>
<td>alboirrorata</td>
<td>Simon</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Araneidae</td>
<td>Araneus</td>
<td></td>
<td>Clerck</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Araneidae</td>
<td>Others</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>Cheiracanthium</td>
<td>mildei</td>
<td>L. Koch</td>
<td>5</td>
<td>7</td>
<td>443</td>
<td>455</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>Clubiona</td>
<td></td>
<td>Latreille</td>
<td>0</td>
<td>0</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>Clubionidae</td>
<td>Others</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Aphantaulax</td>
<td></td>
<td>Simon</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Drassodes</td>
<td></td>
<td>Westring</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Leptodrassus</td>
<td></td>
<td>Simon</td>
<td>0</td>
<td>0</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Poecilochroa</td>
<td></td>
<td>Westring</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Scotophaeus</td>
<td></td>
<td>Simon</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gnaphosidae</td>
<td>Zelotes</td>
<td></td>
<td>Gistl</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Linyphiidae</td>
<td>Meioneta</td>
<td>rurestris</td>
<td>(C.L. Koch)</td>
<td>6</td>
<td>0</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>Linyphiidae</td>
<td>Others</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>Mysmenidae</td>
<td>Mysmenella</td>
<td></td>
<td>Brignoli</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nesticidae</td>
<td>Nesticus</td>
<td></td>
<td>Thorell</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Oxyopidae</td>
<td>Oxyopes</td>
<td>lineatus</td>
<td>Latreille</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Salticidae</td>
<td>Heliophanus</td>
<td></td>
<td>C.L. Koch</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Salticidae</td>
<td>Icius</td>
<td>hamatus</td>
<td>(C.L. Koch)</td>
<td>7</td>
<td>2</td>
<td>126</td>
<td>135</td>
</tr>
<tr>
<td>Salticidae</td>
<td>Others</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Therididae</td>
<td>Theridion</td>
<td>impressum</td>
<td>L. Kock</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Therididae</td>
<td>Theridion</td>
<td></td>
<td>Walckenaer</td>
<td>0</td>
<td>0</td>
<td>194</td>
<td>194</td>
</tr>
<tr>
<td>Therididae</td>
<td>Others</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>57</td>
<td>57</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>Philodromus</td>
<td>rufus</td>
<td>(Walckenaer)</td>
<td>0</td>
<td>1</td>
<td>141</td>
<td>142</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>Synaema</td>
<td>globosum</td>
<td>(Fabricius)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Thomisidae</td>
<td>Xysticus</td>
<td></td>
<td>C.L. Koch</td>
<td>0</td>
<td>0</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>10</td>
<td>1385</td>
<td>1415</td>
</tr>
</tbody>
</table>

Almost 98% of the total collected spiders were represented by spiderlings, thus making the identification of samples at the species level difficult.

As the collected spider species normally had one generation per year, the fluctuations in spider population were partly due to the subsequent occurrence of different spider families.
during the year, and to quantitative variations depending on the gradual hatching of new spiderlings. In order to prevent the influence of the gradual occurrence of different families in the total spiders' population, the relation between the flight trend of *L. botrana* and the populations of each spider family was investigated.

Clubionidae showed a first periodicity (increment, peak and decrement of population density) of two months, starting in mid-June and ending around mid-August, which seemed related to the *L. botrana* second flight (end of May-mid July). A second periodicity of this family started in the second decade of August and extinguished around the end of September. This periodicity followed the third flight of the moth (mid-July/end of August). A new increase in spider populations, starting at the beginning of October, had a less defined ending, but followed, as a whole, the third moth flight (early September-early October). The presence of Clubionidae in the vineyard was therefore related to the availability of preys represented by adults of grapevine moth.

Similar relations emerged comparing *L. botrana* population with Theridiidae and Thomisidae, though the populations of these spider families showed different quantitative and temporal fluctuations from those of Clubionidae.

As to the fluctuations between years, the spider population trend observed in 2000 was quite similar to that of 2001. Some differences emerged when comparing the total number of spiders collected on cv Sangiovese in 2000 (about 300 spiders through the whole year) and 2001 (about 500 spiders), although the general population trend during the year was almost the same in both years.

Moreover, the population trend of spiders was very similar on the two considered cultivars, thus revealing the absence of any preference related to cluster colour, compactness, shape, etc. The total number of spiders collected on Italia and Sangiovese cultivars in 2001 (about 500 spiders/cultivar) was also similar.

No clear and possible influence of climatic factors (temperature, relative humidity, etc.) on spider families emerged in the years being studied.

**Conclusions**

A significant relation was observed between the population trend of single spider families and grapevine moth flight diagram, thus indicating that *L. botrana* could be one of the main preys making up the spider diet. Clubionidae, Theridiidae and Thomisidae, which were the most represented families, showed the best fitting to grapevine moth adult population.

Grapevine moth larvae probably do not represent important preys in spider diet.

The population trend of spiders was very similar on the two considered cultivars, indicating the absence of any preferences related to cluster colour, compactness, shape, etc.

The general spider population trend was almost the same in both years.

**Acknowledgements**

We are very much grateful to Dr. C. Pesarini (Museo Civico di Storia Naturale, Milano) for the identification of some of the collected spiders.

**References**


Laboratory tests of the effect of *Bacillus thuringiensis* on grape berry moth *Lobesia botrana* (Lepidoptera: Tortricidae) and on the pseudococcids’ predator *Nephus includens* (Coleoptera: Coccinellidae)

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Abstract: Some *Bacillus thuringiensis*-based products were tested for their effects on grape berry moth *Lobesia botrana* and on the pseudococcids’ predator *Nephus includens*. The products Agree (*B. thuringiensis* subsp. *kurstaki* / subsp. *aizawai*, 25,000 I.U./mg), Dipel (*B.t. subsp. *kurstaki*), Bactospeine (*B.t. subsp. *kurstaki*), Xentari (*B.t. subsp. *aizawai*) and BMP (*B.t. encapsulated d-entotoxin*) were tested on artificial diet with addition of 1% sugar in order to study the effect on *L. botrana* and all caused >90% larval mortality. The same formulations were tested on the pseudococcids’ predator *N. includens*, reared on *Planococcus citri* (Hemiptera: Pseudococcidae), without to affect the survival of them. Therefore it is suggested in combined infestation by *L. botrana* and pseudococcids, the application with *B.t.* products for the control of the first pest and the releases of the effective predator *N. includens* for the control of the second pest.

Introduction

The control of *Lobesia botrana* and other lepidopterous pests of vineyard by using *Bacillus thuringiensis*-based products is known in laboratory as well in field conditions (Du Fretay and Quenin, 2000, Keil et al., 1998). A problem that follows the use of *B.t.* in vineyards is the unsatisfactory control of other pests like pseudococcids (Baum, 1986). This laboratory-study on the one hand tries to test some *B.t.*-based products to control *Lobesia botrana* and on the other hand to ascertain if these products have any effect on the pest *Planococcus citri* (Hemiptera: Pseudococcidae) and on its natural enemy *Nephus includens* (Coleoptera: Coccinellidae). The mealybugs *Planococcus citri* and *Planococcus vitis* (= *Planococcus ficus*) have been found to infest vineyards in Greece and the pseudococcids predator *N. includens* has been proved as an effective natural enemy (Kontodimas et al., unpublished data).

Material and methods

The products that have been tested were:

- Agree (strain GC-91 *B. t. subsp. kurstaki* / subsp. *aizawai*, 25,000 I.U./mg.),
- Dipel (*B.t. subsp. *kurstaki*, 32,000 I.U./mg),
- Bactospeine (*B.t. subsp. *kurstaki*, 16,000 I.U./mg),
- Xentari (*B.t. subsp. *aizawai*, 15,000 I.U./mg) and
- BMP (*B.t. encapsulated d-entotoxin*, 32,000 I.U./mg)

Three trials of each product have taken place in plastic cylindrical vials 8 cm height and 3 cm width in laboratory conditions (temperature: 26°C and relative humidity: 60%). To study the effects on *Lobesia botrana* the products have been mixture in the recommended doses with artificial diet with addition of 1% sugar (Charmillot et al., 1992). Each trial has been carried out by adding 20 second-instar larvae to the *B.t.*-mixed diet. To study the effects on
Nephus includens the products have been sprayed on 20 second-instar larvae and 20 adults that have been reared on live Planococcus citri. To study the effects on Planococcus citri the products have been sprayed on 20 first-instar larvae that have been reared on potato-germs. Two types of control have been used: the normal diet and the 1% sugar added diet. The vials have been observed daily for one week.

Mortalities have been compared by Tukey – Kramer (HSD) test (Sokal and Rohlf, 1995) using the statistical package JMP (Shall et al., 2001).

The efficacy has been calculated by Abbott’s formula for the two types of control:

\[
Efficacy_1 = \left[1 - \left(\frac{\text{post - spray density in treatment}}{\text{pre - spray density in treatment}} \times \frac{\text{pre - spray density in control}}{\text{post - spray density in control}}\right)\right] \times 100
\]

\[
Efficacy_2 = \left[1 - \left(\frac{\text{post - spray density in treatment}}{\text{pre - spray density in treatment}} \times \frac{\text{pre - spray density in control + sugar1%}}{\text{post - spray density in control + sugar1%}}\right)\right] \times 100
\]

**Results and discussion**

The results are presented in Table1. All B.t.-products caused high mortality (>90%) to L.botrana’s larvae and comparatively to the controls >80% efficacy. In opposition no effect have been observed on N.includens’s larvae and adults and on P.citri’s larvae.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mortality after 10 days (%)</th>
<th>L. botrana larvae Efficacy 1 (%)</th>
<th>Efficacy 2 (%)</th>
<th>N. includens adults Mortality after 7 days (%)</th>
<th>N. includens larvae Mortality after 7 days (%)</th>
<th>P. citri larvae Mortality after 7 days (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agree</td>
<td>95.0 a</td>
<td>90.6</td>
<td>89.7</td>
<td>13.3 a</td>
<td>28.3 a</td>
<td>40.0 a</td>
</tr>
<tr>
<td>Dipel</td>
<td>91.7 a</td>
<td>84.4</td>
<td>82.8</td>
<td>18.3 a</td>
<td>28.3 a</td>
<td>38.3 a</td>
</tr>
<tr>
<td>Bactospeine</td>
<td>93.3 a</td>
<td>87.5</td>
<td>86.2</td>
<td>16.7 a</td>
<td>25.0 a</td>
<td>36.7 a</td>
</tr>
<tr>
<td>Xentari</td>
<td>93.3 a</td>
<td>87.5</td>
<td>86.2</td>
<td>15.0 a</td>
<td>23.3 a</td>
<td>30.0 a</td>
</tr>
<tr>
<td>BMP</td>
<td>91.7 a</td>
<td>84.4</td>
<td>82.8</td>
<td>20.0 a</td>
<td>21.7 a</td>
<td>35.0 a</td>
</tr>
<tr>
<td>Control</td>
<td>51.7 b</td>
<td></td>
<td></td>
<td>13.3 a</td>
<td>26.7 a</td>
<td>33.3 a</td>
</tr>
<tr>
<td>Control + sugar 1%</td>
<td>46.7 b</td>
<td></td>
<td></td>
<td>15.0 a</td>
<td>21.7 a</td>
<td>35.0 a</td>
</tr>
</tbody>
</table>

The above laboratory results certify the effective control of Lobesia botrana by Bacillus thuringiensis. In addition no effects on Planococcus citri and on its predator Nephus includens have been observed. Pseudococcids are serious pests of grapevines and often make a pest-complex with Cryptoblabes gniidiella (Lepidoptera: Pyralidae). It is referred in field trials in vineyards infected by L. botrana, C. gniidiella and Planococcus vitis (=P. ficus), that Bacillus thuringiensis preparations had controlled the lepidopterous pests but not the
mealybug (Baum, 1986). *N. includens* is an effective predator of pseudococcids (Kontodimas et al., unpublished data); therefore it is suggested in combined infestation by lepidopterous pests and pseudococcids, the application with *B.t.*-products for the control of the lepidopterous pests and the releases of *N. includens* for the control of the pseudococcids.

**References**

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European grapevine moth control in a Chianti vineyard by mating disruption technique

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Abstract: In the Chianti district the mating disruption technique against Lobesia botrana (Den. & Schiff.) (Tortricidae) has been investigated in 2001 and 2002 with Shin-Etsu dispensers. Male captures were always absent or extremely limited in the pheromone treated vineyards. Significant differences between treated and untreated areas were pointed out in the two years for each of the three generations. In a plot of the “Sangiovese” variety particularly affected by infestations of L. botrana and situated borderline of the treated area, larval populations of the third generation resulted relatively high, though considerably lower in 2002 than in 2001. On the whole, results of the first two years of investigations are much more satisfying in comparison with those of previous trials carried out with different methods and materials.

Key words: Lobesia botrana, mating disruption, Isonet L dispensers, Chianti, Italy

Introduction

Nowadays, in Central Italy, the European grapevine moth Lobesia botrana (Den. & Schiff.) is still considered as the main pest, in particular for the harmfulness of its carpophagous generations on the varieties with compact bunch and late ripening. The other tortricid Eupoecilia ambiguella (Hb.) is less frequent and noxious (Bagnoli, 1990).

In Tuscany, the mating disruption technique (MDT) against L. botrana has been tested since 1989 both in the Chianti district (Bagnoli et al., 1993; Bagnoli & Goggioli, 1996) and in the province of Pisa (Bagnoli et al., 2001) with Rak2 Basf dispensers. Although mating disruption was applied very differently in the two areas (size of treated area, type of dispensers, target flights, period of application, number of dispensers per ha etc.), results obtained were similar and efficacy of this technique compared to untreated control areas was confirmed; nevertheless the method validity was not always adequate.

Today, this technique is widely used in other European important viticultural regions and is applied on a larger and larger scale (Stockel et al., 1994; Charmillot & Pasquier, 2000; Varner et al., 2001). This fact, together with availability of new dispensers ensuring a more regular and prolonged over time release of pheromones, induced us to investigate again efficacy of this technique in typical and prestigious Chianti vineyards (Bagnoli et al., 2002).

Material and methods

Investigations were carried out in vineyards of Castello di Ama Estate (Gaiole in Chianti, Siena) situated in a hilly area where the population dynamics of the two grape moths have been studied in the last ten years. MDT was applied in a little valley of 22 hectares (Bellavista) in 2001 and in a further little basin of 10 hectares (La Casuccia) in 2002, mainly on “Sangiovese”, “Merlot” and “Chardonnay” varieties. Isonet L Shin-Etsu dispensers, con-
taining 172 mg of active substance each, were placed at the bunch height and before the first flight (end of March) at a density of about 500 dispensers per ha. Other vineyards of the farm having similar agronomical features were considered as untreated control. Pheromone traps (Traptest Isagro) were used to monitor the adult population of *L. botrana* and *E. ambiguella*. The infestation of the first generation was evaluated by field observations. For the carpophagous generations, laboratory observations were carried out on sampled bunches to carefully evaluate density and structure of larval populations and fruit damage. Thus, variables considered for the assessment of MDT effectiveness are the following: a) number of males captured per trap; b) rate of attacked inflorescences or clusters; c) number of nests per inflorescence or number of larvae per cluster; d) number of damaged berries per cluster.

**Results and discussion**

Gas chromatography analysis, performed by Shin-Etsu on samples of dispensers collected during the season, showed that each of the three *L. botrana* flights were affected by a good pheromone release. This is confirmed by data of the traps installed in the pheromone treated plots where the capture of males were always absent or extremely limited, with the following exceptions: Ama19 (Sangiovese) 29/03/01: 1 (specimen); 10/05/01: 1; 05/07/01: 4; Ama12 (Chardonnay) 16/08/01: 1; Ama1 (Canaiolo) 24/04/02: 2; 02/05/02: 1; Ama9 (Sangiovese) 02/05/02: 1; Ama49 (Merlot) 02/05/02: 2.

From Fig. 1, that includes data of two representative untreated plots, it is possible to infer that outside of the MDT area captures of *L. botrana* resulted higher in 2002 whereas those of *E. ambiguella* in 2001.

As concerns the larval population, besides larvae of *L. botrana*, also specimens of *E. ambiguella* (in May-June and July) and of *Ephestia parasitella unicolorella* Staudinger (Pyralidae Phycitinae) (especially during the period of grapes ripening) were found. However, these two species were not considered when comparing the pheromone treated plots to untreated control.

Infestation of the first generation in the pheromone treated plots (Tab. 1) was very low in 2001 and the efficacy rate of the MDT was over 90% in comparison to the majority of the results of the untreated plots. In the vineyard “La Casuccia” where the mating disruption was applied for the first time in 2002, infestation was higher and reached 9% in Ama43 (Sangiovese), a rate very close to that of Ama26 (Sangiovese) of the untreated area (12%).

As regards the second generation (Tab. 2), infestation of the pheromone treated area was, in 2001, rather relevant in some of the plots: in Ama12 (Chardonnay) values of 30 larvae per 100 bunches were found. However, high infestation of untreated plots showed that the effectiveness rate of the MDT is over 64%, even considering the worst scenario. In 2002 effectiveness was everywhere very high, reaching over 87% and peaks of 97%.

Data on the third generation (Tab. 3) highlight, both in 2001 and 2002, a general increase of the infestation in the untreated area that reached a mean value of 2-3 larvae per bunch in Ama58 (Pinot noir) and Ama35 (Sangiovese). On the other hand, infestation resulted successfully controlled in the treated area where the method showed, almost in each plot, an efficacy rate of over 90%.

Results of Ama19 (Sangiovese), where attacks of 0.39 and 0.18 larvae per cluster were registered in 2001 and 2002 respectively, may be attributed to three main factors such as a higher susceptibility of the plot to the *L. botrana* attacks, the site of the plot which was borderline of the treated area, vine plants affected by the Esca disease leading to a loss of over 20% of the vine-stocks and, consequently, to a lower leaf density.
Fig. 1. Male captures of *Lobesia botrana* (Lb) and *Eupoecilia ambiguella* (Ea) in two untreated vineyards of Castello di Ama (Ama 35, “Sangiovese”; Ama58, “Pinot noir”).

Table 1. Infestation data of the first generation of *Lobesia botrana*.

<table>
<thead>
<tr>
<th>Vineyards</th>
<th>N. sampled bunches</th>
<th>% infested bunches</th>
<th>N. nests per bunch</th>
<th>Effectiveness compared to untreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ama58, 26</td>
</tr>
<tr>
<td>2001 “treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama12 (Chardonnay)</td>
<td>650</td>
<td>3.53</td>
<td>0.036</td>
<td>93.48</td>
</tr>
<tr>
<td>Ama20 (Pinot gris)</td>
<td>150</td>
<td>0.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ama24 (Merlot)</td>
<td>1,350</td>
<td>0.52</td>
<td>0.005</td>
<td>99.09</td>
</tr>
<tr>
<td>Ama22 (Sangiovese)</td>
<td>600</td>
<td>0.33</td>
<td>0.003</td>
<td>99.46</td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>450</td>
<td>0.66</td>
<td>0.008</td>
<td>98.55</td>
</tr>
<tr>
<td>2001 “untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama58 (Pinot noir)</td>
<td>250</td>
<td>36.00</td>
<td>0.552</td>
<td>-</td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>400</td>
<td>12.50</td>
<td>0.155</td>
<td>-</td>
</tr>
<tr>
<td>2002 ”treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>260</td>
<td>2.30</td>
<td>0.023</td>
<td>80.83</td>
</tr>
<tr>
<td>Ama43 (Sangiovese)</td>
<td>100</td>
<td>9.00</td>
<td>0.100</td>
<td>16.67</td>
</tr>
<tr>
<td>2002 ”untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama26 (Sangiovese)</td>
<td>300</td>
<td>12.00</td>
<td>0.120</td>
<td>-</td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>200</td>
<td>39.50</td>
<td>0.495</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Infestation data of the second generation of *Lobesia botrana*.

<table>
<thead>
<tr>
<th>Vineyards</th>
<th>N. sampled bunches</th>
<th>% infested bunches</th>
<th>N. larvae per bunch</th>
<th>Effectiveness compared to untreated control</th>
<th>N. damaged berries per bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ama58</td>
<td>Ama35</td>
</tr>
<tr>
<td>2001 “treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama12 (Chardonnay)</td>
<td>50</td>
<td>20.00</td>
<td>0.300</td>
<td>64.29</td>
<td>83.70</td>
</tr>
<tr>
<td>Ama24 (Merlot)</td>
<td>50</td>
<td>10.00</td>
<td>0.100</td>
<td>88.10</td>
<td>94.57</td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>50</td>
<td>10.00</td>
<td>0.100</td>
<td>88.10</td>
<td>94.57</td>
</tr>
<tr>
<td>2001 “untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama58 (Pinot noir)</td>
<td>50</td>
<td>64.00</td>
<td>0.840</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>50</td>
<td>84.00</td>
<td>1.840</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002 “treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama12 (Chardonnay)</td>
<td>50</td>
<td>2.00</td>
<td>0.020</td>
<td>91.67</td>
<td>97.14</td>
</tr>
<tr>
<td>Ama24 (Merlot)</td>
<td>60</td>
<td>3.33</td>
<td>0.030</td>
<td>87.50</td>
<td>95.71</td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>51</td>
<td>1.96</td>
<td>0.020</td>
<td>91.67</td>
<td>97.14</td>
</tr>
<tr>
<td>2002 “untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama58 (Pinot noir)</td>
<td>70</td>
<td>22.85</td>
<td>0.240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>63</td>
<td>49.20</td>
<td>0.700</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Infestation data of the third generation of *Lobesia botrana*.

<table>
<thead>
<tr>
<th>Vineyards</th>
<th>N. sampled bunches</th>
<th>% infested bunches</th>
<th>N. larvae per bunch</th>
<th>Effectiveness compared to untreated control</th>
<th>N. damaged berries per bunch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ama58</td>
<td>Ama35</td>
</tr>
<tr>
<td>2001 “treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama12 (Chardonnay)</td>
<td>50</td>
<td>2.00</td>
<td>0.020</td>
<td>99.17</td>
<td>98.96</td>
</tr>
<tr>
<td>Ama24 (Merlot)</td>
<td>50</td>
<td>2.00</td>
<td>0.020</td>
<td>99.17</td>
<td>98.96</td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>200</td>
<td>25.00</td>
<td>0.390</td>
<td>83.82</td>
<td>79.69</td>
</tr>
<tr>
<td>2001 “untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama58 (Pinot noir)</td>
<td>100</td>
<td>76.00</td>
<td>2.410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>150</td>
<td>76.66</td>
<td>1.920</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2002 “treated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama12 (Chardonnay)</td>
<td>50</td>
<td>2.00</td>
<td>0.020</td>
<td>99.07</td>
<td>99.38</td>
</tr>
<tr>
<td>Ama24 (Merlot)</td>
<td>50</td>
<td>8.00</td>
<td>0.080</td>
<td>96.30</td>
<td>97.53</td>
</tr>
<tr>
<td>Ama49 (Merlot)</td>
<td>52</td>
<td>9.62</td>
<td>0.120</td>
<td>94.44</td>
<td>96.30</td>
</tr>
<tr>
<td>Ama45 (Sangiovese)</td>
<td>51</td>
<td>7.84</td>
<td>0.078</td>
<td>96.39</td>
<td>97.59</td>
</tr>
<tr>
<td>Ama19 (Sangiovese)</td>
<td>50</td>
<td>10.00</td>
<td>0.180</td>
<td>91.67</td>
<td>94.44</td>
</tr>
<tr>
<td>2002 “untreated plots”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama64 (Chardonnay)</td>
<td>50</td>
<td>70.00</td>
<td>1.700</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama58 (Pinot noir)</td>
<td>50</td>
<td>90.00</td>
<td>2.160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama36 (Merlot)</td>
<td>51</td>
<td>41.18</td>
<td>0.921</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama26 (Sangiovese)</td>
<td>50</td>
<td>80.00</td>
<td>1.660</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ama35 (Sangiovese)</td>
<td>50</td>
<td>94.00</td>
<td>3.240</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data concerning the variables “infested bunches rate” and “number of berries damaged per bunch” are substantially aligned with the variable “number of larvae per bunch” which proved to be the most adequate to estimate the degree of infestation and, in this case, to evaluate the efficacy of the method.

In conclusion, significant differences between mean values of the treated and untreated vineyards were observed for each of the three generations of *L. botrana*. In several cases these differences were more relevant in 2002 than in 2001. Nevertheless, in both years, infestation rate progressively increased from the first to the third generation, leading to a decrease of the difference between treated and untreated plots, in particular for certain vineyards of the “Sangiovese” variety (Ama19 and Ama35).

On the whole, positive results of these first two years are definitely more satisfying in comparison with the ones of the previous years when investigations were carried out with different methods and materials. Research is in progress to optimize MDT application in the considered areas.

**References**


Mating disruption using ISONET dispensers to control grape moths

P.J. Charmillot, D. Pasquier and C. Verdun
Swiss Federal Research Station for Plant Production of Changins, CH-1260 Nyon, Switzerland

Abstract: ISONET-L/E dispensers for mating disruption (MD) of the grape berry moth *Eupoecilia ambiguella* and the grapevine moth *Lobesia botrana* were tested in the western part of Switzerland, in 2001 and 2002. Catches of males in pheromone traps were always almost completely inhibited. Damage by larvae on bunches was always greatly reduced in the first generation, in comparison with the nearest located untreated reference vineyards. In the second generation, ISONET dispensers generally achieved better results in reducing damage on berries than classical control using insecticides. A complementary treatment to MD was only necessary on less than 10% of the trial surface. Emission of pheromones estimated by weighing and by GC-analysis of dispensers exposed in vineyards for different durations was consistent throughout the whole season.

Key words: mating disruption, grape berry moth, grapevine moth, *Eupoecilia ambiguella, Lobesia botrana*.

Introduction

In 2001, three trials of mating disruption (MD) using ISONET-L/E twin tube dispensers to control the grape berry moth *Eupoecilia ambiguella* and the grapevine moth *Lobesia botrana* were carried out over a total surface of 40 ha. Six trials were made in 2002, covering a total surface of 67 ha.

Material and methods

**ISONET-L/E dispensers**

According to analysis of 10 dispensers made at Changins, the ISONET-L/E twin tube dispensers contained on average 475.6 ± 41.9 mg of attractant mixture. According to Shin-Etsu data, the dispenser contains 182 mg E7,Z9-12:Ac (75% purity) and 182 mg Z9-12:Ac (92% purity).

**Placing of dispensers**

In 2001, the dispensers were placed between 19 and 26 April, at the beginning of the first flight of the two species. The ISONET-L/E dispensers were distributed at 3 locations, at 2 m intervals along the borders and at 6x4 m inside the vineyard, corresponding to 500 sources per ha (Table 1).

In 2002, the dispensers were put in place between 23 and 25 April. ISONET-L/E dispensers were distributed at 2 m intervals along the borders and at about 6x4 m (24-28 m^2/dispenser) inside the vineyard at 6 locations (Table 1). The density of dispensers varied between 423 and 520 sources per ha.
Table 1. MD trials made in 2001 and 2002 with ISONET-L/E dispensers.

<table>
<thead>
<tr>
<th>Location</th>
<th>Dispenser type</th>
<th>Surface (ha)</th>
<th>Dispensers per ha</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Dézaley</td>
<td>ISONET-L/E</td>
<td>10.0</td>
<td>500 Trial area between RAK dispensers area, a buffer zone and classical control area</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>63.0</td>
<td>500</td>
<td>Vineyard well isolated on 3 sides, buffer on the 4th side</td>
</tr>
<tr>
<td>Aigle</td>
<td>ISONET-L/E</td>
<td>25.0</td>
<td>500</td>
<td>Trial area on a border of a large vineyard</td>
</tr>
<tr>
<td>Bremblens</td>
<td>ISONET-L/E</td>
<td>4.0</td>
<td>500</td>
<td>Vineyard very well isolated</td>
</tr>
<tr>
<td>2002</td>
<td>Aigle</td>
<td>ISONET-L/E</td>
<td>30.0</td>
<td>500 Trial area on a border of a large vineyard</td>
</tr>
<tr>
<td>Yvorne</td>
<td>ISONET-L/E</td>
<td>15.0</td>
<td>500</td>
<td>Vineyard very well isolated</td>
</tr>
<tr>
<td></td>
<td>Bocep Viti</td>
<td>150.0</td>
<td>356</td>
<td>Vineyard well isolated on 3 sides, buffer on the 4th side</td>
</tr>
<tr>
<td>Dézaley</td>
<td>ISONET-L/E</td>
<td>12.0</td>
<td>423</td>
<td>Trial area between RAK dispensers area and classical control area</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>62.0</td>
<td>492</td>
<td>Vineyard well isolated on 3 sides, buffer on the 4th side</td>
</tr>
<tr>
<td>Aubonne</td>
<td>ISONET-L/E</td>
<td>2.5</td>
<td>520</td>
<td>Vineyard very well isolated</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>2.5</td>
<td>508</td>
<td></td>
</tr>
<tr>
<td>Genolier</td>
<td>ISONET-L/E</td>
<td>3.5</td>
<td>486</td>
<td>Vineyard very well isolated</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>3.5</td>
<td>504</td>
<td></td>
</tr>
<tr>
<td>Bremblens</td>
<td>ISONET-L/E</td>
<td>4.0</td>
<td>500</td>
<td>Vineyard very well isolated</td>
</tr>
</tbody>
</table>

Results and discussion

**Damage samplings on bunches in 2001**

*First generation:* In the reference vineyards, which were generally not treated in the first generation, damage varied between 4.9% and 14.8% depending on location (Table 2). In the Bremblens trial, where a preventive treatment was applied over the whole surface to bring down an initially high population, the damage was 1.8%. In the Aigle trial, where 35% of the surface was preventively treated, the average damage rate was 4.8%. In the Dézaley trial, the damage was 3.4% in an ISONET plot where mating disruption was applied for the first year and 1.0% in RAK1+2 area where mating disruption had already been applied the previous year.

*Second generation:* In the reference vineyards treated with classical insecticides in the second generation, damage varied between 0% and 11.0% depending on location. In the trials with ISONET dispensers damage varied between 0% and 0.2%.

**Damage samplings on bunches in 2002**

*First generation:* In the reference vineyards not treated in the first generation, damage varied between 6.4% and 21.5% depending on location (Table 3). In the trials with ISONET dispensers damage varied between 0.2% and 2.3%.

*Second generation:* In the reference vineyards treated by classical insecticides in the second generation, damage varied between 1.0% and 25.1% depending on location. In the trials with ISONET dispensers damage varied between 0% and 0.6%.
Complementary treatments to MD in ISONET Trial area: Upon requests made by the winegrowers in the MD area with ISONET dispensers, a preventive treatment on the second generation was applied over 23% of the surface in the trial at Aigle and over 20% of the trial surface at Dézaley. This treatment was applied on plots where MD was tested for the first time in 2002. No complementary treatment was needed in the other trial vineyards at Yvorne, Aubonne, Genolier or Bremlens.

Table 2. Sampling of damage in 2001 trials, in the first and second generations using ISONET dispensers compared with other dispensers or with reference vineyards using classical control methods in the second generation.

<table>
<thead>
<tr>
<th>location</th>
<th>process</th>
<th>1st generation</th>
<th>2nd generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>preventive</td>
<td>bunches (n)</td>
<td>damage (%)</td>
</tr>
<tr>
<td>Aigle</td>
<td>ISONET L/E</td>
<td>35% 3850</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>reference</td>
<td>1500 14.8</td>
<td>600 0.5</td>
</tr>
<tr>
<td>Dézaley</td>
<td>ISONET L/E</td>
<td>1000 3.4</td>
<td>850 0.1</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>6500 1.0</td>
<td>4230 0.1</td>
</tr>
<tr>
<td></td>
<td>West reference</td>
<td>1200 12.3</td>
<td>500 11.0</td>
</tr>
<tr>
<td></td>
<td>East reference</td>
<td>1200 6.3</td>
<td>400 0.0</td>
</tr>
<tr>
<td>Bremlens</td>
<td>ISONET L/E</td>
<td>100% 1000</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>– 450 4.9</td>
<td>100 0.0</td>
</tr>
</tbody>
</table>

Table 3. Sampling of damage in 2002 trials, in the first and second generations using ISONET dispensers compared with other dispensers or with reference vineyards using classical control methods in the second generation.

<table>
<thead>
<tr>
<th>location</th>
<th>process</th>
<th>1st generation</th>
<th>2nd generation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bunches (n)</td>
<td>damage (%)</td>
</tr>
<tr>
<td>Aigle</td>
<td>ISONET L/E</td>
<td>5400 2.3</td>
<td>23% 4600</td>
</tr>
<tr>
<td></td>
<td>reference</td>
<td>3500 8.7</td>
<td>2040 2.6</td>
</tr>
<tr>
<td>Yvorne</td>
<td>ISONET L/E</td>
<td>2400 0.8</td>
<td>800 0.4</td>
</tr>
<tr>
<td></td>
<td>BOCEP Viti</td>
<td>12700 2.4</td>
<td>4100 1.2</td>
</tr>
<tr>
<td></td>
<td>reference</td>
<td>3500 8.7</td>
<td>2040 2.6</td>
</tr>
<tr>
<td>Dézaley</td>
<td>ISONET L/E</td>
<td>2200 1.0</td>
<td>20% 1000</td>
</tr>
<tr>
<td></td>
<td>RAK 1+2</td>
<td>6900 1.6</td>
<td>3400 0.4</td>
</tr>
<tr>
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<td>West reference</td>
<td>3100 21.5</td>
<td>750 25.1</td>
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<td>1700 8.5</td>
<td>600 1.2</td>
</tr>
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<td>500 0.2</td>
<td>400 0.0</td>
</tr>
<tr>
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<td>500 0.2</td>
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<td></td>
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<tr>
<td></td>
<td>Reference</td>
<td>500 8.6</td>
<td>400 1.0</td>
</tr>
</tbody>
</table>
**Emission of attractant**

*Weighing of dispensers:* Emission was consistent during the whole season (Figure 1). Average emission was 97.4 µg of pheromone mixture per dispenser and hour, corresponding to 48.7 mg/ha.h with a density of 500 dispensers/ha. It varied weekly between 14 and 179 µg/dispenser·h. On 28 August 2002, the amount of product remaining in the dispensers was 193 mg (41% of initial load).

*GC analysis:* Emission was consistent and proportional to weighing. Average emission was 24 mg/ha.h for E,Z-7,9-12Ac and 23 mg/ha.h for Z9-12AC.

![Graph showing the amount of pheromone mixture remaining in ISONET-L/E dispensers during the season 2002.](image)

Fig. 1. Amount of pheromone mixture, E,Z-7,9-12Ac and Z-9-12Ac remaining in ISONET-L/E dispensers during the season 2002.

**Conclusion**

ISONET-L/E dispensers tested in the western part of Switzerland at 3 locations covering approximately 40 ha in 2001 and at 6 locations covering 67 ha in 2002 blocked almost completely the captures of *L. botrana* and *E. ambiguella* throughout the whole season. There was a considerable reduction in damage by larvae on bunches in the first generation, in comparison with the nearest located untreated reference vineyards. In the second generation, MD generally achieved better results than classical control methods using insecticides. A complementary treatment to MD was only necessary on less than 10% of the trial surface. Emission was consistent throughout the whole season and included sufficient reserves of pheromones.

**Acknowledgements**

We would like to offer our grateful thanks to Mr K. Ogawa and to Mr K. Ogura from Shin-Etsu (Japan) who donated the ISONET-L/E dispensers.
Integrating mating disruption techniques against the honeydew moth and the European grapevine moth in vineyards

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Abstract: Lobesia botrana, the European vine moth and Cryptoblabes gnidiella, the honeydew moth are the two major pests in the vineyards of Israel. The pests are routinely controlled using 4-7 chemical applications. In the last 3 years experiments have been conducted to control C. gnidiella together with L. botrana populations using the mating disruption technique in order to reduce the use off toxic chemicals in the vineyards. We applied the mating disruption of C. gnidiella, using Agrisense bend formulation, one month after the onset of L. botrana disruption, using Shin-Etsu ropes. The efficacy of the mating disruption technique against L. botrana alone and the combination of L. botrana and C. gnidiella was evaluated by comparing the percentage of damaged clusters during the season and that of the percentage of rotten clusters at the end of the season, to the percentage of damaged clusters under routine chemical control. During the last year experiments the control measures taken in plots treated with mating disruption techniques against the 2 pests were reduced to zero in Merlot as compared with 4 in the control plots. Rotten clusters in the treated plots were reduced to 1/6 as compared with the control plot.

Introduction

The European vine moth, Lobesia botrana, and the honeydew moth, Cryptoblabes gnidiella, are two major pests of vines in Israel (Ben Yehuda et al., 1992; Anshelevich et al., 1993). Adult L. botrana appears in the vineyard in early spring and 3 to 4 generations are common before harvest. C. Gnidiella males are caught in pheromone traps in low numbers from early spring but only during May a large increase occurs in male catches. The pests are routinely controlled using 4-7 chemical applications. The damage to clusters caused by these pests is two fold: a direct damage is caused to clusters when the larvae feed their ways into the berries, and indirect damage is caused when additional infestation by rot fungi develops in the injured berries (Fermaund and Le Menn, 1992). The female sex pheromone of both pests is known (Roelofs at al. 1973; Bjostad et al., 1981) and both pheromones are commercially used as an attractant in traps in vineyards and deciduous orchards in Israel (Ben Yehuda et al., 1992). In the last 10 years, the "mating disruption" control method against L. botrana has been applied in wine vineyards in Israel. However, the control is not complete since a few (1-3) toxic insecticide applications are still required to reduce the infestation level below the accepted economic threshold. The honeydew moth has been blamed for the failure of the mating disruption in the vineyard. This, however, has never been tested.

The aim of this study was to test the efficacy of the mating disruption methods as control means in vineyard treated with sex pheromone of L. botrana alone and with a combination of the sex pheromones of L. botrana and C. gnidiella.
Materials and methods

The experiment was conducted during 2002 in 0.6 he of Merlot vineyard located in the one corner of a 20 he. vineyard of mixed cultivars. Merlot was chosen for the experiment because of its late maturation, therefore allowing time for the increase of the honeydew moth population. The vineyard was divided to 3 equal plots: the first was treated with L. botrana sex pheromone formulated for mating disruption, the second with the combination of both L. botrana and C. gnidiella sex pheromones formulates for mating disruption and the third was left as control, treated with insecticides as required. The pheromone concentration used for L. botrana was 165 mg/he (Shin-Etsu 750 ropes/ha) and for C. gnidiella 112 mg/he (Agrisense 750 bands/ha).

The pheromone ropes of L. botrana were placed in the vineyard on May 1st at the end of the first generation, while the pheromone bands of C. gnidiella were tied to the higher supporting wire on June 15th. In each of the three treatments we posted 6 traps loaded with L. botrana pheromone dispensers and 4 traps loaded with C. gnidiella pheromone dispensers. All traps were visited once a week and the males were counted. The dispensers were reloaded once in three weeks. In all three plots we sampled once a week 10 replications of 10 intact clusters in 1m. Looking for eggs and larvae of the two pests. We sampled the level of clusters infested by rot fungi on two different occasions: one month before harvest and the other one day before harvest.

Each time we sampled 10 replications of 10 clusters in 1m, at each of the three plots.

Results

When testing the effect of the mating disruption technique on the level of clusters infested by eggs and larvae a significantly higher rates of infestation were found in the control plot and in the plot where L. botrana pheromone had been applied as compared with the level of infested cluster in the plot treated with C. gnidiella and L. botrana pheromones (a total of 22, 25 and 5 infested clusters, respectively. F_{507.2}=5.36, p<0.01, Tukey: p<0.05). No insecticide applications were given in the two plots treated with mating disruption, whereas the control plot was treated 4 times with toxic chemicals (Fig. 1).

![Percentage of infested clusters](image_url)

Fig. 1. Percentage of clusters infested by eggs and larvae in the three plots. In the control plot 4 chemical applications were conducted and none in the plots where mating disruption was applied.
The level of clusters infested by rot fungi was highest in the control plot and lowest in the plot treated with pheromones of the two pests in both sampling occasions. One month before harvest the percentage of rotten clusters was significantly different between the pheromone treated plots and the control (ANOVA: F_{27,2}=5.820, p<0.01; Tukey p<0.01). One day before harvest the percentages of rotten clusters in the control plot and in the plot treated with *L. Lobesia* pheromone were not significantly different, but both were different from the level of rotten clusters in the plot treated with the pheromones of *L. botrana* and *C. gnidiella* (ANOVA: F_{27,2}=6.077, p<0.001; Tukey p<0.01) (Fig. 2).

**Fig. 2.** Percentage of clusters infested by rot fungi in the three plots, one month before harvest and a day before harvest. In the control plot 4 chemical applications were conducted and none in the plots where mating disruption was applied

### Discussion

Mating disruption in European vineyard to control *L. botrana* population has been practiced for more than a decade (Arn and Louis 1989). The standard pheromone concentration in use in Europe is 120mg/ha (500 dispensers/ha.). We chose to increase the pheromone concentration to 165mg/ha. (750 ropes/ha.) in order to reduce the probability of a failure due to high population density or to an insufficient number of pheromone release points (Schmitz et al., 1995; Suckling and Anerelli, 1996). We applied 112mg/ha (750 bands) of *C. gnidiella* pheromone bands after preliminary studies which suggested that this amount of pheromone source points as the optimal.

A few *L. botrana* males where captured in pheromone traps in plots treated with the disruption formulation of the pest pheromone as compared with the control plot. No *C. gnidiella* males were captured in traps located in the plot treated with its disruptive pheromone as compared with a large number of males caught in the other two plots. The low catch of males under mating disruption, a shutoff of the pheromone traps, indicates an even distribution of the airborne pheromones and the effectiveness of the disrupting pheromones in camouflaging the female pheromone in the traps as well as that of live female moths.

The level of clusters infested by larvae and eggs was similar in the control plot and in the plots disrupted by *L. botrana* pheromone alone. However, whereas 4 insecticide applications were performed in the control plot, none was conducted in the disrupted plot. The infestation
levels of these two plots were significantly higher than that in the plot disrupted by the pheromones of the two pests. Sex pheromones are highly species specific and the use of a pheromone of one species is not expected to affect the sexual behavior of another. Therefore, the mating disruption pheromone applied against *L. botrana* although is capable of reducing *L. botrana* populations, does not affect the encounter rate of *C. gnidiella* males and females. Consequently, the population is not reduced. However, the use of the pheromones of the two pests in the same plot did reduce the damage caused to clusters by the larvae.

In another attempt to measure the effect of the disruption technique on grape vine yield we sampled the level of clusters attacked by rot fungi which are associated with the previous damage caused by larvae of moth pests (Fermaund and Le Menn, 1992). The result of sampling the rotten clusters in the 3 plots confirms the conclusion that adding the disruption of *C. gnidiella* to that of *L. botrana* reduces the level of rotten clusters.

To sum up, a better control is achieved with the application of the pheromones of both pests for mating disruption in vineyards prone to the attack of *L. botrana* and *C. gnidiella*.

**References**


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**Bunch extracts of *Vitis vinifera* at different development stages stimulate or deter oviposition in *Lobesia botrana* females**

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**Abstract:** The effect of chemicals produced at the surface of grapevine fructiferous organs on the oviposition of *Lobesia botrana* was evaluated throughout the season. Bunch extracts of three different grape varieties (Chasselas, Merlot and Sauvignon) obtained at four different development stages (15-17, 23, 33 and 38) were tested by a laboratory bioassay at two different concentrations. For all three varieties, flower extracts (stage 23) deterred oviposition at the highest doses tested and mature berry extracts (stage 38) stimulated oviposition at both doses tested. Chasselas extracts at stages 15-17 and 33 stimulated and deterred oviposition whereas the corresponding Merlot and Sauvignon extracts had no significant effect. These findings indicate that the flowering stage of grapevine seems to be chemically protected from oviposition of *L. botrana* by antixenosis corroborating previous observations that this stage is less favourable to larvae survival.

**Keywords:** Lobesia botrana, host plant selection, oviposition, stimulants, deterrents, Vitis vinifera.

**Introduction**

The European grapevine moth (EGVM), *Lobesia botrana* (Lepidoptera: Tortricidae), which is a serious pest in European vineyards, develops in 2 to 4 generations per year depending on the latitude (Roehrich & Boller, 1991). Females lay single eggs from spring to autumn mainly on the fructiferous organs (i.e. flower buds and developing berries) of its main host plant *Vitis vinifera* and on those of various other host plants (Bovey, 1966). In laboratory conditions, EGVM females can adapt the number of eggs they lay to the host species quality (Stavridis & Savopoulou-Soultani, 1998; Maher et al., 2000). In phytophagous insects, decisions made by the female to accept a plant for oviposition are crucial for larval feeding stages and depend greatly on chemical information detected on the plant surface just before egg deposition (Schoonhoven et al., 1998). For EGVM, we have found that non-volatile polar compounds produced by mature host fruit play a role in host plant recognition and oviposition preference (Maher et al., 2001; Maher, 2002). Throughout the grapevine’s maturation, the chemical composition of fructiferous organs changes greatly between the pre-flowering and the maturity stages. Therefore the question arises if the different generations of EGVM use the same chemical information to identify their oviposition sites at different grapevine development stages. In a previous study, Gabel & Roehrich (1995) suggested that EGVM larvae could be affected by the biochemical changes of grapevine clusters since their feeding behaviour and survival differed in relation with the grapevine’s development stage. Indeed, these authors observed a reduction in larval attack and survival during the flowering stage of different grapevine varieties compared to preceding and following stages. In addition, Thiéry & Gabel (2000) observed that oviposition was also affected by repulsive or deterrent compounds produced only during this flowering stage of the variety Muller-Thurgau. They concluded that the flowering stage of grapevine could be chemically protected against EGVM attack, or at least oviposition.
In this work, we evaluate the effect of compounds produced by the fructiferous organs of three grapevine varieties throughout the season on EGVM oviposition.

Materials and methods

**Insects:** Moths used were from our laboratory stock culture which originated from larvae collected in Bordeaux vineyards during 1998 and 1999, and mass reared on a semi-synthetic diet. To obtain moths of same age and experience for experiments, females and males were allowed to emerge together and mate the same day (D0) in a plexiglass cage (25x25x25 cm). Isolated females having laid eggs the following day (D1) were selected for bioassay experiments that took place on D2.

**Bioassay:** Oviposition responses to grapevine extracts were evaluated by a binary choice laboratory bioassay consisting of two glass oviposition substrates placed in a synthetic felt lined box (18.5 x 12.5 x 4 cm) (Maher & Thiéry, submitted). Individual gravid females (or a group of 5 females in the case of Chasselas extracts) had the choice to oviposit between an extract treated substrate and a methanol treated substrate for one night (D2). Extracts were sprayed on substrates at the doses of 2 or 4 ge / substrate. From egg numbers counted the following morning (D3), an oviposition discrimination index (ODI) was calculated. ODI = [(no. eggs on extract treated substrate – no. eggs on solvent treated substrate) / total no. eggs] x 100. Insect rearing and bioassays were performed at the controlled environmental conditions of 24 ± 1°C and 60 ± 10% r.h., under a L15:D9 photoperiod including 1 hour of artificial twilight occurring between the photo- and scotophase.

**Extraction of compounds from grape clusters:** Fructiferous organs of three grapevine varieties (Chasselas, Merlot and Sauvignon) were collected from INRA’s experimental vineyard of Latresnes (Bordeaux) at four different development stages according to (Eichhorn & Lorenz, 1977): 15-17 (Inflorescence swelling – Inflorescence fully developed), 23 (Full flowering), 33 (Beginning of berry touch) and 38 (Ripe for harvest). The fresh bunches, placed in a glass jar and covered with methanol (SDS, Purex grade) and left to soak for 20 minutes. The extracts obtained were then filtered through glass wool and concentrated by rotaevaporation to 4 gram equivalents (ge) per ml and conserved at -30°C until use.

Results and discussion

The grapevine extracts had different effects on the oviposition of EGVM females depending on the development stage tested. Throughout the season, the global picture of extract activity was, considering all grapevine varieties tested (fig. 1, 2 and 3), no or a stimulant activity at the pre-flowering stages (15-17), appearance of a strong deterrent activity during the flowering stage, and then a gradual increase of the stimulant activity reaching its maximum at ripeness.

These different oviposition responses to extracts were the most contrasted with the **Chasselas extracts** (fig. 1). At stage 15-17, the extract significantly stimulated oviposition (more eggs laid on the extract treated substrate then on the control substrate) at both doses tested. Stimulant compounds are thus present on the inflorescences of Chasselas at this stage probably playing a role in the identification of these organs by females of the 1st flight. The flower extract (stage 23) had a strong deterrent effect on oviposition at both doses tested, confirming earlier results obtained by Thiéry & Gabel (2000) with a CH₂Cl₂ extract of Muller-Thurgau flowers at an identical stage. At stage 33, when occurs the 2nd flight of EGVM in Bordeaux vineyards, the extract had no effect on oviposition at the lower dose tested (2 ge) and a significant deterrent effect at the higher dose (4 ge). This deterrent activity
was nevertheless reduced compared to the flower extract’s activity. Finally, the extract obtained from ripe grapes (stage 38) had a significant stimulant activity at both doses tested. The slight reduction in stimulation at the highest dose was possibly due to an increase in the sticky nature of this extract at 4 ge caused probably by the concentration of sugars.

Fig. 1. EGVM oviposition responses to methanol extracts of Chasselas fructiferous organs at different development stages in two choice situations. The oviposition discrimination index (-100 < ODI < 100) represents the oviposition choice of females between an extract treated and a control substrate. Asterisks indicate a significant difference (* at $P<0.05$ and ** at $P<0.01$) and ns a non significant difference in egg numbers laid on these two substrates according to the Wilcoxon test. N represents the number of females.

Fig. 2. EGVM oviposition responses to methanol extracts of Merlot fructiferous organs at different development stages. See fig.1 for details.

Fig. 3. EGVM oviposition responses to methanol extracts of Sauvignon fructiferous organs at different development stages. See fig.1 for details.
The activities of the Merlot and Sauvignon extracts were quite comparable to each other (fig. 2 and 3). No significant activity was observed at stage 15-17. A significant deterrent activity of flower extracts (stage 23) was only observed for these varieties at the highest dose tested of 4 g, the other dose being inactive. Extracts of stage 33 had no significant effect on oviposition at both doses, although a slight stimulant effect was noticeable with the Merlot extract. This stimulant activity of extracts became significant for both varieties and doses at the ripening stage (38).

The results indicate that different development stages of grapevine fructiferous organs do not provide the same chemical information to females throughout the season. Changes in the balance between oviposition stimulants and deterrents at the surface of the fructiferous organs might explain the different extract activities we have observed. At the pre-flowering stages (15-17) the balance could be in favour of oviposition stimulants explaining stimulant activity of the Chasselas inflorescence extract. At the flowering stage (23) the proportion of oviposition deterrents might increase rapidly rendering the overall signal unfavourable to oviposition, thus protecting this crucial stage from EGVM attack. Then during the development and maturation of berries the production of oviposition stimulants could gradually increase, reducing the proportion of oviposition deterrents. The chemical balance would then become in favour of stimulation which seems to be maximal on ripe berries (38). Such biochemical changes protecting the flowering stage could also exist in other host plants with a reduction of oviposition on flowers and not on fruits (Stavridis & Savopoulou-Soultani, 1998). The identification of compounds involved in the oviposition deterrence and stimulation could lead to the development of push and pull strategies that could be useful for integrated vine protection.

Acknowledgements

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References


Study on the strategies of control against the Grape Berry Moths (*Lobesia botrana*) on table grape in the South-East of France

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**Abstract:** Moths (*Lobesia botrana*) are an important sanitary problem on table grape: damages affect the grape quality which requires until now lot of treatments. Tests were made in order to compare the efficiency of one and two insecticide application per generation against *L. botrana*. The observations show that, in second generation, the treatments renewed did not improve the control and thus are not necessary. In third generation, the results were not convincing enough.

**Key words:** *L. botrana*, table grape quality.

**Introduction**

On table grape, the control of *Lobesia botrana* is problematic: We accept a level of 0 damage, means no. *Botrytis* development, no incidence on visual quality, on grape conservation …). Therefore, several treatments are applied: in the South-East of France (Vaucluse), two or three applications per generation are commonly performed.

We, however, do not know exactly how many applications are really necessary to harvest a table grape of good quality. The objective of that work is to evaluate the function of one or two treatments per generation. To answer this question, we started experiments 3 years ago about the assessment of the significance of only one or two treatments by generation of *Lobesia botrana* on table grape.

**Material and methods**

These experiments compared:

- an ovicid in only one treatment with an ovicid followed of a larvicid three weeks later (6 locations during 3 years),
- or, a larvicid in only one treatment with a larvicid renewed fifteen days later (4 locations during 3 years)

The moth damages on table grape berries (var. Muscat de Hambourg) was observed after treatments and classed in 3 categories:
Results

On the second insect generation

In second generation, the test shows a fluctuation of the results and the treatments renewed did not show an increased efficiency.

If the first application is an ovicid (fig. 1), the second application is not necessary.

![Fig. 1. Relative efficiency in the second generation](image)

If the first application is a larvicid (fig. 2), the second application is also not necessary.

![Fig. 2. Relative efficiency in the second generation](image)

On the third insect generation

In the third generation, during the three years, the number of grape berry moths was very low. So, the results are not concluding.
If the first application is an ovicid (fig. 3), a second application is possible.

Fig. 3. Relative efficiency in the third generation

If the first application is a larvicid (fig. 4), the results are not concluding.

Fig. 4. Relative efficiency in the third generation

**Conclusion**

The observations show that, in second generation, the treatments renewed do not lead to an increased efficiency and are therefore not necessary. In third generation, the results are not convincing enough: when the first treatment is an ovicid, a second application is possible; when the first treatment is a larvicid, the tests show variable results.

The results presented here suggest that a reduction in the number of application may be considered in table grape production, and this with a good quality of harvest.
Integrated control of grape berry moth *Lobesia botrana* Den. & Schiff. (Lepidoptera: Tortricidae) in Greece – present status and perspectives

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The grape berry moth *Lobesia botrana* Den. & Schiff. consists the major pest of vines in Greece. The total area of vineyards in Greece and allocation of vine crops is presented in Table 1.

Table 1. The acreage of vineyards, allocation of vine crops and Integrated Crop Management (ICM) in ha in 2002 in Greece

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<th>% certified</th>
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</tr>
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* Source Ministry of Agriculture year 2002, + Source “AGROCERT”

Last two years the certification of Integrated Crop Management (ICM) was started. There are one governmental organization “AGROCERT” and two private ones “EUROCERT” and “CHEQMATE” which inspect the application of ICM. Despite that *L. botrana* consist the major pest problem, protocols of IPM programmes have not constructed yet. Taking under consideration that the growers involved in ICM, usually apply more or less IPM programmes on *L. botrana* based mainly on Bt applications, we consider that the certified areas consist the area subjected in IPM. We know also that some growers involved in ICM apply good agricultural practice on chemical control of pests and diseases, which is quite far from IPM, but it is a good step to become familiar easier to IPM methods should follow later.

It is known that an IPM is based on a suitable combination of means aiming to minimize the use of insecticides and harmful effects on beneficial fauna and human health. There are 30 registered insecticides for *L. botrana* but there is not any selection of those suitable for IPM methods. Concerning biological agents only *Bacillus thuringiensis* is registered. From biotechnological means only mate disruption has been tested with successful results in many cases. It is evident that there are not many choices on biological and biotechnological tools. We need much more cases than one to choose in order to construct reliable IPM protocols suitable to diverse climatic conditions or microclimatic ones. For this purpose we need firstly adequate national funds plus a national plan in purpose. Priority should be given to ecological studies, insecticide resistance management to start to “build” IPM programmes. In this IOBC
meeting the role of biodiversity and push–pull strategy were pointed out as a perspective means on IPM programmes. Further research is necessary to be performed and recent results have to be implemented.

Another weak point is the absence of a tight cooperation between research Institutes, universities, agricultural advisory services and growers. Market conditions, the growers constitution and up today system of information of parts involved and consumers consist essential tools for a better dissemination and exploitation of IPM methods and promotion of final products. The organic vine crops are the best example. Their acreage (3500 ha -2.9%) is almost 10 fold higher than IPM because certain prepositions in production and market process are much better than those of IPM.

There are an EU policy favouring IPM research activities and implementation, IOBC groups focusing their efforts on IPM, hundreds of scientists dealing with IPM but the implementation of IPM methods are varied between 1-70% in EU countries. It is evident that the implementation of IPM is related with the conditions maintained in each country. One key reason is the cost of biological and biotechnological methods proposed depending mainly on market conditions and consumer demands.

The perspectives of implementation of cost effective and very competitive IPM methods on L. botrana are excellent in Greece especially in S. Greece. There is a dry and hot climate not favouring many insecticides applications. The use of Bt as a dust at 1000 IU is well documented that it is equal effective with conventional insecticides on wine grapes and raisins which consist 90% of vine area. The price of biological insecticide is rather double compared with the conventional ones e.g. carbaryl 10%, malathion 5%, but the cost of final product is less than 5%. Its use is restricted to family size local wineries, which enjoy very good prices due to a net of special shops. Under suitable conditions a 40% of total area is an effective target, which could be achieved in future under the prepositions mentioned above.
Relative abundance of several larval parasitoids of *Lobesia botrana* on different varieties of grapes

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**Abstract:** We have checked the natural parasitism of *Lobesia botrana* by Ichneumonids on our experimental vineyard which is planted as a patch of 5 grapevine cultivars (merlot, cabernet franc, cabernet sauvignon, semillion and sauvignon) during 2 consecutive years. We first collected in first generation of *L. botrana* all the nests containing worms and checked the parasitism. Then an artificial infestation was done during the beginning of July in order to constitute an artificial 2nd generation. In 2001, the population of worms produced by ovipositing females varied from 1.8 to 8.5 larvae per stock. We collected 4 species *Campoplex capitator*, *Itoplectis maculator*, *Dicaelotus inflexus* and *Scambus elegans* and they were present on each grape cultivar. Between 17 and 31% of larvae were parasitized, not clearly related to the cultivar. *C. capitator* was the most abundant species and was particularly active during the first generation of *L. botrana*. The parasitism appeared to be mainly correlated to the host density per bunch and per stock.

**Key words:** Tortricidae, biological control, *Lobesia botrana*, Ichneumonidae, parasitoid, grapevine.

**Introduction**

Several parasites and predators can be efficient as biological control agents against the several grape berry moths which damage the grapes in Europe, from which *Lobesia botrana*, *Eupoeclia ambiguella*, *Sparganothis pilleriana* and *Argyrotaenia pulchellana*. Parasitoids present the advantage to kill the host and may be used then as control agents. In the parasitoids, few species of Trichogrammas are active against the eggs, and several species of Ichneumonids, Braconids or Pteromalids which are naturally present in most European vineyards can kill the larvae or the pupae (Marchesini & Dalla Monta, 1994; Shirra and Louis, 1998; Thiéry et al, 2001).

In the present work we tested the hypothesis that larval parasitoids of *L. botrana* may distribute according to the grape cultivar, or at least that their parasitic activity may vary according to the grape cultivar. We have then tested this hypothesis on an experimental vineyard planted in a patchy arrangement of 5 grape cultivars classically cultivated in Bordeaux.

**Material and methods**

**Experimental vineyard**

Experiments were conducted from June to September in 2000 and 2001 in our experimental vineyard (INRA Bordeaux, La Grande Ferrade). The yard was planted 30 years ago with 5 cultivars of grapes (merlot, semillion, cabernet franc, cabernet sauvignon and sauvignon) arranged in 8 rows of 35 stocks each. The patches are constituted of 7 stocks of each variety along each row, each patch of cultivar being then replicated 8 times (figure 1).
Insects

All the nests were collected in the 1st generation in 2000 and 2001. Because of the extensive collection and in order to obtain a sufficient number of larvae per bunch in 2nd generation, we released during the first week of July (2000 and 2001) mated females of *L. botrana* from our laboratory stock culture (strain Vineyard 1999 reared on artificial diet), and larvae of 2nd generation resulting from our infestation were all collected during the first week of August. Parasitism was measured *a posteriori* by checking emergences. Number of worms and parasites were batched by cultivar (in 2000) and separately by stock and patch in 2001.

Identification of parasitoids was made based on the adult morphology and was confirmed by C. Villemant ‘Laboratoire d’Entomologie’ MNHN Paris.

Data were compared by parametric ANOVA, and non parametric Mann Witney U test and spearman correlation were used for comparisons between cultivars and correlations.

Results

*Distribution of the hosts (larvae of L. botrana)*

Both years, the mean number of *L. botrana* larvae per stock varied a lot. However, we could not find statistical differences (ANOVA) and no correlation between the attack of *L. botrana* and the cultivar (table 1). Semillion, however appeared to be often the more attacked cultivar but a more detailed analysis and complementary experiments are needed to confirm this point.

Table 1. Lowest and highest average numbers of larvae of *L. botrana* per stock for each of the 2 generations studied in 2000 and 2001. Letter equivalents, see fig.1.

<table>
<thead>
<tr>
<th></th>
<th>lowest</th>
<th></th>
<th>highest</th>
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<tbody>
<tr>
<td>2000 G1</td>
<td>CS = 0.05</td>
<td>S = 0.39</td>
<td></td>
</tr>
<tr>
<td>2000 G2</td>
<td>CF = 0.38</td>
<td>SEM = 1.47</td>
<td></td>
</tr>
<tr>
<td>2001 G1</td>
<td>CF = 0.02</td>
<td>CS = 0.18</td>
<td></td>
</tr>
<tr>
<td>2001 G2</td>
<td>CS = 1.84</td>
<td>SEM = 8.45</td>
<td></td>
</tr>
</tbody>
</table>
Parasitism

Four species of parasitoids were recorded: C. capitator, S. elegans, D. inflexus and I. maculator. C. capitator was the more abundant (fig.2) and the only one present at each of the four collects. The parasitic efficiency varied according to generations and cultivars from 17 to 78 %, no relation to the cultivar. The highest percentage of 78 % does not mean that much, being recorded with a low number of larvae.

![Fig 2. Numbers of each of the 4 species (including L. botrana) collected at each generation. In G2 2001, L.botrana = 870.](image)

Table 2. Lowest and highest ratio of parasitized larvae per cultivar for each of the 2 generations studied in 2000 and 2001. Letter equivalents, see fig.1.

<table>
<thead>
<tr>
<th></th>
<th>lowest</th>
<th>highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 G1</td>
<td>M = 20 %</td>
<td>CS = 67 %</td>
</tr>
<tr>
<td>2000 G2</td>
<td>CF = 19 %</td>
<td>CS = 35 %</td>
</tr>
<tr>
<td>2001 G1</td>
<td>M = 25 %</td>
<td>CF = 78 %</td>
</tr>
<tr>
<td>2001 G2</td>
<td>SEM = 17 %</td>
<td>M = 31 %</td>
</tr>
</tbody>
</table>

Discussion and conclusion

In this study, several parasitoids of L. botrana were naturally present, even though in this area previous population of L. botrana were always low. This parasitism can be considered as rather efficient for natural populations of parasitoids. The most efficient species was C. capitator which is described as a possible specialist of grape tortricid. We may however question this specialisation since it is always present in several areas where low populations of L. botrana (and almost no Eupoecilia ambiguella) exist. It appeared from this study that C. capitator is probably more active in G1 as compared to G2. This was already suspected in this species and also related in Crete by Roditakis & Roditakis.
Our study shows that there was no clear preference of ovipositing females for each of the 5 cultivars tested, though the highest attacks were found on semillion. This result has however to be confirmed by complementary studies.

The grape cultivar did no affect that much the distribution of the parasitoid (at least *C. capitator*) and its activity. The clearest relation found was a positive relation of the parasitism and the host density for *C. capitator*. Such a relation could not be studied with the 3 other species because of their low occurrence.

Fig. 3. Total percentage of *L botrana* larvae parasitized by *C. capitator* on our experimental vineyard.

Acknowledgments

to Dr Claire Villemant for her help with the identification of parasites, to Emmanuelle Mautrait, Pascale Xuéreb and Guillaume Thébaut for the field work, and to Marc Etienne Toulouse for the production of moths.

References


Can we expect *Lobesia botrana* to distribute its eggs partly using differential exposure of bunches to light?

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**Abstract:** In Israeli vineyards, damages to clusters by *Lobesia botrana* are often not evenly distributed. In rows of cabernet sauvignon planted along a north-south axis, 2 fold damages was found in bunches facing east as compared to west facing clusters ones. On the other hand with Merlot grapes (Bordeaux), no clear difference was found between numbers of eggs on exposed and shaded grapes from the same cluster (though there was a small tendency towards the exposed berries). We studied here if these differences are due to differences in berry quality or to the exposure to light. Cage experiments indicated a clear tendency, more eggs on clusters that were facing east in the vineyard as compare to west ones. To check an eventual effect of physical properties, females were held in cellophane pockets near a window and the no. of eggs on each half of the pocket was recorded. In this experiment a high preference to the window facing side was found. The result is somehow in contradiction with the preference for the ‘east facing clusters preferences’, indicating that light is possibly not the only factor involved. Therefore, work is needed to measure berry temperature and light intensities at the two sides of the vine rows during the relevant hours (dusk) as well as repetition of the choice experiments in the two distinctly different areas (Israel and Bordeaux) following the same protocols.

**Key words:** *Lobesia botrana*, Lepidoptera, oviposition behaviour , temperature, exposure to light.

**Introduction**

*Lobesia botrana* is the key pest in vineyards in the Mediterranean region and some European countries. In Israel, adults of the first generation appear early in spring, usually before bud burst and lay single eggs, first on canes and spurs and later on the young shoots. The emerging larvae move to the flower clusters and weave flower buds together to form a shelter in which they are relatively protected. They feed on flowers and later in the season on young berries, causing some thinning of the clusters that is usually not considered as damage. The second (usually starts after fruit set) third and in some places fourth generations oviposit on the grape berries and the hatching larvae quickly enter the berry causing both direct and indirect damage as they make the berries susceptible to invasion by rot causing fungi. Once inside the berries the larvae are relatively protected from chemical pesticides, therefore it is important to accurately time the application of insecticides to the relatively short period when larvae are exposed to open air.

In vineyards under IPM programs in Israel, decisions on control action are based on field monitoring for eggs. As trained people are needed for this work it is important to increase searching efficiency and reduce the time needed for monitoring. The definition of certain ovipositing sites preferred by the females would increase monitoring efficacy when searching for eggs and would reduce the time needed to make a sound decision on taking control action.
Furthermore, elucidating the factors promoting egg laying in certain sites may lead to a better understanding of the pest biology and behavior.

The aim of the present work was to test the preference of *L. botrana* females to “oviposition sites” and elucidate the probable reasons for the observed preferences.

**Materials and methods**

*Field monitoring (Israel)*

Monitoring was done in a highly infested Cabernet sauvignon vineyard in the north Golan height in July 2002. Rows are planted north south, trained to a bilateral cordon and shoots are divided and positioned up and down with moveable wires to form “ballerina” shape. Shaped this way part of the clusters are exposed to east and the rest to the west, afternoon sun. We monitored 10 clusters at each side (10 facing east and 10 facing west) of 12 rows with a total of 240 clusters. The number of damaged berries and live larva in each cluster was recorded.

*Cage experiment (Bordeaux)*

This experiment was conducted in boxes (10X15X5) lined with felt tissue to avoid oviposition on the box walls. A single mated female was put in the afternoon in each box with two small clusters (3 berries) that were picked one from the west and the other from the east side of Merlot rows, grown in “La Grande Ferrade”. The number of eggs per “cluster” was counted in the following morning. The experiment was repeated 4 times and the results from all females (40) were pooled together for statistic analysis. Choice index (CI) for each female was calculated as: “east” minus “west” divided by “east” plus “west” eggs.

*Bag experiment*

Individual, gravid females were put in cellophane bags next to a north facing window. The number of eggs was counted separately for the window and the room facing sides. The experiment was repeated twice with 4 and 8 replicates respectively.

**Results**

*Field experiments*

The number of larvae per cluster was higher in east (mean=4) compared to west facing clusters (mean=1.25). Respectively, the number of damaged berries per cluster from the east facing side of the row (mean=21.17) was nearly twice the number on clusters from the west side (mean=11.08). Using t-test to compare the means, both differences were found significant (N=12, P=.0029 and .00076 for damages and larvae respectively).

<table>
<thead>
<tr>
<th>Total fecundity</th>
<th>Eggs number/cluster</th>
<th>Choice Index¹</th>
<th>Preference²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>east</td>
<td>west</td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>11.53</td>
<td>8.93</td>
<td>0.11</td>
</tr>
<tr>
<td>&gt;5</td>
<td>12.35</td>
<td>9.49</td>
<td>0.12</td>
</tr>
<tr>
<td>&gt;11</td>
<td>15.65</td>
<td>11.88</td>
<td>0.14</td>
</tr>
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<td>&gt;17</td>
<td>17.66</td>
<td>13.80</td>
<td>0.22</td>
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<tr>
<td>&gt;20</td>
<td>22.29</td>
<td>15.07</td>
<td>0.26</td>
</tr>
</tbody>
</table>

¹ CI=(“east” minus “west”) / ( “east” plus “west”)
² Ratio between the number of females that oviposit more than 75% of the eggs on “east” berries to those that preferred (>75%) the “west” berries.
Laboratory experiments on small clusters
Individual females lay between 0 and 58 eggs. However, no difference was found between the average number of eggs on clusters taken from east and west sides of the row (mean and Standard error 11.5 (1.67) and 8.9 (1.41) respectively) but the number of females that strongly preferred the east side (CI>0.5) was 1.8 times more than the number that preferred to oviposit on clusters from the west side. This preference increased with the increase in the fecundity of the female, though not significantly (table 1).

Laboratory experiments using transparent bags
The difference between eggs number on the 2 sides of the bags was clear on each day of sampling (Fig. 1) and accumulated to 73 eggs on the “window” side and 36 eggs on the “room” side (p=0.002).

Discussion
The results indicate that *L. botrana* females do not oviposit randomly but have preferences to certain niches. More eggs were found on east facing clusters in the vineyard trials in Israel and a tendency in that direction was also found in laboratory experiments in Bordeaux, even if it was not statistically different, hinting that the difference is due to different characteristic of the berries themselves. Because of the importance of uniform maturity in wine grapes, many works world wide deal with differences in characteristic of clusters from the two sides of the row. North-west planting (resulting in rows facing east and west) is considered better than east-west planting because of more uniform exposure of the two faces of the row to the sun, but “west” clusters are exposed to afternoon sun and therefore get more warmth than “east” facing ones (Pieri et al 2001). This may lead to differences found in chemical composition of clusters from the two faces (Spayd et al., 2002). Maher et al. (2001) have shown that *L.*
*botrana* females prefer to oviposit on certain grape cultivars. It is thus assumed that differences in temperatures may affect the biochemical characteristics of certain parts of the bunch, and thus contribute to the differences in egg distribution that we found.

‘East’ clusters are shaded in the afternoon thus light intensities are lower in their surroundings at dusk (when *L. botrana* adults start moving) as compared to “west” clusters. In the lab experiment conducted in cellophane bags females clearly preferred to oviposit at the exposed side of the bag, which is in agreement with most of the observations made on *L. botrana* in laboratory conditions. This seems however in contradiction with the preference for the ‘east facing clusters’ observed in the vineyard, but this behaviour could be balanced by a tendency of the female to avoid a direct exposure of their eggs to the sun. We also do not clearly know how females displace in the vineyard, and their location before dusk (when oviposition starts) is probably crucial for the egg distribution. A fine study comparing the distribution of the eggs within the ‘east’ and ‘west’ bunches, and especially between the inside and the outside of the bunch, would also be certainly very informative. Also, no measurement was done to define the two sides (e.g. light intensity, spectral quality, temperature) but since the differences are important in the vineyard, more work should be done in this direction.

In Israel, several of the more experienced population monitors already use the knowledge acquired on *L. botrana* female preferences (cultivars and row sides) in their work (Harpaz, pers. com.), especially at the beginning of each generation, when trying to find the first eggs.

Defining the exact factors that attract or deters the females to the special niches will help in IPM programs in the future.

**References**


Control of *Lobesia botrana* (Lepidoptera: Tortricidae) by the mating disruption technique in two vineyards in central Greece

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**Abstract:** Mating disruption was evaluated as a control strategy of *Lobesia botrana* (Lepidoptera: Tortricidae) in vineyards in Tsaritsani, Larissa, during 1992-95 and in Atalanti during 1992-1997. However, in the latter region detailed data were recorded only in 1997. Four treatments were carried out in the experimental vineyard in Tsaritsani. A four ha central plot was treated only with pheromone dispensers and the 10 ha buffer zone around with both pheromone dispensers and insecticides. The treatments were compared with an untreated control (0.4 ha) and with a nearby vineyard where only chemical control was applied (60 ha). A similar experimental design was followed in Atalanti, and the corresponding size of experimental plots were 10, 15, 1 and 5 ha. *L. botrana* populations were monitored using pheromone traps. Infestation was evaluated visually and by counting live larvae at harvest. In Tsaritsani, during the four year experimentation, the captures in pheromone traps after the installation of the dispensers were higher in the vineyard protected only with insecticides than in plots treated only with pheromone dispensers or both pheromone dispensers and insecticides, where captures were practically nil. The corresponding yearly captures ranged from 27 to 454, from 1 to 98 and from 0 to 17 males/trap. The visual examinations revealed higher infestation rates in the vineyard protected only with insecticides (1-35%) and in untreated control (0-80%) than in plots treated only with pheromone dispensers (1-13%) or both pheromone dispensers and insecticides (2-17%). In Atalanti, infestation rates were 3, 16, 10 and 5%, respectively. In both regions, counting of live larvae revealed different infestation levels than those obtained visually. This was attributed to the fact that visual observations at the harvest time revealed also early infestation without any live larvae. The results of the present study revealed that confusion occurred in the test area and the effectiveness of mating disruption, as an alternative and ecologically compatible control strategy, was equal or better than the chemical control method.

**Key words:** mating disruption, vines, *Lobesia botrana*

**Introduction**

The vine moth or grape berry moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae), is a serious pest in vineyards in Greece. Larvae cause either direct damage by attacking grape berries creating cavities or secondary damages caused by fungal diseases. In many cases, damage on grapes leads to production reduction and great loss of growers’ income. In Greece, the pest appears with 3-4 generations per year and has been successfully controlled by using classical insecticides (organophosphates or pyrethroids) or, less often, by applying microbial agents (e.g. *Bacillus thuringiensis*) (Tzanakakis & Katsoyannos, 1998 and references there in). However, in the framework of ecologically compatible control strategies, novel methods such as the mating disruption technique (Charmillot et al., 1996) against the
grape berry moth have been evaluated lately aiming at the improvement of the product quality and the reduction of the adverse effects on the environment. Thus, the current study aimed at evaluating the mating disruption technique in two vineyards in central Greece.

**Materials and methods**

Experiments were carried out in vineyards in Tsaritsani, Larissa, during 1992-95 and in Atalanti during 1992-1997. However, in the latter region detailed data were recorded only in 1997. In both regions *L. botrana* populations were monitored by pheromone traps (‘Pherocon’-type). Infestation was evaluated in situ visually and by counting live larvae at harvest by immersing whole grapes into salt solution for 30 mins. Four treatments were carried out in the experimental vineyard in Tsaritsani. A four ha central plot was treated only with pheromone dispensers and the 10 ha buffer zone around with both pheromone dispensers and insecticides. The treatments were compared with an untreated control (0.4 ha) and with a nearby vineyard, where only chemical control was applied (60 ha). The dispensers (BASF, 500/ha) were established in mid-May until early-June in the four years of monitoring. Totally, six visual inspections in the field were performed, while at harvest period live larvae were counted four times. A similar experimental design was followed in Atalanti. A 10 ha central plot treated only with pheromone dispensers and a 15 ha buffer zone treated with dispensers and insecticides were compared with a completely unprotected (1 ha) control and another treated only with insecticides (5 ha).

**Results and discussion**

**Tsaritsani**

During the four year experimentation, the captures in pheromone traps after the installation of the dispensers were higher in the vineyard protected only with insecticides than in plots treated only with pheromone dispensers or both pheromone dispensers and insecticides. The corresponding total yearly captures ranged from 27 to 454, from 0 to 17 and from 1 to 98 males/trap (figure 1). The visual inspections revealed higher infestation rates in the vineyard protected only with insecticides and in untreated control than in plots treated only with pheromone dispensers or both pheromone dispensers and insecticides (figure 2). Counting of live larvae revealed different infestation levels than those obtained visually. This was attributed to the fact that visual observations at harvest time revealed also old damages from early infestation without any live larvae (figure 3).

**Atalanti**

In Atalanti, the infestation rates at harvest in 1997 were 3, 16, 10 and 5% for the plots treated with insecticides, untreated control, plots treated with pheromone dispensers and both dispensers and insecticides, respectively.

Conclusively, population monitoring revealed three generations of the moth with lower populations in plots treated with dispensers. The set up of dispensers early in the season (before 2nd generation) proved successful, as very low populations appeared thereafter. Furthermore, visual inspections of grapes revealed lower infestation rates in the plots treated with dispensers or dispensers and insecticides than in those treated only with insecticides or in the untreated control. It is worth mentioning, however, that counting live larvae and visual inspections of damage symptoms did not always coincide, since visual observations at harvest revealed also old damages from early infestations without any live larvae on berries. The present study showed that mating disruption provides sufficient protection against the vine moth and should be considered as an alternative and ecologically compatible control strategy.
Fig. 2. Evaluation of infestation level after visual inspections in Tsaritsani, central Greece in mid summer (a) and at harvest (b) during the years 1992-95.
A: dispensers, B: dispensers + insecticides, C: insecticides, D: untreated control

Fig. 3. Evaluation of infestation level after visual inspections in Tsaritsani, central Greece in mid summer (a) and at harvest (b) during the years 1992-95.
A: dispensers, B: dispensers + insecticides, C: insecticides, D: untreated control

References

Seasonal abundance of *Otiorhynchus schlaeflini* Stierl. adults
(Coleoptera: Curculionidae) on soultanina grapevines

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**Abstract:** The seasonal abundance of *Otiorhynchus schlaeflini* Stierl. adults on grapevines of the table cultivar Soultanina (currant raising) was studied in a vineyard, located at the coastal area of Zacharo in western Peloponnese. Grapevines of cv. Soultanina were grown in a corner of that vineyard. Population abundance of adults on the foliage of each grapevine was studied by assessments performed during the night (3h after sunset) on 10 randomly selected plants, on weekly intervals, in the periods between March 6 to May 15 in 1993 and March 5 to May 21 in 1994. In 1993, adults were firstly recorded on grapevines on March 13 (0.3 adults/grapevine) and the peak of their population occurred on April 3 (average 1.8 adults/grapevine); the highest number of adults found on one plant was 4. In 1994, adults were recorded from March 13 (0.4 adults/grapevine) until May 7. Their population reached a remarkable peak on March 19 when an average of 4.3 adults per grapevine was recorded; the highest number of adults found on one plant was 6. The results of the present study show that *O. schlaeflini* adults are present on the vineyards of cv. Soultanina in western Peloponnese in the period between middle of March until the beginning of May. Adults of *O. schlaeflini* on grapevines of cv. Soultanina were recorded at much higher densities and peaked much earlier than on grapevines of wine varieties which were grown in the same field. The preference of adults of *O. schlaeflini* to feed on cv. Soultanina rather than wine cultivars, is of particular importance and should be taking into consideration in the framework of integrated pest management.

**Key words:** *Otiorhynchus schlaeflini*, grapevine, Soultanina, abundance, currant raisin.

**Introduction**

*Otiorhynchus* species may cause severe damage on vineyards, ornamental and horticultural crops (Smith, 1932; Cone, 1968; Warner and Negley, 1976; Moorhouse, 1992; Buxton, 1996; Labuschagne, 1999). The black vine weevil, *Otiorhynchus sulcatus* Fabricius, feeds on buds and new growth of the vine, *Vitis vinifera* L., and it is considered as an important pest which can seriously decrease yield (Cone, 1963 and 1968; Phillips, 1989). Its larvae feed on roots and may be destructive on vines (Eglert, 1996). In Greece nine species of *Otiorhynchus* have been recorded, named *O. excelens* Kirsch, *O. bisphaericus* Reiche, *O. lavandus* Germ., *O. lugens* Germ., *O. scitus* Gyll., *O. ovalipesinis* Boh., *O. schlaeflini* Stierl., *O. subfilum* Reitt. and *O. longirostris* Stierl. (Pelekassos, 1962). Damages by *Otiorhynchus* species have been repeatedly observed in vineyards of western Peloponnese. There are cases where adult feeding on buds had caused high decrease in vine production but the severity of these damages depends on year and region.

In a study concerning *Otiorhynchus schlaeflini* Stierl. temporal population abundance and distribution in the soil in a vineyard in w. Peloponnese, it was found to be more abundant in a distance of 15-45 cm from the grapevine trunk and at the upper 5cm from the soil surface. Larvae were met mainly in winter and early spring, pupation took place in April and adults are recorded from late spring to October (Lykouressis *et al*., 2001).
The aim of the present work was to investigate the abundance of *O. schlaeflini* adults on the foliage of the table grape cultivar cv. Soultanina (currant raisin).

**Materials and methods**

This work was conducted in a 0.3 ha vineyard located at the coastal area of Zacharo in western Peloponnese, where severe damages by vine weevils had been observed in the last years. In that vineyard the currant raisin cv Soultanina was grown together with other cultivars which were used for wine production. In that vineyard the cultivating practices, normally applied in that area were conducted. During this study no pesticide sprayings were done.

The adult population abundance on the foliage of grapevines was assessed by weekly observations from 6th March to 22nd May 1993 and from 6th March to 22nd May 1994. In each sampling 10 randomly selected plants were inspected. The observations were taken place 3h after sunset.

![Graph](image)

Fig. 1. Number of adults of *Otiorhynchus schlaeflini* (mean ± SE) found during night on grapevines of Soultanina in 1993 (a) and 1994 (b) in a vineyard in the area of Zacharo, western Peloponnese.
Results

Adults of vine weevils found in this work identified as *O. schlaeflini*. They were firstly encountered on Sultanina grapevines on March 13 in 1993 (0.3 adults/grapevine) (Fig. 1a). The peak of their population occurred on April 3 (average 1.8 adults/grapevine). They were present in foliage until May 1. During the sampling period, the highest number of adults found in one grapevine was 4.

In 1994, adults on grapevines were recorded from March the 13th (0.4 adults/grapevine) until of May 8 (Fig. 1b). Their population reached a remarkable peak on March 20 when an average of 4.3 adults was recorded per grapevine; the highest number of adults found on one grapevine was 6.

Discussion

The night assessment of *O. schlaeflini* adults indicated that they were present on Sultanina grapevines in the period between middle of March until the beginning of May.

In both years 1993 and 1994, adults were recorded on grapevines of cv. Sultanina earlier and with much higher numbers from these on wine cultivars grown in the same vineyard. The higher numbers and the earlier emergence of adults on cv. Sultanina, may be due to the fact that the emergence of adults was synchronised with the development of buds in cv. Sultanina, which occurs earlier than the development of the buds in the wine cvs of the area. In addition the damage degree on cv. Sultanina was much higher compared to that observed in wine cvs. In a similar study, Phillips (1989) reported that adults of *O. sulcatus* were firstly recorded on grapevines that developed earlier; simultaneous adult emergence to bud development led to high losses on the yield. Therefore, it seems that *O. schlaeflini* adults shows a feeding preference to the table cv. Sultanina than on wine cultivars buds.

The higher number of adults recorded on Sultanina than on wine cultivars early in the season, apart from the existed population in the soil under the grapevines of cv Sultanina, could be also due to the movement of adults emerged in the soil under grapevines of wine cultivars to the grapevines of Sultanina on which buds had already been developed. Such a kind of behaviour is very likely to occur in *O. schlaeflini* since it was observed that adults had a considerable walking activity among grapevines during the night. Adults of *O. sulcatus*, in a shortage of food, has been referred that move about 60 meters in a 3 days period (Phillips, 1989). The preference of adults of *O. schlaeflini* to feed on one or more grapevine cultivars needs further investigation and is a very important aspect within the framework of integrated pest management in vineyards.

In conclusion, the results show that adults of *O. schlaeflini* start feeding on buds of the grapevines of cv Sultanina from the beginning of spring. The preference of adults of *O. schlaeflini* to feed on cv. Sultanina rather than wine cultivars, is of particular importance for integrated pest management. Therefore, grapevines of cv. Sultanina, scattered in vineyards, on which sticky rings around the trunk have been applied early in the season, they could consist natural traps of *O. schlaeflini* adults a method particularly beneficial to the environment. Local sprays also, could be applied on grapevines of cv. Sultanina when the first adults emerge. Further studies are necessary to examine not only the existing feeding preferences of *O. schlaeflini* on grapevine cvs, but also the aspect of the synchronised adult emergence with the stage of bud development.
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Observations on the efficacy of different traps in capturing *Tropinota squalida* (Scopoli)*1*

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**Abstract:** In order to develop an efficient trap for *Tropinota squalida* mass-trapping and the best trap position in the vineyard, a research was conducted in Sardinia in 2001-2002. The highest capturing efficacy was obtained with a trap composed by a white funnel placed in the neck of a transparent bottle, baited with either trans-anethol or cinnamyl alcohol. Regarding trap position, the best results were obtained when the trap was placed in the vineyard fence and slightly above the spontaneous vegetation.

**Key words:** *Tropinota squalida*, traps, grapevine, mass-trapping.

**Introduction**

The adults of *Tropinota squalida* (Coleoptera, Scarabaeidae) feed on buds, sprouts and clusters of grapevines and, as a consequence, can cause great damage to the crop (Mineo, 1964; Ortu et al., 2001).

In Sardinia, *T. squalida* control is usually based on mass-trapping of adults using traps composed by white-plastic cups half-filled with water. However, these traps lose their ability of killing insects whenever water is no longer present or the trap is full of insects. Therefore, periodic trap maintenance is needed to eliminate captured insects and replenish the water to the initial level.

The aims of this research were: 1) to develop a trap model efficient throughout the period of *T. squalida* presence that does not require periodic operator work to maintain its efficiency; and 2) to determine the best trap position in the vineyard.

**Materials and methods**

The research was conducted in a vineyard of about 12 ha located in Central-Western Sardinia. Vines were double-Guyot trained.

The study of capturing efficacy regarded three treatments composed by cone-trunk shaped white-plastic containers of the following dimensions: 1) small (65 mm diameter and 70 mm height); 2) medium (200 mm diameter and 200 mm height); and 3) large (270 mm diameter and 270 height). Containers were half-filled with water (on a volume basis) and placed 1 m above the soil along the vineyard fence. The distance between traps was 8 m. Each trap-size treatment was replicated 5 times. Every week, captured insects were removed from the trap and counted. Following that, water that had evaporated or had poured down was replenished to its initial level.

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In order to determine the best height to hang the traps, white containers of 200 ml were half-filled with water and placed along a central row of the vineyard at 4 different heights as follows: 1) at soil level; and 2) at 85 cm, 3) 115 cm, and 4) 150 cm above the soil. The four treatments were replicated six times. Insect counting and removal, replenishment of water to the initial level and measurement of the vegetation height along the espalier were performed weekly.

During the trial, it was observed that plants located near the traps were more damaged by the insect adults than the others. That was attributed to a greater concentration of adults in that area that were attracted but not captured by the traps. In order to clarify this aspect, damage was determined on 10 plants located near the traps and in 10 plants far from the traps. In April, the percentage of attacked sprouts was determined on each plant. At harvest, the number and medium weight of clusters, and grape total soluble solids content (°Brix) were determined for each plant.

In addition, in order to determine capture efficacy of traps located far from the plants, the number of captured insects was compared between 5 medium-size white traps located in a central row of the vineyard and 5 identical traps placed at vineyard fence (about 3 m far from the border vines).

An important part of the study consisted in developing a trap model able to capture the insect throughout the whole period of its presence. In order to avoid periodic operator work, a trap formed by a funnel and a container large enough to hold all the insects captured during the season was proposed. Traps were hand-made by placing a white funnel in the neck of a transparent plastic bottle. The Scarabeidae, which are attracted by the white colour, reached the funnel, fell down inside the bottle and could not fly away anymore. In order to increase the white-coloured attractive surface and to ease the fall of insects inside the trap, two crossed white plastic panels (20 x 15 cm each) were placed perpendicularly to the top of the funnel. The white panels were placed also at the top of green funnels in order to simulate the contrast observed between the attractive white colour and the green vegetation. The white- and green-funnel traps were also baited with 15 g of either trans-anethol or cinnamyl alcohol kept in an open test tube inserted inside the plastic bottle. The eight treatments are reported in Fig. 1. Traps were hanged on the second wire of the espalier, at 1 m height, in three adjacent rows. In each row, the different traps were placed randomly at a minimum distance of 15 cm.

Results

Trap size influenced significantly capture efficacy. In fact, from 4 April to 8 June, large traps captured a higher number of insects (209.5 ± 57.9, mean ± standard deviation) than medium and small traps (118.4 ± 41.6 and 78.4 ± 43.0, respectively).

Capture efficacy was closely related to trap height position in relation to vegetation height. At each sampling date, the highest number of captured insects was observed in the traps placed slightly above the vegetation (Tab. I).

The evaluated plant parameters showed that the plants on which the traps were placed suffered a higher insect infestation and damage. These plants showed a 48.04 ± 20.66 % of sprouts attacked by the insect in comparison to 11.89 ± 11.90 % of attacked sprouts in the plants located far from the traps. As a consequence of a greater insect attack, a lower mean yield per plant (1862 ± 1190 g vs. 2213 ± 1368 g) was observed, due to the feeding damage of the young clusters caused by the insect. Mean cluster weight (184.28 ± 107.13 g vs. 176.64 ± 1177.69 g) and total soluble solids content (18.34 ± 1.27 °Brix vs. 18.35 ± 1.55 °Brix) did not differ significantly between more attacked and less attacked grapevines.
Regarding trap positioning along the vineyard, insect number did not differ significantly between traps positioned in a central row and those placed in the vineyard fence (90.6 ± 22.6 and 77.6 ± 30.9, respectively) during the period of greater presence of the insect. Only from May on, the first traps showed a greater capture efficacy than the second ones (152.6 ± 87.9 vs. 40.8 ± 16.4).

The capture efficacy of the white-funnel trap was higher than that of the traditional white vase (112 ± 35 vs. 34 ± 15, respectively) (Fig. 1). Neither placing white panels on the white funnel nor using white panels on green funnel (to simulate white trap on the green vegetation) had positive effects. On the other hand, when traps were baited with either trans-anethol or cymnammol alcohol, insect capture in both white and green funnels doubled.

Table 1. Number (mean ± standard deviation) of adults of *T. squalida* captured by white traps placed in the central rows of a vineyard on 28 March at 4 different heights above the soil, and mean vegetation height at each sampling date (San Vero Milis, 2001).

<table>
<thead>
<tr>
<th>Captured insects (mean ± standard deviation)</th>
<th>Vegetation height</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cm</td>
<td>85 cm</td>
</tr>
<tr>
<td>4 April</td>
<td>3.2 ± 2.3</td>
</tr>
<tr>
<td>11 April</td>
<td>5.0 ± 3.9</td>
</tr>
<tr>
<td>20 April</td>
<td>-</td>
</tr>
<tr>
<td>17 May</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 1. Number of adults of *T. squalida* captured from 30 April to 29 May by various trap models: A) white vase (20 x 20 cm filled with water); B) white funnel (20 cm diameter) without panels; C) white funnel with two white panels on the top; D) white funnel with white panels baited with 15 g of cymnammol alcohol; E) white funnel with white panels baited with 15 g of trans-anethol; F) green funnel with two white panels; G) green funnel with two white panels baited with 15 g cymnammol alcohol; H) green funnel with two white panels baited with 15 g trans-anethol. (Mean ± standard deviation).
Discussion and conclusions

This study allowed both the development of a trap model efficient in mass-capturing *T. squalida* adults and the determination of the best trap position in the vineyard.

Since insect capture was positively correlated with trap size, large traps are preferred to small ones. The greater efficacy of traps placed slightly above the vegetation height was in accordance with the results of Tesic (1971) for *E. hirta* and *O. funesta* in cereal fields.

This research showed that the greater damage of *T. squalida* to the plants near the traps was related to a partial capture of the insects attracted by the traps. In order to avoid this problem, it is suggested to place the traps at a certain distance from the vineyard. Fortunately, in the period in which the sprouts are more susceptible to the attack of *T. squalida*, no differences were observed between the captured insect number of the traps positioned in the centre of the orchard and those placed in the vineyard fence.

Finally, the trap model formed by a white funnel, baited with either trans-anethol or cinnamyl alcohol, was ideal for mass trapping of adults because it showed higher capture efficacy and required less operator work. In our study, the presence of attractive compounds increased the attractive effect of the white colour. On the other hand, when these products were used in transparent containers, they showed a weak attractive power (Ortu et al., 2001). We hypothesise that these different effects are due to the fact that the white colour can be seen by the insects from longer distances, while the attractive substances are perceived by the insect only near the trap.

Further studies should be performed to test if the capture efficacy of the developed trap can be further increased by using trans-anethol and cinnamyl alcohol simultaneously as already shown for *E. hirta* (Tóth et al., 1998).

In conclusion, mass-trapping of *T. squalida* in vineyards can be performed using funnel traps, baited with either trans-anethol or cinnamyl alcohol, placed in the vineyard fence and slightly above the spontaneous vegetation height.

References


Using pheromone multisurface traps in the mass trapping of pyralid moths in stored sultanas

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Abstract: A pheromone-baited multisurface trap was tested for the detection of pyralid moths in stored sultanas, in an horizontal-type warehouse in the island of Crete (Greece). The pheromone used was the TDA, which is the male attractant for several stored-product pyralid species. The baited multisurface trap was compared with an unbaited multisurface trap, a baited single trap and an unbaited single trap. All trap types were inspected for captured pyralid adults, at weekly intervals, from January to December 1999. The most abundant species in traps were Ephestia cautella and Plodia interpunctella. E. cautella was found in traps from March to December, while P. interpunctella from May to November. For both species, the baited multisurface trap caught significantly more adults than the other three trap types. In addition, the unbaited multisurface trap and the baited single trap was statistically equivalent, but both trap types caught significantly more adults than the unbaited single trap.

Key words: stored sultanas, Pyralidae, Ephestia cautella, Plodia interpunctella, TDA, multisurface trap

Introduction

About 90 % of the Greek sultanas is produced in the region of Heraklion, Crete, while the larger part of its production is exported in Europe, mainly in Britain and Germany. The most important Lepidoptera species infesting stored raisin in Greece are Ephestia cautella (Walker), Plodia interpunctella (Hübner) and Corcyra cephalonica (Stainton) (Buchelos 1985). Among the traps used for monitoring or suppressing stored product insect populations, the multisurface trap was designed for the mass trapping of Coleoptera and Lepidoptera. This trap was tested, in full scale, for the first time in tobacco stores against Lasioderma serricorne F. (Coleoptera: Anobiidae) (Buchelos and Levinson 1993). For this species, this trap was proved cost-effective, and has a high capture potential of adults, a characteristic which can be utilized for mass trapping. Similar experiments also, taken place in floor mills and raisin stores, showed that this type of trap has a considerably elevated efficacy inducing sensory stimulation of moths and flying beetles, after simultaneous exposure to figural and pheromonic stimuli; the trap aims at fully exploiting the “insect cloud” flying around the pheromonic source (Athanassiou et al. 2003). In the present work, we compare the efficacy of multisurface traps and single traps in order to evaluate their capability to mass-trap stored raisin moths as well.

Material and methods

The store-room employed in this study is part of a large horizontal (flat) warehouse belonging to Co-operative Sultanas Association and located near Heraklion, Crete. The room measures approx. 30X10X4 m. About 500-600 tons of sultanas, in linen and plastic sacs forming piles
of up to 1.40 m height, were stored throughout the experimental period, which lasted 12
months (January to December 1999). The average temperatures of the above store were
approx. 13 °C in winter and approx. 28 °C in summer. No insecticide treatments took place
during the trapping period. The multisurface trap, as designed from Buchelos and Levinson
(1993) consists of 5 white rectangular adhesive cardboard stripes of equal size (27 x 8 cm),
vertically suspended from a cruciform device in such a manner that the central stripe is
provided with a pheromone capsule containing 100 µg of TDA [(Z, E)-9,12-tetradecadien-1-
yl acetate], the sex attractant for male Phycitidae (Buchelos and Levinson 1985, Athanassiou
et al. 2003). Two multi surface traps of which one was baited with TDA and one was left
unbaited (control) were suspended in the diagonal corners of the store, while the remaining
corners were occupied by one baited and one unbaited single-stripe traps. All traps were hung
approx. 1 m above the piles of sacs. Inspection of traps and counting of insects, by renewing
and replacing the strips, was performed at weekly intervals. On each trap-check date, baited
and unbaited traps were rotated clockwise from one corner to the next, in order to minimize
the influence of the individual trapping location. For the most abundant species found, the
trap counts were submitted to analysis of variance to examine the significance of the trapping
device. Means were separated by using the Tukey-Kramer (HSD) test at \( p = 0.05 \).

Results and discussion

Most of the individuals caught in traps were *Ephestia cautella* (Walker) and *Plodia
interpunctella* (Hübner) adults, corresponding to more than 90 % of the total number of
individuals found. However, small numbers of other pyralid species were occasionally found
in traps. These species were *Ephestia kuehniella* Zeller, *Ephestia elutella* (Hübner) and
*Ephestia figulilella* Gregson, corresponding to less than 1.5 % of the total. In addition, several
species of Coleoptera were found in traps, mainly *Carpophilus* spp. (Nitidulidae), *Crypto-
estes* spp. (Cucujidae) and *Oryzaephilus* spp. (Silvanidae). Finally, Diptera and Hymenoptera
(not identified), were also recorded.

![Fig. 1. Mean number of *E. cautella* adults per trap (± SE) for each trap type (means followed
by the same letter are not significantly different; HSD-test at \( p = 0.05 \)).](image-url)
Fig. 2. Mean number of *P. interpunctella* adults per trap (± SE) for each trap type (means followed by the same letter are not significantly different; HSD-test at $p = 0.05$).

*Ephestia cautella* and *Plodia interpunctella* moths were found in relatively equal numbers, among the total number of 2685 moths caught on all trap types. Significant differences were noted among different trap types (df = 3,188; $F = 45.39$; $P < 0.0001$ for *E. cautella*, df = 3,188; $F = 19.06$; $P < 0.0001$ for *P. interpunctella*). For both species, significantly more adults were found in the baited multisurface traps, compared to the other three trap types; however, captures between baited single and unbaited multisurface traps did not differ significantly (Figs. 1-2).

The main differences observed between the two species are limited to their flight periods and population peaks. The first *E. cautella* flights were recorded during the third week of
March and the last during the second week of December. High populations were recorded from June to and including October, with maximal trap catches during August (Fig. 3). *P. interpunctella* moths were first recorded on traps two months later, during the first days of May, while the last catches were recorded in the first days of November. High populations were observed mainly from July to October, with population peaks in August and September (Fig. 4). This seasonal occurrence of *P. interpunctella* adults in pheromone-baited traps, confirms previous reports from flour mills, in Central Greece (Buchelos 1980, Levinson and Buchelos 1981), on which *P. interpunctella* “appears” later and “disappears” earlier than *Ephestia* species, in pheromone-baited traps.

![Fig. 4. Captures of *P. interpunctella* on each trap type, during the trapping period.](image)

Concerning *E. cautella*, single traps without pheromone caught 5.4 %, multisurface without pheromone 14.4 %, single traps with pheromone 19.2 % and pheromone-baited multisurface traps 61.0 % of the total number of captured moths, respectively. In the case of *P. interpunctella*, trapping data were comparatively very similar to those of *E. cautella*; single traps without pheromone caught 4.8 %, multisurface without pheromone 15.1 %, single traps with pheromone 16.6 % and pheromone-baited multisurface traps 63.5 % of the total, respectively. Evaluating the efficacy of the different trap types used we may conclude that pheromone-baited multisurface traps caught approx. 3.5 times more insects than single traps with pheromone, approx. 4.2 times more than the unbaited multisurface ones, and approx. 12.2 times more than single traps without pheromone; the pheromone baited multisurface trap is thus, able to catch large numbers of storage insects, by its increased sticky surface and with only one pheromone lure per trap. However, these figures are lower than those which recorded for *L. serricorne* (Buchelos and Levinson 1993, Athanassiou and Buchelos 2002). In the case of the tobacco beetle, the adults captured were 8 times more numerous, as compared to the single baited trap. Hence, in our case, the benefit is smaller as compared to the 800 % increase for *Lasioderma*. This is partially due to the different way of the insects’s approach the pheromone source; Lepidoptera do not fly directly to the trap, at least as some Coleoptera do.
Surprisingly, the unbaited multisurface trap was statistically equivalent to the baited single trap, despite the absence of a pheromonic source on this trap type. This may be attributed to the fact that, even an increase of the trapping surface, can cause a significant increase of captures, and this certain trap design may provide a visual stimuli for males. Similar observations for the unbaited multisurface trap have been reported in a previous study for _E. kuehniella_ by Athanassiou et al. (2003). Apart from the visual stimuli, air permeation of pheromone may increase captures even in unbaited traps. However, the argument of the stimuli that is provided by this trap design, is supported from observations for _L. serricorne_; Levinson and Buchelos (1993) and later Athanassiou and Buchelos (2002) reported that four times more adults were found in the unbaited multisurface trap in comparison with the baited single trap.

Traps with high capture potential (high load capacity) and efficiency (numbers captured vs numbers attracted), as the multisurface trap, have some certain advantages for use in mass trapping. Mass trapping is a target that can be achieved in enclosed storage facilities, because in these cases the limiting influence of the immigration of mated females does not exist (Jones 1998). Levinson and Buchelos (1988) report a considerable level of suppression of _L. serricorne_ population in stored tobacco, after a three successive year trapping period. However, in the case of moths, several female pyralid moth species mate with more than one male, which constitute an additional implication when assessing the multisurface trap for mass trapping (Trematerra 1997, Buchelos and Trematerra 1998). If these parameters are not taken into account, suppression via mass trapping may not be achieved (Trematerra 1997). Hence, apart from monitoring, the feasibility of using high capacity trap designs for mass trapping must be further investigated.

References


Species spectrum, dominance relationships and population dynamics of egg parasitoids (Mymaridae) of the Grape Leafhopper (Empoasca vitis Goethe) in the Franconian wine region

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Abstract: Parasitoid wasp species (Mymaridae) are the most important antagonists of the grape leafhopper (Empoasca vitis Goethe). In Franconia, besides Anagrus atomus and Stethynium triclavatum, a hitherto unreported species, Anagrus avalae, with a high antagonistic potential occurs. For the past five years the population dynamics of these egg parasitoids and their hosts were monitored at five Franconian vineyards. The dominance relationship of the mymarid species were site-specific, but could not be related to the kind of management or surrounding habitat structures. Shrubs, especially Rosa canina, in the close vicinity of vineyards are essential overwintering sites for mymarids. In contrast, common cover crops do not seem to promote any of the relevant mymarid species. The increasing use of strobilurines is suspected to decrease the populations of the grape leafhopper in Franconia.

Key words: Empoasca vitis, Mymaridae, Anagrus avalae, Anagrus atomus, Stethynium triclavatum, dominance relationship, overwintering sites, strobilurine fungicides.

Introduction

In the nineties, the grape leafhopper (Empoasca vitis Goethe) turned into a pest species in German wine areas, causing stress symptoms as discoloured intercostal leaf areas as well as rolled leaf edges. The most important antagonists of the grape leafhopper are mymarid egg parasitoid species. For Anagrus atomus, the most efficient known parasitoid, parasitation rates of 50% (Vidano et al. 1987) to 80% (Cerutti et al. 1990) are reported.

In our study we focused on the species spectrum, the dominance relationship and the population dynamics of the egg parasitoids occurring in the Franconian wine area. We investigated if cover crops promote mymarids within vineyards and which shrub plants are preferred as overwintering sites.

Material and methods

Monitoring mymarids
From 1998 to 2001, at five representative sites of the Franconian wine region the population dynamics of the grape leafhopper and its antagonistic mymarid species were monitored. During the growing season two yellow sticky traps per site were installed and changed weekly. At one site, over a 3 yrs period (1998-2000) three additional rows were equipped with sticky traps (2 traps/row) to study the relative distribution of the different mymarid species within the vineyard. In 1999, surrounding habitat structures were included in the weekly monitoring.
**Hatching experiments**

In February and March, 2001, hatching experiments were carried out to determine favourite overwintering sites of the mymarids. Plant material of different shrub plants as well as of winter wheat were cut into pieces fitting large petri dishes (Ø 20 cm). The petri dishes were kept at room temperature. Hatching mymarids got caught on either white sticky tape lining the lid (February) or on a yellow sticky trap in the lid (March). After three weeks the petri dishes as well as the sticky tapes or traps were examined carefully for mymarids.

During the season of 2002, we examined a number of common cover crop plant species for hatching mymarids as shown in tab.1. The plant material was kept in small waterfilled vessels in dark hatching boxes (30x19x12 cm) for three weeks. At the upper end the hatching boxes had transparent collecting tubes filled with 70% alc.

Table 1. Cover crop plant species used in hatching experiment.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>no. of hatching experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Convolvulus arvensis</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Malva silvestris</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Matricaria chamomilla</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Medicago lupulina</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Medicago sativa</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Mercurialis annua</em></td>
<td>1x</td>
</tr>
<tr>
<td><em>Plantago lanceolata</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Rumex acetosa</em></td>
<td>1x</td>
</tr>
<tr>
<td><em>Senecio vulgaris</em></td>
<td>2x</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em></td>
<td>4x</td>
</tr>
</tbody>
</table>

**Field trial with a strobilurine fungicide**

A continuous decrease of leafhopper numbers was observed over the years, that coincided with the introduction of a new functional group of fungicides: the strobilurines. To clarify if strobilurines have a side effect on leafhoppers, a field trial was conducted with a sequential strobilurine application as shown in tab.2. Adult grape leafhopper were monitored weekly with two yellow sticky traps/experimental row. To assess leafhopper larval densities in the different treatments, once a week, 20 leaves per experimental row were examined for larvae.

Table 2. Experimental design of the field trial to test the impact of strobilurines on the grape leafhopper.

<table>
<thead>
<tr>
<th>application sequence (experimental row)</th>
<th>1</th>
<th>1+2</th>
<th>1+2+3</th>
</tr>
</thead>
<tbody>
<tr>
<td>application date</td>
<td>24/05/02</td>
<td>12/06/02</td>
<td>20/06/02</td>
</tr>
<tr>
<td>phenological stage of the vine</td>
<td>appearance of the 8th leaf</td>
<td>end of emergence of inflorescence</td>
<td>end of flowering</td>
</tr>
<tr>
<td>BBCH</td>
<td>18</td>
<td>57-60</td>
<td>69</td>
</tr>
<tr>
<td>treatment</td>
<td>0.04 % active agent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>experimental unit</td>
<td>1 row/treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results and discussion

Population dynamics, species spectrum and dominance relationships
Over the whole study period, the population dynamics of the mymarids paralleled in their temporal pattern at all study sites with high grape leafhopper densities, as exemplified for 1998 (fig.1): with the invading grape leafhopper also mymarid ♀♀ appeared in the vineyards, regardless which mymarid species dominated. Six weeks later, the new generation of mymarids emerged, generating a distinct population peak. This was the only time that mymarid ♂♂ could be observed. As the grape leafhopper produces only one generation/year in Franconia, in contrast to all other German wine regions, host eggs were no longer available and mymarid numbers dropped and remained on a low level till the end of the season.

Fig. 1. Population dynamics of *Empoasca vitis* and antagonistic mymarids in 1998.
* insecticide treatment
Over the whole study period the observed mymarid:leafhopper ratios of the first generation were extremely high (Böll & Herrmann 2003), indicating that the mymarids seem to control the first leafhopper generation so effectively, that no second leafhopper generation is produced in the Franconian wine region.

Aside from *Anagrus atomus* and *Stethynium triclavatum*, mymarid species known to parasitize eggs of the grape leafhopper, a third mymarid egg parasitoid occurred in Franconian vineyards, hitherto unreported in German wine regions: *Anagrus avalae*. A hatching experiment confirmed its high antagonistic potential (Böll & Herrmann 2003). Over the whole study period all three species occurred at all monitoring sites though in different dominance relationships (fig.2): contrary to other studies (Vidano et al. 1987, Cerutti et al. 1990, Remund & Boller 1996), *Anagrus atomus* did not dominate at all sites. However, the observed differences could not be related to the kind of soil management or the natural surrounding of the vineyards. On the other hand, the observed species composition remained fairly constant over the whole study period and, as such, seems to be typical for each site.

Within a chosen experimental site the dominance relationship of mymarid species remained highly constant, even over larger distances (row 15 vs. row 110, i.e. 200 m approximately, fig.3). But it differed significantly from that found in a nearby hedge (10 m) or an
apple orchard (50 m), despite the small distances (fig.3). The results indicate, that apple orchards are the preferred habitat of *Anagrus avalae*, while *Anagrus atomus* dominates in hedges.

![Dominance relationship of three mymarid species in different habitats, 1999.](image)

**Fig. 3.** Dominance relationship of three mymarid species in different habitats, 1999.

![Suitability of different shrub plant species as overwintering sites.](image)

**Fig. 4.** Suitability of different shrub plant species as overwintering sites.

**Habitat management**
To promote mymarids in vineyards, suitable overwintering sites should be in close vicinity of the vineyard. Hatching experiments confirmed results from Switzerland (Remund & Boller...
1996): *Anagrus atomus*, and to some extent *Anagrus avalae*, hibernated preferably in eggs of cicadellidae deposited under the epidermis of roses (fig.4, 5). Whereas none of the shrub species served as overwintering sites for *Stethynium triclavatum* at the study site, Remund & Boller (1996) found on rare occasions that *Stethynium triclavatum* hatched from roses and blackberrys. The main overwintering site is not known for this species.

Contrary to results from Switzerland (Remund & Boller 1995), a species rich cover crop in vineyards does not seem to advance the establishment of mymarids in Franconian vineyards: first results of hatching experiments showed, that none of the common cover crops examined (tab.1) produced any of the relevant mymarid species.

![Fig. 5. Suitability of different shrub and other plant species as overwintering sites.](image)

![Fig. 6. Effect of the strobilurine on the grape leafhopper after the first application sequence](image)

*Strobilurines – potential insecticides?*

Grape leafhopper numbers decreased continuously over the study period for unknown reasons (Böll & Herrmann 2003). This trend is observed in the whole Franconian wine area.
First results of a field experiment indicate that the increasing use of a new group of active agents, the strobilurines, has a negative impact on the grape leafhopper. Both the number of adults and of larvae decreased significantly after the application of strobilurines (fig. 6-8).

Lab studies are in preparation to find out if strobilurines have a repellent or an insecticide effect on grape leafhoppers.

Fig. 7. Effect of the strobilurine on grape leafhopper larvae after the second application sequence. Different letters above the columns indicate statistical significances (Kruskal-Wallis ANOVA: $H=41.11, p<0.0001$)

Fig. 8. Effect of the strobilurine on grape leafhopper larvae after the third application sequence. Group 4 = untreated control row. Different letters above the columns indicate statistical significances (Kruskal-Wallis ANOVA: $H=45.4, p<0.0001$)

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Intra-plot distribution of the Green Leafhopper *Empoasca vitis* in a Bordeaux vineyard

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Abstract: A study of the spatial-temporal distribution of the green leafhopper (*Empoasca vitis* Goethe) inside the vineyard, combined with detailed observations of vineyard plants and topography, has been performed in the Bordeaux area. Analysis by statistical, geostatistical methods, and cartography, permitted us to show that (i) adult and larval distribution are aggregated (ii) the adult and larval distributions for a given generation are similar, but differ when considering the two generations of the same year (iii) summer generations have distributions which correspond to plant vigour and vineyard slope and exposition to the sun.

Key words: Cicadellidae, intra plot distribution, geostatistics, cartography.

Introduction

New geo statistical tools used in the development of precision agriculture permit to adapt agricultural management techniques to intra plot variability. We decided to attempt a study on the spatial temporal distribution of the green leafhopper (*Empoasca vitis* Goethe) using these techniques. A better understanding of the eco-ethology of this insect in relation with intra plot characteristics might contribute to develop new management strategies. We therefore monitor *E. vitis* spatial temporal intra plot distribution, and compared those distributions with vineyard’s and vine plant characteristics inside the plot.

Adults of *E. vitis* over winter outside of the vineyards on coniferous trees and evergreen trees and shrubs. Immigration occurs during early spring in vineyards, where it remains during 3 or 4 generations, before returning to winter host plants, outside of the vineyard (van Helden, 2000). The adult stage of this insect appears to be highly mobile. Recombinations of the population inside and among neighbouring vineyards located in the same wine-growing area are systematically observed (van Helden & Decante, 2001). Similar adult and larval distributions for a given generation are observed between consecutive years, and differences between different generations in a given year, have been shown in a previous study , performed in a low dimension vineyard (1.7 ha), with a low density plot (9 x 12 m) (Decante & van Helden, 2001). Summer generation distributions were strongly correlated with vine plant vigour (Decante & van Helden, 2001). This also correspond to observations made in the United States, where *Erythroneura variabilis* (Beamer) population are more important on high nitrogen containing vine plants (MAYSE et al. 1991).

Materials and methods

Vineyard

Two neighbouring vineyards (Fig.1) were studied in the Bordeaux region. Their surfaces are 12 and 7 ha respectively. They both contain two varieties, Merlot and Cabernet-franc, planted at a density of 3300 plants/ha (3m x 1m), and of approximately thirty years of age. The
foliage extends from 0.5 to 2m in height. Those vineyards topography are highly variable (ranging from 50 to 80m above sea level).

The vineyards surrounding is made of hedges containing intermediate host plants (Rosa sp. and Rubus sp.) on the south-eastern and north-western borders, and by a forest containing both intermediate and winter host plants (mainly Pines and Junipers) on the north-eastern border. Dominant wind of the area is highly variable in speed, but quite constant in direction, and mainly from the south-eastern sector. A small stream, mainly covered by blackberries (Rubus sp.) separates the two neighbouring plots.

**Parameters measured**

The following parameters have been measured during 2001 on 225 points, uniformly distributed on the 19 ha, on a 27 x 30 m (9 rows x 30 plant) distance (Fig. 1). For each parameter, the abbreviation used throughout text and figures is indicated.

**Insects**
- Adult distributions are measured using Yellow Sticky Traps (YST) of 5 x 15 cm, which are fixed on the top wire of vine rows, inside the foliage. They are collected every 2 to 5 weeks, depending on E. vitis population dynamics. The adults numbers caught (EvA1 to 9) are expressed as number per trap per week.
- 2 larval counting (EvL1 and 2) are performed on 40 leaves per data point. Larval numbers are expressed as individuals per leaf and per plot.
- Adult dynamics are also measured weekly in between rows, on 17 tubes trap, as used by Fishpool et al. (1988) uniformly disposed on a 114 x 120 m (38 rows x 120 plant)
distance net in the vineyards. Such a trap is composed of a vertical PVC tube, of 5 meters high and 125 mm in diameter. Circular 10 cm high yellow sticky traps are disposed at five different heights, from 0.5m to 4.5m, and divided in 8 sectors corresponding to N – NE – E – SE – S – SW – W and NW directions.

**Vineyards characteristics**
The plant characteristics were measured on 5 plants next to each YST, in the same row.
- **Vigour:** pruned wood weight (PWW) recorded in winter, chlorophyll leaf content (Ntest) measured with an Hydro Ntester during summer (Hebrard, 1999). At harvest: total harvest weight (Weight), weight per bunch (W/Bunch) and 100 berry weight (W100);
- **Phenology:** average number of leaves per bud, measured, 3 weeks after the bud burst (BBD). At harvest: grape sugar content (Sugar), pH (pH) and Total Acidity (TA).
- **Topography:** Altitudes (Alti) and slopes (Slope) of each measure point. Slopes in degrees are measured in the rows direction (NE – SW), and are positive if oriented on the SW – negative if oriented on NE.

**Data analysis**
Following statistical and geo statistical treatments are consecutively performed:
- Test for Normal distribution; non-normal characteristics are removed.
- Aggregation; Coefficient of dispersion are calculated for each insect measure.
- Insect distributions; comparison between insect distributions , and also with vineyard characteristics, are performed by calculation of correlation matrices. Only the significant correlation coefficients, with a confident interval of 95 %, are presented into those matrix (Snedecor & Cochran, 1957).
- Principal Component Analysis (PCA) (Philippe au, 1986) are performed at first only on insect distributions, and in a second time with vineyard characteristics (as primary parameter) and insects (as secondary parameter). Vineyard characteristics that did not correspond to any insect distribution are removed.
- Variograms (Freycon & Sebastien, 1991) are calculated, and permit to detect spatial structure for each parameter, and to judge the sampling quality.
- Cartography: Spatial distributions of parameters are represented by cartography (only for those with acceptable nugget effects) using the Kriging method (Chauvet, 1992). This permits to visualize the spatial distribution, and therefore to confirm or infirm the PCA results.

![Population dynamics of E. vitis](Fig. 2. Population dynamics of E. vitis.)
Results and Discussion

Population Dynamic

The green leafhopper population dynamics (Fig. 2), measured in 2001, shows that spring adults (over wintering adults immigrating in the vineyards; first generation) are clearly separated from the two summer (second and third) generations. On the other hand, those summer generations partly overlap, and will be considered as a single generation for our analysis.

Dynamics as measured on tube traps in between rows is very different from the one measured on YST inside the foliage. Numbers of adults caught inside the foliage are predominant during early summer (start of 2\textsuperscript{nd} generation), and gradually decrease, whereas those caught in between rows show the opposite trend. This observation probably reflects an increase in leafhopper dispersion during summer.

![Fig. 3. Coefficients of dispersion.](image1)

![Fig. 4. PCA graph for insects](image2)
Insect distribution

The examination of coefficients of dispersion (Fig. 3) reveals that adults aggregation, low during the spring generation, gradually increase until a maximum during the early summer dispersion period (second flight period, maximum catches), and then starts to decrease.

Positions of the insect distribution measures inside the PCA correlation circle (Fig. 4) (and also in the correlation matrix, not presented), show a very strong similarity among distributions (of adults or larvae) observed during the same generation. This is particularly clear for the summer generation (EvA4 to 8, EvL2), but less evident during the spring period (EvA1 to 3, EvL1). As observed previously (Decante & van Helden, 2001), the spatial distributions are clearly different when comparing spring to summer generations.

Table 1. Correlation matrix.

<table>
<thead>
<tr>
<th></th>
<th>Merlot</th>
<th></th>
<th>Cabernet Franc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ntest</td>
<td>PWW</td>
<td>Alti</td>
</tr>
<tr>
<td>EvA1</td>
<td></td>
<td>-0.28</td>
<td></td>
</tr>
<tr>
<td>EvA2</td>
<td></td>
<td>-0.17</td>
<td></td>
</tr>
<tr>
<td>EvA3</td>
<td>0.45</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>EvL1</td>
<td></td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td>EvA4</td>
<td>0.46</td>
<td>0.58</td>
<td>0.16</td>
</tr>
<tr>
<td>EvA5</td>
<td>0.41</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>EvA6</td>
<td>0.40</td>
<td>0.45</td>
<td>0.18</td>
</tr>
<tr>
<td>EvA7</td>
<td>0.41</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>EvA8</td>
<td>0.49</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>EvA9</td>
<td>0.38</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>EvL2</td>
<td>0.36</td>
<td>0.46</td>
<td>-0.22</td>
</tr>
</tbody>
</table>

Insect distribution governing factors

Such spatial temporal distributions, with period showing high aggregation levels, and similar for a given generation, let us suppose the existence of distribution governing factors. Two PCA (Fig. 5) are performed for each of the two varieties (Merlot and Cabernet-Franc).

According to the correlation matrix (Tab I), and also to previous results (Decante & van Helden, 2001), spring population distributions are rarely correlated with vineyard characteristics, whereas summer population distributions strongly correspond to vigour distribution (PWW and Ntest). We can distinguish Merlot, where insect distribution corresponds to chlorophyll content (Ntest) and Pruned Wood Weight (PWW), and Cabernet-Franc, where Pruned Wood Weight is a predominant factor for insect distribution. To a lesser extent, we can also observe that ‘Slopes’ are correlated with insect distribution.

We notice that the positions of vineyards characteristic in the PCA correlation circle (Fig.5) are coherent. Indeed, vigour measures are grouped together with slopes, and clearly separated from phenological (BBD, pH, AT, Sugar) and topography (Alti) characteristics.

Summer population distributions do again correspond to vigour characteristics (PWW, Ntest, Weight, W/Bunch), and to a lesser extent to slopes.
Cartography
The observation of distribution maps (Fig. 6) confirms the previous conclusions of correlation matrix and PCA:

- Early spring adult distribution (EvA1) reveals a strong aggregation in the proximity of overwintering plants (Decante and van Helden, 2001). Moreover, this distribution corresponds to larval distribution of the same generation (EvL1).
- Early summer generation adults and larvae distribution (EvA4 and EvL2) are both clearly aggregated in the same areas.
- Those areas do correspond to high vigour levels, dominated by Ntester values in the Merlot part (left side of maps), and by Pruned Wood Weights (PWW) in the Cabernet-Franc part (right side of maps).

Conclusion
The green leafhopper distribution is strongly influenced by intra plot characteristics. Insect aggregation, quit weak during the spring generation, suddenly increases during summer generations. The adult and larval distributions for a given generation are similar, particularly during summer generations, but differ when comparing spring (immigrant) and summer generations of the same year. Those summer generations have distributions which correspond to plant vigour and to a lesser extent to vineyard slope and therefore sun exposure. Finally, plant phenology and topography does not seem to have a direct influence on those distributions.

We can also mention that most of the results obtained by way of the present study corroborate preliminary results (higher density trapping and measuring device (9 x 12m)) (Decante & van Helden, 2001).

Further studies would permit to confirm the distribution’s constancy from one year to another. Measures of water stress, and foliage density may allow further understanding of *E. vitis* ethology, and will probably allow a better adaptation of protection strategies, through both conservation biological control and precision agriculture.
Fig. 6. spatial distribution of insects and plant vigour.

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Green leafhopper (*Empoasca vitis* Goethe) migrations and dispersions

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**Abstract:** A preliminary study on green leafhopper (*Empoasca vitis* Goethe) migrations has been conducted in 2001 and 2002 in a Bordeaux vineyard. Population dynamics and spatial temporal distribution were also studied in the same vineyard (Decante and van Helden 2003, this symposium). Cylindrical yellow sticky traps, positioned at 5 heights (from 50cm to 4,5m) in 17 places inside the vineyard, allowed us to measure migration heights and directions of different flight periods.

The insects showed migration phases at the beginning of each adult population. Those migrations, remarkable by inside foliage to in between rows trappings comparison, occurred mainly above vine canopy height. Despite local variations attributed to vineyard topography and surrounding vegetation movements of the insects mainly seem to follow prevailing winds.

**Key words:** Cicadellidae, migrations, topography, winds.

**Introduction**

Knowledge of insect dispersions and migrations is essential for precise and efficient pest management strategies. In viticulture, many harmful insects, such as leafhoppers and moths, are active flyers, capable of dispersion over relatively long distances. Prediction of population dynamics (modelling) becomes less precise, and pest management strategies less efficient when migration process are important for such pest insects.

The green leafhopper *Empoasca vitis* Goethe (Homoptera: Cicadellidae), a widespread vine pest of Europe is well known for his ability to migrate (van Helden *et al.*, 2000) as many other leafhoppers (Della Giustina, 2002). Even if *E. vitis* appears to complete his whole cycle on vine plants, many exchanges of *E. vitis* adults between vineyards and surrounding vegetation are observed during spring, summer and autumn populations (van Helden and Decante, 2001). Moreover, this insect is often qualified as polyphagous (van Helden, 2000, Della Giustina, 2002).

Adults of *E. vitis*, mainly females in reproductive diapause, overwinter on appropriate host plants, mainly coniferous trees such as *Juniperus communis* L. (Cerutti, 1989; van Helden and Decante, 2001). During the first generation, in early spring, overwintering *E. vitis* migrate from coniferous to intermediate host plants, such as *Rosa sp.* and *Rubus sp* (van Helden and Decante, 2001), present in the vineyard perimeter. After bud burst, they gradually colonize the inside of the vineyard (Decante and van Helden, 2003) (phenological stage BBCH 11-18, Baggioni F and G) (Lorenz *et al.*, 1995), and lay their eggs inside the vine leaves (Lorenz *et al.* 1995; van Helden, 2000). The second (the most important) and third generation develop inside the vineyard during summer, respectively in June and July-August. A partial forth generation is frequently observed during September-October. Because no relation exist between first generation of larvae and subsequent adult flight numbers, van Helden (2000) concluded that emerging adults of *E. vitis* disperse either inside the same plot or to other vineyards. Moreover, the intra plot distribution is very different for the first generation immigrating adults in spring, and the second, third and forth generations during
summer. The first generation distribution appears to be slightly aggregated and seems to reflect winter host plants proximity to the vineyard perimeter, whereas the second and third generations are strongly correlated with the vine plant vigour (Decante and van Helden, 2003). During September and October, *E. vitis* gradually migrate out of the vineyard toward to the same intermediate host plants as in spring, and subsequently to coniferous hosts (van Helden, 2000; van Helden and Decante, 2001).

There is no information available concerning height, altitude, frequency, and distance of *E. vitis* dispersion or migrations. But migrations of the potato leafhopper *Empoasca fabae* (Harris), have been studied in detail in the United States. This insect, a serious pest of many crops, is clearly a migratory insect that overwinters in the southern US (Taylor et al., 1992; Taylor and Shields, 1995), migrates northward each spring, using the spring weather systems (Carlson et al., 1992), and return to the overwintering area in reproductive diapause, assisted by the movement of the fall weather systems (Taylor et al., 1995). Leafhoppers thus appear to have passive long-distance migrations in nature (Taylor, 1985) flying above a few meters high. Shields and Testa (1999) showed that the onset of southward autumn migration of *E. fabae* was correlated with the passing of weather fronts.

The current study aims to investigate dispersions and migrations of *E. vitis* by trapping them at different heights and directions during the supposed dispersion and migration periods, inside and around the vineyard. We use for this purpose special traps type, elaborated for insect movements monitoring inside and above the crop canopy (Fishpool et al., 1988).

**Material and methods**

**Vineyard**

The two neighbouring vineyards were studied in the Bordeaux region. Their surfaces are 12 and 7 ha respectively. They both contain two varieties, Merlot and Cabernet-franc, planted at a density of 3300 plants/ha (3m x 1m), and of approximately thirty years of age. The foliage extends from 0.5 to 2m in height.

The vineyards surrounding is made of hedges containing intermediate host plants for *E. vitis* (*Rosa* sp. and *Rubus* sp.), and by a forest containing both winter and intermediate host plants (mainly Pines and Juniper). A small stream, mainly covered by blackberries (*Rubus* sp.) separates the two neighbouring plots (See Poster or publication “Intra plot distribution of the green leafhopper *Empoasca vitis* in a Bordeaux vineyard, Decante and van Helden”, this symposium). Dominant wind of the area is highly variable in speed, but quite constant in direction, and mainly from the south-eastern sector.

**Measures**

Two different trapping systems are placed in those two vineyards, from April to October in both 2001 and 2002.

The first one consist in 225 Yellow Sticky Traps (YST) of 15 x 5 cm uniformly distributed on the vineyard’s 19 ha, that are fixed on the top wire of vine rows, inside the foliage. They are collected every 2 to 5 weeks, depending on *E. vitis* population dynamics. These traps permitted to reconstitute, by Kriging (Freycon and Sebastien, 1991), the spatial-temporal distribution of the adults during the vine-growing period (Decante and van Helden, 2003). In this study, this system is mainly used to monitor the insect aggregation and dispersion.

The second trap type (Fishpool et al., 1988), is composed of 17 vertical PVC tubes uniformly disposed in the vineyards, in between the vine rows. They are 5 meters high and 125 mm in diameter. Circular 10 cm high yellow bands covered with transparent sticky traps are disposed at heights of 0,5m and 1,5m (foliage height), and 2,5m, 3,5m and 4,5m (above
canopy height), and divided in 8 sectors corresponding to N - NE – E – SE – S – SW – W and NW directions. These traps are collected every week (2001 season) or every 2 weeks (2002 season).

**Data analysis**

Coefficients of dispersion (Var. X / Avg. X) (Sharov, 1995) are calculated for the 225 YST plots at every date.

_E. vitis_ adult dynamics measured on the 225 traps disposed inside the foliage are compared with the catches on the 17 vertical tubes located in between the rows. _E. vitis_ numbers are expressed as total per trap.

Dynamics of trap catches measured on tubes at each height and separated according to directions are also compared, and examined with respect to the supposed dispersion and migration periods, based as well on literature (van Helden, 2000) as previous observations. Because numbers of adult caught at the foliage level (0,5 - 1,5m) are very superior to catches above canopy height (2,5 - 3,5 - 4,5m), catches per height for each date are expressed as a percentage of the total catches.

Likewise, dynamics measured on tubes in each cardinal direction at foliage (0,5 - 1,5m) and above canopy (2,5 - 3,5 - 4,5m) levels are compared, and examined with respect to dominant wind (SE) and previous indications of dominant dispersions and migration periods.

**Results and discussion**

**Dispersion coefficients**

Fig. 1 shows the dynamics of trap catches on YST and the Dispersion coefficient calculated for each date. Adult’s population dynamics (measured on YST, so inside foliage) reveals that the second generation peak occurs on the 4th of July in 2001, whereas it occurs on the 17th of July in 2002.

![Dispersion Coefficients vs Numbers of E. vitis](image)

**Fig. 1. Dispersion coefficients vs numbers of E. vitis.**

This comparison between the average numbers of _E. vitis_ and its level of aggregation shows clearly that the first overwintering adults immigrating in the vineyard (Immig) are strongly aggregated in spite of low population levels. The supposed dispersion of the second
generation adults (Disp) could generate the considerable increase of the population at that time, which is associated with an important aggregation level. Surprisingly, we also observe an increase in aggregation during the gradual emigration period (Emig) of the third and fourth generation.

At the beginning of the spring immigration period, the important aggregation of the adults confirms *E. vitis* arrival in the vineyard perimeter, and its progressive colonization (Decante and van Helden, 2003). This phenomenon is particularly discernible in this large vineyards (12 an 7 ha). The sudden aggregation of the summer generation and then emigrating populations shows that *E. vitis* preferentially moves to specific areas (*i.e.* the more vigourous areas; Decante and van Helden, 2003). The shift between these two, very different, spatial distribution, supports the idea of a dispersion period between the first (overwintering) and second generation. During the emigration period, vine plants become less and less favourable to *E. vitis*. This could explain both the massive emigrations and the important aggregation of residual populations remaining in the vineyard (more vigourous areas longer maintain there foliage).

**Distribution inside foliage vs. in between vine rows**

Fig. 2 compares results from the YST with the tube traps. When compared to YST, trappings on tubes in between vine rows are most important during the first generation immigration (Immig), very important during the onset of the second generation peak (Disp) and also important during the last part of the third-fourth generation emigration phase (Emig, most visible in 2001).

![Fig. 2. Numbers of *E. vitis* inside foliage (YST) vs. in between rows (Tubes).](image)

Our observations confirm the supposed increase of the foraging dispersion or migration activity during those periods. These observations lead us to conclude that dispersion and migration do occur inside the vineyard, and at least partly under 4,5 m height. The increasing catches observed in between vine rows from mid September to November 2001 may be attributed to the development of an important forth generation under favourable weather conditions, gradually emigrating to intermediate or winter host plants.
Fig. 3. Percentage of *E. vitis* per height between vine rows

**Height distribution**

When the relative importance of trappings at different heights is compared (fig 3), the proportion of adults trapped at 2.5, 3.5 and 4.5m is high during the supposed migrating (Immig and Emig) and dispersion periods (Disp). Between those periods, the numbers caught at 1.5m, and to a lesser extent 0.5m are more important. In addition, when 1.5m’s trapping are decreasing, those at 2.5m simultaneously increase, and conversely (circles). We can finally notice that, at the end of the emigration period, the few adults remaining in the vineyard are mainly present at 0.5m.

During the supposed dispersion (Disp) and migration periods (Immig and Emig), adults are effectively more present above the foliage height (2.5 - 3.5 - 4.5 m), confirming that they migrate or disperse above the leaf canopy during those periods. Between those periods, low height important catching may signify that they mainly move about by flying essentially under or at foliage heights (0.5 – 1.5m) and remain inside the row. The shifts between 1.5 and 2.5m trappings seem to be a good indication for migration periods. Indeed, though a first to second generation dispersion is clearly identified in literature (van Helden, 2000), a dispersion period is not mentioned between second and third generation, which are partly overlapping. The comparison between mid foliage height trapping (1.5m) to top foliage height trapping (2.5m) (bold circles) clearly suggest its existence.

**Migrating direction**

As mentioned previously, the dominant wind direction is SE. *E. vitis* trappings are represented for each of the eight sector (N - NE – E – SE – S – SW – W and NW), for two situations; foliage height (0.5 and 1.5m) (Fig. 4A) and above canopy height (2.5, 3.5 and 4.5m) (Fig. 4B).

At foliage height, dynamics of trapping on each sector/direction are very similar, whereas catches above foliage heights are predominant on the E, SE, S and SW sectors during dispersion and migration periods. Dispersion inside the vineyard seems random. Boundary layer limits seem to correspond with canopy height and migration therefore seems mainly to occur through passive wind transport.
Conclusion

The parallel study of spatial temporal distribution of *E. vitis* and his dispersions and migrations, with a new trap type elaborated for insect movement monitoring, permitted to underline some characteristics of population’s relocation during the vine growing season.

The analysis of trappings at different heights and directions, based on supposed migration and dispersion periods, shows that they alternate with phases of high aggregation (Coefficient of dispersion) and low dispersal activities (Distribution inside foliage vs. in between vine rows). Those periods also coincide with an increase of above canopy catching (Height distribution), which obviously correspond to dispersion and migration behaviour. Those high altitude movements are related with wind direction, suggesting that dispersions and migrations occurs through passive wind transport.
Even though immigrant population is at first strongly aggregated in vineyard periphery (corresponding to the intermediate host plants and therefore “entry points” towards the vineyard, the immigration flight heights and direction do not differ from others dispersion and migration flights. The shorter flying distances during spring migration, can be explained by unfavourable weather conditions (low temperatures and abundant rains). Shifts between 1.5 and 2.5m trapings seem a good indication for migration and dispersion periods, and permitted to distinguish a second to third generation dispersion phase.

Further investigations, such as more precise local studies, with respect to topography, local exchanges with the species present in the surrounding vegetation, and surrounding landscapes, could permit us to learn more about *E. vitis* movements and behaviour.

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**References**


Control of phytoplasma vectors in organic viticulture

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Abstract: A comparative study is carried out in 2002 and 2003 in the Mosel viticultural area in order to evaluate the specific risk of infection by Bois noir (Vergilbungskrankheit, VK) in organic viticulture. Occurrence, abundance and infestation of the disease vector Hyalesthes obsoletus is monitored in organic vineyards and compared to data from conventional plots. The organic vineyards under investigation are characterized by a wide variety of herbaceous weed species. Immature H. obsoletus were found on the roots of known host plants like Convolvulus arvensis, Urtica dioica, and Ranunculus bulbosus. Calystegia sepium was identified as a new host plant for the vector. It is, like C. arvensis, a widespread and problematic weed in organic viticulture. Different isolates of the VK-phytoplasma were detected in host plants and vectors, but their biological and phytopathological significance is not yet clear. While no significant differences in the abundance of H. obsoletus could be observed between organic and conventional vineyards, the infestation of the populations was significantly higher in the latter. Disease incidence was generally lower in organic vineyards compared to adjacent conventional plots. Hibernating larvae of H. obsoletus move to a depth of approximately 18 cm during winter. Ploughing brought them up to the surface where they were killed by frost. The efficiency of this method needs to be evaluated during the flight of adult planthoppers in 2003.

Key words: Vector, phytoplasma, grapevine yellows, organic viticulture, control

Introduction

Two types of grapevine yellows occur in German viticulture. Grapevine Palatinate Yellows (GPY) has no economic significance but Bois noir (Vergilbungskrankheit, VK) is widespread in Germany. Severe damage, however, is restricted to vineyards on the steep slopes of the valleys of Middle-Rhine, Mosel, and Nahe rivers where climate and soils provide favorable conditions for the thermophilic Cixiid planthopper Hyalesthes obsoletus Signoret, the only known vector of Bois noir. This species depends on common weeds in vineyards like Convolvulus arvensis, Ranunculus spp. and Urtica dioica, on which it completes its whole life cycle. Larvae and nymphs acquire the VK-phytoplasma by feeding on infected weeds. The pathogen is subsequently transmitted to grapevine by the adult vectors that are active for about ten weeks in June/July.

Due to the biology of H. obsoletus, the epidemiology of Bois noir is significantly influenced by soil cultivation and management of green cover. We are therefore interested to evaluate whether the specific conditions in organic viticulture influence infection pressure. Studies are carried out in organic vineyards as well as in adjacent conventional plots. Occurrence and abundance of the planthopper as well as the infestation of the vector populations with the VK phytoplasma and the disease incidence on grapevine were assessed.

Disease incidence could be decreased by two strategies: control of the alternative hosts plants of the phytoplasma or reduction of the population density of the vector. Control strategies have to consider the role of fallow vineyards that provide ideal conditions for both the herbaceous host plants and the vector.

The data presented here are results of the first year of a still ongoing project.
Material and methods

The study is carried out in organic vineyards of private growers along the river Mosel. Vineyards on slopes were chosen because VK and its vector are associated with those areas. However, no information was available about the incidence of VK and the presence of *H. obsoletus* in those vineyards. In some of the vineyards a more or less permanent green cover has been established, but most plots show a spontaneous herbaceous flora of varying intensity.

The occurrence and relative abundance of *H. obsoletus* was monitored with yellow sticky traps (13x26 cm²) that were exposed close to the soil surface and in the height of the canopy. Adult planthoppers were captured alive from specific host plants by sweep net or a motorized suction device. To monitor the movement of *H. obsoletus* larvae in the soil, a minimum of 20 larval instars was dug from the roots of host plants in monthly intervals and the average depth was calculated.

The infestation of the vector populations was analyzed by PCR-tests of individual insects using primers specific for the stolbur-group of phytoplasmas to which the VK-phytoplasma belongs (Maixner et al., 1995). For a further characterization of the pathogens detected in insects and plants, amplification products achieved with the primers fTufAy/rTufAy (Schneider et al., 1997) were digested with the restriction enzyme *Hpa*I.

Disease incidence was measured in the experimental plots and in adjacent conventional vineyards by visual inspection of each individual grapevine for symptoms of VK.

A section of a fallow field with a high abundance of infected *C. arvensis* was planted with seedlings of *Hieracium pilosella* in 1999. This creeping plant is well adapted to the xerothermic conditions of steep slope vineyards, doesn’t require mowing and covers the soil through runners. We tested the ability of this plant to reduce the density of *C. arvensis* by competition. Soil coverage by *C. arvensis* was estimated by repeated estimation of the coverage in a 1m by 1m frame that was randomly thrown to the ground with 25 repeats.

In order to decrease the population density of *H. obsoletus* fields were grubbed in August or ploughed in December during severe frost with the objective to damage the larval instars either mechanically or by freezing.

Results and discussion

Host plants and phytoplasma isolates

The major host plants of *H. obsoletus* such as *Convolvulus arvensis*, *Urtica dioica*, and *Ranunculus* spp. were commonly found in the vineyards. *C. arvensis* or *Ranunculus* covered the soil almost completely in some vineyards but they were rare in other plots, mainly where a closed green cover had been established. Another common weed, *Calystegia sepium*, was identified for the first time as a host plant of *H. obsoletus*. We detected both the vector and the phytoplasma in all of these plants. A further characterization revealed differences between phytoplasma isolates from *U. dioica*, *C. arvensis* and *C. sepium* (data not shown), and corresponding results could be achieved by the analysis of phytoplasma isolates from planthoppers that were caught on these plants. *C. sepium* was found to be infected by a so far unknown isolate of the VK phytoplasma. It is not yet clear whether these differences have a biological significance.

Relative abundance of *H. obsoletus*

The trapping results of *H. obsoletus* are presented in figure 1. The relative abundance of the vector shows a wide variation both in organic and in conventional vineyards that doesn’t allow to identify significant differences. In one organic vineyard, however, a ten-
twentyfold number of planthoppers was caught compared to all other plots. This vineyard was characterized by *Ranunculus sp.* as the predominant weed that was densely colonized by *H. obsoletus*.

**Fig. 1.** Trapping of *Hyalesthes obsoletus* in conventional and organic vineyards

**Infestation of vector populations**

Data on the infestation of the vector populations are presented in figure 2. Compared to data of conventional vineyards from previous years, the infestation of *H. obsoletus* populations in the organic vineyards was significantly lower than in the conventional ones (U-Test, p= 0.029). This might be an effect of sampling design, because the organic sites were chosen without information about their VK history, while conventional plots were chosen because of their already known high infection pressure.

The function of host plants is emphasized by the comparison of the first three conventional plots and the first two organic vineyards shown in figure 2. In these vineyards, *Ranunculus* spp. was the predominant host plant of *H. obsoletus*. Previous studies revealed that this weed dies off quickly when infected by the VK phytoplasma. Therefore, infected *Ranunculus* doesn’t play a role in VK epidemiology because it doesn’t allow the vector to hibernate and acquire the pathogen.

**Fig. 2.** Infestation of *Hyalesthes obsoletus* populations with the VK phytoplasma.
**Disease incidence**

The incidence of VK was assessed in both organic and adjacent conventional plots. Data for vineyards of cv. Riesling are presented as figure 3. The low levels of incidence indicate that our experimental vineyards are not situated in foci of the disease. The proportion of symptomatic vines was usually lower in organic vineyards but no statistical significant difference could be observed. It is not clear yet, whether these differences point towards a lower risk of infection in organic vineyards. Other factors that influence the development and distinctness of VK, e.g. differences in plant nutrition and vitality, could play a role.

![Fig. 3. Incidence of Bois noir in organic and adjacent conventional vineyards of cv. Riesling.](image)

**Control**

A field trial gave evidence for the ability of *Hieracium pilosella* to suppress *C. arvensis* and to decrease thereby the infestation of the vector population and infestation (figure 4). This plant might be used on fallow fields and other risky areas but, due to its susceptibility to mechanical damage, it is less suitable for vineyards. Another disadvantage is the fact that this plant needs to be planted instead of sown. However, once established, it doesn’t need any more care or protection. Additional experiments have been initialized to check the ability of fast growing crops such as *Phacelia tanscetifolia*, oil raddish (*Raphanus sativus oleiferus*) and grasses to suppress *C. arvensis* on fallowed vineyards.

Short after hatching the larvae of *H. obsoletus* are still close to the soil level. They move down into the soil during winter, presumably to avoid frost damage (figure 5). The maximum average depth was 18 cm in December (9 to 26 cm). While the mean depth of the larvae remained quite constant until April we recorded a steady increase of the variance which indicates an intensifying mobility of the larvae in early spring.

We tried to damage the young larvae mechanically by grubbing the soil in an abandoned vineyard in August. Furthermore, the hibernating nymphs were brought to the soil by ploughing in December during a period of severe frost. First observations confirmed that this treatment caused a high mortality. However, the efficiency of both treatments in the reduction of *H. obsoletus* populations has to be evaluated by a comparison of the relative abundance of the planthopper on treated and untreated plots during the flight of adult vectors.
Fig. 4. Influence of green cover by *Hieracium pilosella* on the soil coverage by *Convolvulus arvensis* and the rate of infestation of the *Hyalesthes obsoletus* population in the years 1999-2001.

Fig. 5. Movement of *H. obsoletus* larvae in the soil. Average depth was calculated from data of at least 20 insects per date. Arrows indicate the time of soil cultivation.

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**References**


Harmfulness of the green leafhopper *Empoasca vitis* Goethe on the grape variety Pinot noir grown in Valais

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**Abstract:** After a first study conducted in 2000 on the harmfulness of the green leafhopper *Empoasca vitis* Goethe in a Pinot noir vineyard plot, new observations were made in 2002 on the same variety. Leaf damage, vegetative growth, yield and berry quality of a treated plot were compared with those of an untreated plot. Population dynamics were observed during the growing season in the two plots. A relationship was calculated between the load on the basis of a recognised sampling method used by growers and the load obtained by sampling two complete shoots per stock. Loads are expressed in leafhopper-days/leaf. Results obtained allow the use of this "practical" sampling method to estimate the mean pressure of the insect in a plot. The percentage of damaged leaf surface was correlated to the cumulated leafhopper-days. The lower sensitivity of the variety Pinot noir to feeding punctures of the leafhopper is confirmed. The first generation of the insect contributes only very little to the final damage. There was no difference in yield parameters between the two plots. Plants of the untreated plot with densities of up to 90.8 leafhopper-days/leaf and maximal losses of 25% of mean leaf surface did not show any vegetative compensation suggesting mobilisation of the reserves. For the variety Pinot noir, abandoning treatment of the first generation and raising threshold levels for the second generation to a minimum of 2 leafhoppers/leaf is proposed, provided that pruning of the lateral shoots is not too severe.

**Key words:** vineyards, *Empoasca vitis*, harmfulness, growth, yield, fruit quality

**Introduction**

Several studies have already been made in Switzerland on the harmfulness of the green vineyard leafhopper *Empoasca vitis* (Baillod *et al*., 1990; Candolfi *et al*., 1993; Remund & Boller, 1995; Linder & Jermini, 2001). Insect punctures induce discolouring of foliage which in turn leads to a reduction in photosynthetic activity of the damaged leaves and compensatory phenomena, such as the development of lateral shoots (Candolfi *et al*., 1993, Remund & Boller, 1995). Nevertheless, in an initial study carried out in 2000 on small populations of the insect, no evidence of these compensatory growths was found in a Pinot noir vineyard in Wallis, Switzerland (Linder & Jermini, 2001). Results from recent experiments conducted in 2002 on larger populations of the leafhopper are herewith presented. In addition, at the same time, a comparison of two methods of population monitoring was made.
Materials and methods

The trial was conducted on a Pinot noir vine "Wädenswil" (planted in 1987) grafted onto 5BB. The vineyard is located at St-Pierre-de-Clages (Wallis, Switzerland). Vines are planted at distances of 1.2 x 0.8 m and trained in a permanently low ribbon. The plot (2000m²) was divided into two parts: one part was treated to provide maximum protection against the development of *Empoasca vitis* (EV) (500 m²; treated with indoxacarb); the other part was left untreated (1500 m²) in order to allow development of the leafhopper. At the phenological stage F (BBCH 15-16), 10 plants were chosen in the treated part of the plot and 20 plants in the untreated part. To provide the greatest homogeneity possible, each of these plants was regulated to 6 shoots per stock. Two vigorously representative shoots of the stock were chosen from each plant. During the month of July, the main shoots of each stock were cut back to 15 main leaves and 10 clusters per plant. A comparison of two methods of monitoring populations was made. Every week, EV larvae and nymphs on all the principal leaves of the selected shoots per plant were counted ("all leaves": AL). At the same time, sampling on 4 series of 25 leaves per plot was carried out (Cerrutti, 1989, Baillod et al., 1993). More rapid, this method is regularly employed in practice to estimate population densities of EV ("practical method": P). EV load is expressed in EV-days per leaf using results from both monitoring methods. Growth of shoots and lateral leaves was estimated at different occasions six times during the season, using the same population sampling shoots. An estimation of damage was made, according to the Horsfall & Cowling scale (1978), at six different occasions during the season on the leaves of the shoots selected for population density sampling, as well as on lateral leaves. At harvesting, each vine plant was harvested individually and the following parameters measured: total harvested weight, average weight of berries (50 berries per plant), and number of berries per cluster. After the grapes had been pressed, analysis of principal must ingredients was made by the laboratories at Changins. Growth data were analysed using the Mann-Whitney test (p < 0.05) and relationships between leafhopper loads and damage together with harvest parameters were tested using linear regression analysis.

![Fig. 1. Population dynamics of *E. vitis* larvae and pupae in the untreated plot, according to two methods of monitoring (P = practical method, AL = all leaves).](image-url)
Results and discussion

Population dynamics and leafhopper loads

Results of population dynamics obtained from both tested sampling methods are expressed in Figure 1 for the untreated plot. The P method gave a much larger and slightly displaced 1st generation peak, whereas the 2nd generation peaks were practically identical in both methods. The difference in the 1st generation results can be explained by the fact that in the P sampling method, leaves are sampled from the zone where a greater number of EV shelter. In the AL method, on the other hand, the number of insects per shoot is divided by a higher number of leaves. Observations of populations made using the P method were within tolerance limits currently acceptable in Wallis, that is, 1 to 3 leafhopper/leaf.

After transformation of data into EV-days per leaf, Figure 2 gives a picture of the regression of one method over the other.

These results allow use of the P method to be envisaged for estimating leafhopper loads per plot, thus enabling more specific tests to be started up with a good estimation of EV population dynamics and insect pressure in EV-days on a larger scale.

Damage and load-damage relationships

The average percentage of main leaves coloured in red at the end of the 1st generation (31 July) was 0.6% in the untreated plot (with a peak of 1.5%) and less than 0.03% in the treated plot. The average rate of foliage damage reached 17.9% at harvest in the control plot (with a peak of 25.1%) and was less than 3.7% in the treated plot (Fig. 3). It becomes obvious that the 1st summer generation is responsible for only a very small part of the final damage, even though it peaked higher than the 2nd generation. Damage on lateral leaves reached an average of 5.9% in the treated plot and 2.3% in the untreated vines. These low values are due to the fact that leafhopper colonisation of lateral leaves is low. Since lateral leaves developed after application of insecticide treatment in the treated plot, they were untouched and this would explain the absence of any significant difference between the two plots.
Fig. 3. Percentage of main leaf surfaces coloured in red at the end of the first and second generations. The first 20 plants represent the untreated control plot, and the ten last plants, the treated part of the vineyard.

Regression curves between EV load and foliage damage during the experimentation years 2002 \( (y = 0.27x - 2.41; r^2 : 0.76) \) and 2000 \( (y = 0.24x - 0.35; r^2 : 0.50) \) were similar, even though, in 2000, low populations gave a rather low \( r^2 \). This tendency allows a relationship to be calculated, considering the results from the two years of experimentation.

Fig. 4. Linear regression between load expressed in EV-days / leaf, according to the AL method, and percentage of main leaf surface coloured in red. Grouping of data from 2000 and 2002.

Symptoms can clearly be correlated to load in accumulated EV-days (Fig. 4). On the basis of the equation obtained by grouping results from the two years of trials, theoretical damage of the Pinot noir vine can be calculated and compared with that of Merlot vines measured in Ticino by Candolfi et al. (1993) (Table 1). It would appear that Pinot noir is
much less sensitive than the Merlot to red leaf colouring induced by the insect. This result demonstrates that generalisation of results obtained from one variety to another is risky and implies that further studies of this kind on other representative vine varieties of Wallis should be followed up.

Table 1. Comparison between theoretical susceptibility of the Pinot noir and Merlot (Candolfi et al., 1993) as a function of load in EV-days / leaf.

<table>
<thead>
<tr>
<th>Load EV-days / leaf (AL)</th>
<th>% of redishness Pinot noir</th>
<th>% of redishness Merlot</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>3.9</td>
<td>9.4</td>
</tr>
<tr>
<td>40</td>
<td>9</td>
<td>21.4</td>
</tr>
<tr>
<td>60</td>
<td>14</td>
<td>33.4</td>
</tr>
<tr>
<td>80</td>
<td>19</td>
<td>45.4</td>
</tr>
<tr>
<td>100</td>
<td>24.1</td>
<td>57.4</td>
</tr>
</tbody>
</table>

**Relationship between load and parameters of yield and quality**

None of the measured harvest parameters was affected by EV (Table 2). The average production per vine plant of 1.4 kg is slightly above production limits imposed in Wallis for the Pinot noir variety (1.2 kg/m²). Only malic acid showed a significant deviation from the 0 slope but the r² value was very poor.

Table 2. Linear regression between load in EV-days/leaf and parameters of yield and quality measured at harvest (26.09.02).

<table>
<thead>
<tr>
<th>Yield &amp; Quality</th>
<th>Regression</th>
<th>r²</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (kg / stock)</td>
<td>y = 0.008x + 1.5</td>
<td>0.004</td>
<td>0.7381</td>
</tr>
<tr>
<td>Berries / cluster</td>
<td>y = -0.114x + 90.9</td>
<td>0.049</td>
<td>0.2384</td>
</tr>
<tr>
<td>Berries weight (g)</td>
<td>y = -0.0002x + 1.9</td>
<td>0.001</td>
<td>0.8705</td>
</tr>
<tr>
<td>Must soluble contents (°Brix)</td>
<td>y = 0.001x + 20.7</td>
<td>0.00006</td>
<td>0.8958</td>
</tr>
<tr>
<td>Total acidity (g/l)</td>
<td>y = -0.010x + 11.5</td>
<td>0.099</td>
<td>0.0803</td>
</tr>
<tr>
<td>Tartric acid (g/l)</td>
<td>y = 0.002x + 7</td>
<td>0.038</td>
<td>0.2689</td>
</tr>
<tr>
<td>Malic acid (g/l)</td>
<td>y = -0.014x + 6.6</td>
<td>0.194</td>
<td>0.0156</td>
</tr>
<tr>
<td>Formol index</td>
<td>y = -0.039x + 23.1</td>
<td>0.115</td>
<td>0.0643</td>
</tr>
</tbody>
</table>

**Plant compensatory capacities**

Under present trial conditions, no compensatory increase of leaf surface was noted on Pinot noir. The number of main leaves remained stable after shoots had been pruned to 15 leaves and only one single significant difference was recorded between the two plots, and in favour of the untreated control plot. Occasional occurrences of statistically significant distinctions in the number of lateral shoots and lateral leaves between the two plots were observed but not in any systematic way and always to the advantage of the treated part of the vineyard. Thus, at the end of July and at the end of September, a greater number of shoots and lateral leaves were measured in the plot treated with insecticide. Generally speaking, the untreated plot did not compensate for the 17.9% loss of main leaf surface by producing supplementary foliage, unlike the phenomenon observed by Candolfi et al. (1993) on Merlot. Figure 1 shows that the
second EV generation, which was responsible for final leaf damage, exercised maximum pressure from the time of grape ripening to mid-September. Compensation for this potentially stressful situation in the plant may be made by mobilising root reserves, as demonstrated by Jermini et al. (2001) for grapevine mildew (*Plasmopora viticola*).

**Conclusions**

A simple and rapid method of sampling can be used for estimating the average load of populations in vineyards, thus facilitating future studies on other varieties. Under conditions studied in Wallis, the 2nd EV generation was responsible for the formation of leaf damage on Pinot noir. Damage occasioned by the 1st generation was negligible. It is thus proposed to abandon forthwith phytosanitary treatments of this generation. Threshold intervention limits for the 2nd generation can be increased to 2 leafhoppers / leaf for the Pinot noir variety, provided that lateral shoots are not too severely pruned and that there is a balanced production. In view of the varying susceptibilities observed between varieties, further studies on the principal varieties should be made before theses proposals can be generalised. A deeper understanding of plant-insect relationships could be gained from a study of the mobilisation of plant reserves in cases of insect attack.

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A sequential sampling procedure for *Empoasca vitis* Goethe (Homoptera: Auchenorrhyncha)

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**Abstract:** Spatial distribution of grape leafhopper *Empoasca vitis* larvae on grapevine leaves was determined based on data obtained by weekly sampling in three vineyards of the Mosel valley in Germany in 2001. Taylor’s power law was used as a regression model with 67 data sets to analyze the sampling data. The slope $b$ of this model was significantly $> 1$ which indicated an aggregation of *E. vitis* larval populations. The parameters of the Taylor’s power law were used to calculate minimum sample sizes and sampling stop lines for different levels of precision. Stop lines for fixed precision levels were tabulated on sheets to be used in field sampling. Repeated simulated sampling for a range of mean population densities showed the suitability of the sequential sampling procedure.

**Key words:** grape leafhopper, spatial distribution, sequential sampling

**Introduction**

The grape leafhopper, *Empoasca vitis* Goethe, is an important pest in viticulture all over Europe. It causes hopper-burn symptoms that become evident at levels of larval density that are far from economic damage. Sampling of *E. vitis* is mostly done by counting numbers of larval instars on 50 or 100 grapevine leaves. However, little is known so far about optimum sample sizes which are a function of pest distribution. The objective of our study was the development of a procedure that allows the definition of optimum sample size with regard to labor efficiency a sufficient precision. The latter depends on the particular purpose of sampling. High levels of precision may be necessary for scientific studies, while efficiency could be more important if sampling is done as a tool for decision making on control measures.

We studied the dispersion of *E. vitis* larval instars on grapevine in order to design specific and efficient sampling procedures. Commonly used statistical methods were applied that allow the estimation of indices of aggregation based on sample mean and variance. Based on these parameters we calculated optimal sample sizes with respect to different levels of sampling precision as well as a sequential sampling procedure for *E. vitis* larvae.

**Methods**

Observations of *E. vitis* larvae were carried out in 2001 on three different vineyards of cv. Riesling in the Mosel valley of Germany. Each vineyard was divided into three sections. Every week from June to August eight or nine grapevines were chosen randomly in each section of the three plots and three leaves of the lower, middle, and upper insertion level, respectively, were sampled on each of three randomly chosen shoots. All larval instars of *E. vitis* were counted on the leaves. Thus a total of 675 leaves on 225 shoots of 75 vines in 9 sections of three vineyards were sampled every week.
A nested analysis of variance (nested ANOVA) was carried out in order to identify the contribution of the different sampling units of the hierarchical sampling scheme to the total variation and to choose an appropriate sampling unit for calculation of means and variances of larval densities (Hutchinson, 1994).

The mean density (m) of *E. vitis* larvae per leaf and variance (s^2) were calculated separately for each section every week. Taylor’s power law (Taylor, 1961) was used to model the relationship between mean and variance as s^2 = a m^b. A linear regression was computed using the linearized form of this equation as ln(s^2) = ln(a) + b ln(m). While the parameter a was described as a scaling factor that is related to sample size, b has been considered as a constant for a particular species (Davis, 1994). It can be used as an aggregation index with b>1 indicating an aggregated dispersion pattern.

To determine necessary sample sizes for specific levels of precision (expressed as a certain proportion of the standard error (SE=s/√n) to the mean) we substituted Taylor’s power law equation into the formula for the standard error of the mean. Rearranging leads to N = a m^{b/2} / D^2 where N is the required sample size, a and b are the coefficients of Taylor’s equation and D is the specific level of precision (SE/m) (Buntin, 1994). If precision is expressed as a certain proportion of the confidence interval (t_{α/2}(SE)) to the mean the equation becomes N = a m^{b/2} (t_{α/2} / D)^2, with t_{α} as the t value for a particular probability (Buntin, 1994).

The cumulative number of leafhoppers (T_n) after which sampling can be terminated with a defined precision is calculated using Green’s formula (Green, 1970) as

\[ \log T_n = \log \left( \frac{D^2}{t_{α/2}^2 a} \right) / (b-2) + \left[ \frac{(b-1)(b-2)}{(b-2)} \right] \log(n), \]

where n is the sample size. Stop lines were constructed by plotting T_n against n.

For practical use in the field, stop lines for different levels of precision were tabulated on sampling sheets so that it was possible to decide after sampling of each leaf whether the required precision was reached or sampling had to be continued. A computer program was written that outputs tables of required sample sizes and of stop values after putting in the parameters of Taylor’s power law.

The suitability and precision of the sampling procedure was tested using a Monte-Carlo procedure. Leafhopper populations of different population densities were simulated assuming a negative binomial distribution (NBD) of the insects on grapevine leaves. The parameter k of the NBD was estimated using the parameters of Taylor’s power law (Buntin, 1994) k = m / ((a m^{b-1} ) -1). For each population density the sampling was simulated 100 times.

**Results and discussion**

**Distribution of *E. vitis* larvae in the canopy**

The maximum density of *E. vitis* larvae was low in all three vineyards. Maximum levels were 0.4, 0.8 and 2.1 larvae/leaf, respectively. Two maxima could be clearly identified (Fig. 1), representing the two larval generations that are usually present in the Mosel area. With the exception of the first two sampling dates when the leafhoppers were mainly found on the basal leaves the distribution between the three insertion levels was quite uniform throughout the season. On average, approximately 40 % of the total count of larvae was present on the central portion of the canopy.

A nested ANOVA procedure was used to identify the principal sources of variation in the hierarchical sampling plan. Considerable variation was found between vineyards, between grapevine stocks and between levels of leaf insertion (Fig. 2). Since ‘Sections within vineyards’ did not noticeably contribute to the total variation, this stratum was chosen as
appropriate to calculate means and variances of larval density. Sampling should be carried out separately for individual vineyards and both stocks and leaves should be chosen randomly. Except for the early sampling dates, leaves of the middle insertion level should be chosen.

Fig. 1. Density and distribution of *E. vitis* larvae on grapevine. Data of 2001, combined from three different vineyards.

Fig. 2. Nested analysis of variance results for the larvae of *E. vitis* on grapevine leaves.
**Spatial distribution**
The relationship between variance and mean described by Taylor’s power law was highly significant with $r^2 = 0.92$ (Fig. 3). The slope $b$ of Taylor’s model was significantly $> 1$ ($t = 12.49; df = 65; p < 0.00001$) which indicates an aggregated distribution of *E. vitis* larvae on grapevine leaves. Possible reasons for this distribution could be differences between individual grapes within vineyards with regard to vigour (Decante & vanHelden, 2001) or aggregated deposition of eggs on individual leaves. According to data published by Delrio et al. (2001) the distribution of *Jacobiasca lybica* in Sardinia ($a = 1.79; b = 1.13$) is similar to *E. vitis* although they differ in average population density and geographical distribution.

![Fig. 3. Regression analysis of Taylor’s power law for *E. vitis* larval populations.](image)

![Fig. 4. Stop lines for the sequential sampling plan for *E. vitis* larvae on grapevine leaves with fixed precision levels so that m can be estimated with 90 % probability within a confidence interval of ± D.](image)
**Sampling plan**

We developed a computer program ‘SeqSamp’ that tabulates the required sample sizes and stop values. Regression parameters are either calculated by the program from means and variances of sampling data or they are put in from other sources. The regression parameters of Taylor’s power law were used to calculate sample sizes and stop lines for different levels of precision (Fig. 4). Sample sizes rise extremely with increasing levels of precision. For example, to reach a precision of $D=25\%$ with 90% probability, 42 leaves have to be sampled compared to 262 leaves for $D=10\%$ and 1049 leaves if a precision of 5% is required. However, for practical purposes of leafhopper management decision making a precision of 25 to 20% appears to be sufficient.

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Fig. 5. Excerpt from a sampling sheet with tabulated stop values for field sampling. Field data are written into the 2nd and 3rd column (simulated sampling from a population with $m=2.8$ larvae/leaf as an example). Sampling can be stopped as soon as the cumulative number in the third column reaches or exceeds the stop value of the required precision level. The estimated mean can be read from the appropriate column. For example, sampling can be stopped after 36 leaves (estimated mean: 2.4 larvae/leaf) if a precision of 25% is required and after 50 leaves (estimated mean: 2.8 larvae/leaf) if a precision of 20% is needed.
The stop lines for fixed precision levels are presented in Figure 4. Sampling can be terminated as soon as the plot of the cumulative number of leafhoppers and number of sampled leaves crosses the particular stop line of the required precision level. To facilitate this procedure, the data are tabulated by ‘SeqSamp’ and used as sampling sheets in the field (Fig. 5). In this case, writing down the counts of leafhoppers for each leaf in one column and summing up the counts in a second column is all that has to be done. As soon as the cumulative number reaches or exceeds the tabulated value for the appropriate precision level, sampling can be terminated and the estimated mean density can be read from the sheet.

![Graph showing estimated means vs. defined mean density](image)

Fig. 6. Simulation of sequential sampling with population densities from 0.25 to 5 larvae/leaf. Sampling was repeated 100 times for each level of density. Required precision is 90% probability for the confidence interval within 25% of the mean.

**Validation of the sampling procedure**
We simulated sequential sampling from populations with different mean densities and checked for the correspondence of defined and estimated population densities and the compliance of the results with the required precision level. An example is presented in Figure 6. The correlation between expected and estimated means was highly significant. The achieved precision corresponded well with the required values. In the example shown it varied between 24.3 and 25.3% with an expected value of 25%.

**Conclusions**
A sequential sampling plan for *E. vitis* was developed based on counts of larvae on grapevine leaves. It is an advantage of such a sampling method that time and labor afford can be adjusted to the purpose of the sampling by choosing different levels of precision. Even during the sampling procedure in the field the actual precision reached so far can be assessed. If sampling is done by growers or advisers in order to make control decisions, a precision of 20% to 25% appears to be sufficient. For research purposes, however, higher precision may
be required that results in rapidly increasing sample sizes. While basal leaves should be sampled early in the season, leaves of the middle part of the canopy are most suitable later on.

The database that was used to establish this sampling scheme is too small yet to establish a generalized sampling sheet. More data from different regions and cultivars as well as other viticultural systems should be analyzed.

References


Preliminary notes on the biodiversity of egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) in vineyards of Southern Italy

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Abstract: The biodiversity of egg parasitoids in vineyards of Southern Italy has been investigated in the framework of a biennial project started in 2002. In 4 vineyards, managed with different agronomic techniques, located in Campania (3) and Basilicata (1), a total of 11 yellow sticky traps (13x24 cm) have been placed from end of May to November. The traps have been replaced weekly and the egg parasitoids counted, removed and identified. An additional short term sampling (28 sticky traps, placed in 11 vineyards) has been made in Aprilia (Latium) from 13 to 27 September 2002.

Other data have been obtained by sampling grapevine leaves, identifying the parasitized insect eggs and maintaining them until the parasitoid emergence.

A total of 8204 egg parasitoids have been trapped, all belonging to the Mymaridae (91%) and Trichogrammatidae (9%) (Hymenoptera), not including very few specimens of Scelionidae. The genus Anagrus has been the most represented (71%). Other mymarid genera rather common have been Alaptus, Anaphes, Camptoptera, Erythmelus, Gonatocerus and Stethynium; rare Litus, Mymar and Polynema. The specimens of Anagrus collected, belong in great majority to the species Anagrus ustulatus Haliday; all those of Stethynium represent Stethynium triclavatum Enock. Both Anagrus and Stethynium have been caught in vineyards from late May to November.

An impressive number of Anagrus have been trapped in a short term sampling in 11 vineyards in Aprilia. On a total of 4482 individuals, the average per trap has been of 160, corresponding to 1 individual/4 sq. cm/trap; the maximum individuals/trap has been of 1095 with a density of 7 individuals/4 sq. cm/trap.

Among the trichogrammatid genera recorded (Chaetostricha, Lathromeris, Megaphragma, Monorthochaeta, Oligosita, Paracentrobia, Trichogramma, Ufens and Uscana) the most common in all sites has been Trichogramma (2.18%), except in Rivello (PZ), where the highest catch was that of a very small species of Megaphragma (about 0.2 mm in length), probably undescribed and egg parasitoid of thrips.

Moreover, from eggs of Zygina rhamni Ferrari and Empoasca vitis (Goethe), the most common species of grapevine leafhoppers in the experimental sites, the mymarids Anagrus ustulatus Haliday and Stethynium triclavatum Enock have been reared. The first species was not previously recorded from the mentioned hosts.

Key words: Anagrus, Stethynium, leafhoppers

Introduction

One of the most interesting components of the vineyard biodiversity is represented by the egg parasitoids, which are retained to play a role in keeping some pests, like leafhoppers, under the economic threshold. Most of available studies are limited to the egg parasitoids of the green vine leafhopper Empoasca vitis (Göthe) in Northern Italy (Vidano et al., 1987; 1988; Picotti & Pavan, 1991), in Switzerland (Cerutti et al., 1989; Genini, 2000), in Germany (Herrmann & Eichler, 2000) and in France (Van Helden & Decante, 2001). No data are
available on the biodiversity of the egg parasitoids in vineyards of Southern Italy, where leafhoppers are considered minor pests. A biennial project started in 2002 to study the system vineyard-leafhoppers-egg parasitoids-alternative hosts for general and applied purposes.

The present paper reports on some preliminary results.

**Material and methods**

In 4 vineyards, managed with different agronomic techniques, located in Campania (3) and Basilicata (1), a total of 11 yellow sticky traps (13x24 cm) have been placed from end of May to November. The traps have been replaced weekly. An additional short term sampling (28 sticky traps placed in 11 vineyards) has been made in Aprilia (Latiun) from 13 to 27 September 2002.

The egg parasitoids trapped have been counted, removed from the traps and identified, making slides when necessary.

Other data have been obtained by sampling grapevine leaves, identifying the parasitized insect eggs and maintaining them until the parasitoid emergence.

Table 1. Egg parasitoids (Mymaridae and Trichogrammatidae) collected by using yellow sticky traps in vineyards of Southern Italy in 2002.

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<th>Rivello (PZ)</th>
<th>S.Giorgio a Cremano (NA)</th>
<th>Aprilia (LT)</th>
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<tr>
<td><em>Trichogramma</em></td>
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<td><em>Uscaena</em></td>
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<td>0</td>
<td>1</td>
<td>0.01</td>
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<tr>
<td>Totals</td>
<td>741</td>
<td>547</td>
<td>1030</td>
<td>997</td>
<td>4889</td>
<td>8204</td>
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</tr>
</tbody>
</table>
Results and discussion

A total of 8204 egg parasitoids have been caught, all belonging to the Mymaridae (91%) and Trichogrammatidae (9%) (Hymenoptera), not including very few specimens of Scelionidae (Table 1).

The genus *Anagrus* has been the most represented (70.58 %). Other mymarid genera rather common have been *Alaptus*, *Anaphes*, *Camptoptera*, *Erythmelus*, *Gonatocerus* and *Stethynium*; rare *Litus*, *Mymar* and *Polynema*. The specimens of *Anagrus* collected, belong in great majority to the species *Anagrus ustulatus* Haliday (*sensu* Chiappini,1989); all those of *Stethynium* represent *Stethynium triclavatum* Enock. Both *Anagrus* and *Stethynium* have been caught in vineyards from late May to November (Figure 1).

An impressive amount of *Anagrus* has been collected by using 28 sticky traps in Aprilia (LT) in a short term sampling from 13 to 27 September 2002. On a total of 4482 individuals, the average per trap has been of 160/trap and of 1 individual/4 cm²; in the latter case with a maximum of 7 individuals/4 cm².

Among the trichogrammatid genera recorded (*Chaetostricha*, *Lathromeris*, *Megaphragma*, *Monorthochaeta*, *Oligosita*, *Paracentrobia*, *Trichogramma*, *Ufens* and *Uscana*) the most common in all sites has been *Trichogramma* (2.18%), except at Rivello (PZ), where the highest catch was that of a very small species of *Megaphragma* (about 0.2 mm in length), probably undescribed and egg parasitoid of thrips.

![Graph of Anagrus and Stethynium captures](image-url)

Fig. 1. Catches of *Anagrus* and *Stethynium triclavatum* in three experimental vineyards (Prata U. P., Castelvenere, Rivello) in 2002.

Moreover, from eggs of *Zygina rhamni* Ferrari and *Empoasca vitis* (Göt he), the most common species of grapevine leafhoppers in the experimental sites, the mymarids *Anagrus ustulatus* Haliday and *Stethynium triclavatum* Enock have been reared. The first species has not been previously recorded from the mentioned hosts.
References


Experiences for vector control of grape golden flavescence in Lombardia and Emilia Romagna (Northern Italy) vineyards

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Abstract: Starting from spring and summer 1999 in various vineyards of Piacenza and Pavia provinces (Northern Italy) the presence of severe symptoms of “Golden Flavescence” was reported. Following these outbreaks, in 2000 and 2001, to evaluate the best control strategies against the vector, the planthopper *Scaphoideus titanus* Ball, but with a reduced impact against mites populations, some experimental tests were carried out. Two different approaches were adopted: efficacy trials in randomized blocks (3-8 products compared) and strategy comparison on whole vineyards (2 couple of products or 2 products compared). In 2000 2 treatments were done while in 2001 in randomized block test and in some vineyards only 1 insecticide was applied. Results were quite satisfactory: generally a good efficacy was achieved without any negative effect on mites populations.

Key words: *Scaphoideus titanus*, mites, side effects

Introduction

During the last years, severe symptoms of vegetative and productive withering linked to the phytoplasma of the “Golden Flavescence” were reported in many vineyards of Piedmont, Lombardia and Emila-Romagna. Till then the most serious symptoms were present in Veneto, that is the area where this disease diffused first (Mori et al., 1999; Cravedi & Nicoli Aldini, 2000; Posenato et al., 2001). To face this emergency, now widely diffused in Northern Italy, a special decree law of the Italian Ministry of Agriculture (D.M. n. 32442 del 31/05/2000 – G.U. n. 159 – 10/07/2000) was promulgated. According to this decree-law regional phytosanitary services must check the presence and distribution of the disease and of its vector, the leafhopper *Scaphoideus titanus* Ball. Moreover they must define the necessary phytosanitary practices. New strategies for integrated plant protection in vineyards had to be developed and tested to evaluate the efficacy, rapidity and application time of various active ingredients and/or formulations, also taking in account the production strategies usually adopted like conventional, integrated or organic farming. Moreover in many areas as the use of insecticides was usually very low, the side effect on mites populations naturally occurring in vineyards had to be evaluated too.

Material and methods

Tests have been carried out in 2000 and 2001 adopting two main approaches. In the first one a series of “efficacy trials”, that is randomised complete block layout tests, to evaluate the efficacy of some active ingredients in comparison with an untreated control, were conducted.
In this case plots of 14 plants and 3 rows were used. The second approach was based on “strategy trials”, that is vineyards were split in two similar large plot and each one was treated with different products without any untreated plot. Different experimental designs were adopted in 2000 and 2001. This was done to: a) have a better evaluation of some product without the interference of any following application; b) compensate for reduced abundance of *S. titanus* populations in the second year of the experimentation; c) compensate some difficulties recorded in 2000. In fact, in 2000, because of the mobility of the pest inside vineyards and in spite of the use of special shields to avoid interference among the thesis during the applications, *S. titanus* population in untreated plots decreased dramatically and in many cases it was reduced to zero (Graph no. 1a). To avoid, or at least to reduce this phenomenon in 2001, as recommended by ANPP (1989) only the 10 central plants of central row of each plot were treated. The number of vineyards, of applications and type of assessments are reported in table 1. The locations in which the tests were carried out are shown in figure 1. The list of insecticides used in 2000 and 2001, in different combinations, is the following: buprofezin, flufenoxuron, acrinathrin, chlorpyrifos-ethyl, chlorpyrifos-methyl, fenitrothion, methyl-parathion, malathion (2000), etofenprox, indoxacarb (2001), azadirachtin (2001), pirethrum + piperonil-butoxide, rotenon (2000). In the case of chlorpyrifos-ethyl and fenitrothion standard and micro-encapsulated formulations were used. The insecticides were usually applied at the mid of June and at the beginning of July. The number of assessment varied between year and the type of test and ranged from 4 to 6. As a rule a pre-treatment and a final assessment (in September) were carried out.

**Results and discussion**

Most of the insecticides registered and used for *S. titanus* control in Italy are quite efficacious. A difficulty observed in the evaluation of efficacy tests is given by a significant reduction of...
S. titanus populations that occurs naturally (Fig. 2). Insecticides used can grouped in two categories: “IGR” and “neurotoxic”. The use of product of the former category is allowed by IPM guidelines and they are appreciated for their reduced environmental impact but they kill the planthopper quite slowly even if their efficacy 10-15 days after the application is comparable with that of more classical insecticides (Fig. 2, 3). Neurotoxic products showed to be very effective (Fig. 2, 3) without particular impact on mites populations, especially on Phytoseids (Fig. 4a).

Table 1. List of tests carried out in 2000 and 2001. In 2001 in “strategy test” each plot was subdivided in 9 subplots for sampling.

<table>
<thead>
<tr>
<th>Year</th>
<th>tests</th>
<th>place</th>
<th>management</th>
<th>thesis</th>
<th>treatments</th>
<th>Sampling (leaves /plot)</th>
<th>S. titanus</th>
<th>mites</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>efficacy</td>
<td>Oltrepo' pavese</td>
<td>IPM/conventional</td>
<td>4</td>
<td>2</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piacenza</td>
<td>IPM/conventional</td>
<td>5</td>
<td>2</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>strategy</td>
<td>Lombardia</td>
<td>IPM/conventional</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lombardia</td>
<td>organic farming</td>
<td>2</td>
<td>3</td>
<td>100</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>efficacy</td>
<td>Brescia / Mantova</td>
<td>IPM/conventional</td>
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<td>1</td>
<td>100</td>
<td>20</td>
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<tr>
<td></td>
<td></td>
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<td>IPM/conventional</td>
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<td>1</td>
<td>100</td>
<td>20</td>
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<tr>
<td></td>
<td>strategy</td>
<td>Lombardia</td>
<td>IPM/conventional</td>
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<td>1</td>
<td>135</td>
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<td></td>
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<td>2</td>
<td>135</td>
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<td>Lombardia</td>
<td>organic farming</td>
<td>2</td>
<td>3</td>
<td>135</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Efficacy tests in 2000 (a) and 2001 (b). Two treatments in 2000 and only one in 2001. In 2001, IGRs and “indoxacarb” were applied one week before. (Arrows: time of applications. Bars with the same letter do not differ significantly. Duncan test. p>0.05).
Fig. 3. Strategy tests. Efficacy against *S. titanus* in 2000 (a) and 2001 (b) in the same vineyard. In both years two treatments/plots were done (Arrows: time of treatments).

Many doubts still remain on the efficacy and best use for products allowed in organic farming. In fact the most applied product in these vineyards is pirethrum but it has shown a little efficacy and persistence even when synergized with piperonil-butoxide (Graph 3b). Azadirachtin, used only in 2001, has proved to be a promising product but only if it is used in preventive strategies. From this point of view it is very important in many cases of organic farming to modify also agronomic practices to change microclimatic conditions and plant vigour that seems to be very favourable for *S. titanus* populations.

Fig. 4. Strategy tests. (a) Specimens of Phytoseid mites observed in the same vineyard as in graph 2. (b) Efficacy against *S. titanus* in an organic vineyard. (Arrows: time of treatments. None of the treatments gave significant differences. Duncan test. p>0.05).

**Conclusions**

The results of these experiments point out that a significant reduction of *S. titanus* populations is possible without dramatic changes in vineyard environment. Moreover if leafhopper populations have been reduced it is possible to get a very good control also with only one application. However this must be carefully evaluated and certainly it must be applied only where the disease have been greatly reduced. On the contrary, in areas where the disease is
still in an epidemic phase it is greatly recommended to use all the available procedures to control the vector of the phytoplasma and two insecticide application, according to phytosanitary guidelines, must be applied. Nevertheless according to the experiences accumulated in the last years a third application during summer it is not necessary.

References

ANPP 1989: Methode d’essai d’efficacité au champ de produits destines a combattre la cicadelle *Scaphoideus titanus* (*littoralis* Ball.) (Vecteur de la Flavescence Dorée). – Methode n. 147.
First remarks on the leafhopper population in a vine-growing area of South-Western Sicily

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Abstract: The structure and dynamics of the leafhopper populations in some vineyards of Feudo Arancio (Sambuca di Sicilia, Agrigento) were investigated in 2002 by periodical leaf samplings and by means of yellow sticky traps. The most recurrent species resulted Empoasca vitis (Goethe), Zygina rhamni Ferrari and Jacobiasca lybica (Bergevin & Zanon). The latter showed the highest peaks of population density, which increased from the first week of July to the end of September and resulted strictly related to the onset and development of chromatic alterations and heavy drying of the leaves. Other 16 species of leafhoppers were collected, with Zygina nivea (Mulsant & Rey) and Ribautiana cruciata (Ribaut) firstly recorded from Sicily.

Key words: Jacobiasca lybica, Empoasca vitis, Zygina rhamni, leafhoppers, vineyards, Sicily

Introduction

In recent years large and economically important vine growing areas of Spain, Portugal, Sardinia and Sicily showed heavy outbreaks of the cotton leafhopper Jacobiasca lybica (Bergevin & Zanon) (López et al., 1998; Raposo et al., 2000; Lentini et al., 2000; Delrio et al., 2001; Manzella et al., 2001; Tsolakis, 2003).

This species, described in 1922 as Chlorita lybica from material associated with Vitis vinifera L. from Cyrenaica and Tripolitania, was soon regarded as an important pest of several cultivated plants. For this reason, its biology, ecology and host relationship, were investigated in various Mediterranean and African areas (Klein, 1948; Joyce, 1961; Evans, 1965; Habib et al., 1972). J. lybica was firstly recorded in Italy on cotton by Russo (1942) and later on vine by Vidano who pointed out its trophic activity and the consequent leaf alterations (1962a, 1962b, 1963).

The observation of a huge outbreak of leafhoppers, occurred in 2001 in some vineyards of Feudo Arancio (Sambuca di Sicilia, Agrigento), followed by striking leaf reddening and drying, stimulated the present investigation aimed to improve the knowledge of the structure and dynamics of the leafhopper populations in that area.

Material and methods

In 2001 symptoms of leafhoppers outbreak were visible in various plots of the Feudo Arancio farm including different varieties of vines such as “Cabernet”, “Merlot”, “Chardonnay” and “Nero d’Avola”. The latter was particularly affected by reddening and represented therefore
the main target of our investigation in 2002. This vineyard, aged 15 years, is located in a flat area with a surface of 12 hectares and a density of 1,111 plants per hectare.

The monitoring of the nymph population was carried out by periodical sampling of 100 leaves per thesis (treated and untreated control). Adults were monitored with yellow sticky traps (24x13 cm) replaced every two weeks. Taxonomic identification of the collected specimens was done in laboratory using the papers of Ribaut (1936, 1952) and Della Giustina (1989). In order to improve the knowledge of the overwintering population of \textit{J. lybica} several yellow sticky traps were distributed in the “Nero d’Avola” vineyard, in its surroundings and in some other wild areas of the farm. Moreover, two days were spent in January 2003 for a direct field survey.

**Results and discussion**

The sampling of the leaves indicated that the density of the whole leafhopper nymph population slightly increased from May to the end of August (0.61 individuals per leaf), reached the highest peak in the third decade of September (4.1 individuals per leaf) and regressed to lower levels at the end of October (0.43 individuals per leaf) (Fig. 1).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Leafhopper nymph populations in a vineyard of “Nero d’Avola” variety (Feudo Arancio, Sicily, 2002).}
\end{figure}

As regards the imaginal population, a careful examination of the traps highlighted that the most recurrent species were \textit{Empoasca vitis} (Goethe), \textit{Zygina rhamni} Ferrari and \textit{J. lybica} (Typhlocibinae) (Fig. 2).

\textit{E. vitis} was always caught at low density levels from the end of May to the end of August: the species did not show any relevant peak of captures, being clearly relegated to less prominent importance than in Northern Italy, where it is considered the main grapevine leafhopper (Vidano, 1958; Vidano \textit{et al.}, 1988; Pavan \textit{et al.}, 1992). This may be compatible with its scarce thermophily that makes it, in Sicily, less competitive for feeding sites compared to the other ampelophagous species.
Z. rhamni was constantly present from May to October with a population density increasing until the third decade of August. Actually this species proved to have well adapted to this climatic context, as it was able to develop high levels of population density throughout the vine-growing season.

![Graph showing population density of leafhoppers](image1)

Fig. 2. Leafhopper adult population in a vineyard of “Nero d’Avola” variety (Feudo Arancio, Sicily, 2002).

![Graph showing sex ratio of J. lybica](image2)

Fig. 3. Jacobiasca lybica: sex ratio (Feudo Arancio, Sicily, 2002).

J. lybica appeared only during the first decade of July and, from that moment on, its population density gradually augmented up to a peak of 270 individuals per trap in the last decade of September. It is noteworthy to stress that the Empoascini observed before July
belonged exclusively to other species, mainly *E. viti*. Furthermore, the increase of the *J. lybica* population seemed strictly related to the appearance of a massive number of nymphs and the onset and development of chromatic alterations and heavy drying of the leaves.

As concerns the sex ratio (males/females) of the population collected by means of sticky traps, two different groups were observed: one with a proportion constantly over 4:1 until the first half of August, and the other, from that moment on, with a proportion between 3:1 and 1:1 (Fig. 3). The trend may be only apparent and occasioned by both a greater mobility of males and a higher longevity of females.

During the field survey carried out in January on different grasses, various bushes and conifers, several females and a pair of males of *J. lybica* were exclusively collected on *Angelica* sp. (Umbelliferae) and *Rubus fruticosus* (Rosaceae). Given the high number of specimens still present at the end of October, it may be assumed that the leafhopper underwent high mortality probably because of low winter temperatures. The possibility of seasonal migrations from and to distant territories reported by some authors (Joyce, 1961; Tsolakis, 2003), appears, in such a context, in contrast with the progressive but not abrupt increase of population. Nevertheless the leafhopper periodically undergoes strong skips of population density, which makes it a dangerous pest for the Sicilian viticulture.

As regards the other leafhoppers occurring in the surrounding areas and collected just occasionally, the following species have been identified: *E. decipiens* Paoli, *E. alsiosa* Ribaut, *Asymmetrasca decedens* (Paoli), *Arboridia parvula* (Boheman), *Haptidia provincialis* (Ribaut), *Zygina nivea* (Mulsant & Rey), *Zyginita servadeii* Vidano, *Eupteryx rostrata* Ribaut, *Linnavaurioriana sexmaculata* (Hardy), *Ficocyba ficaria* (Horvath), *Ribautiana cruciata* (Ribaut), *Ribautiana tenerrima* (Herrich-Schäffer) (Typhlocybinae), *Grypotes staurus* Ivanoff, *Euscelis lineolatus* Brullé, *Exitianus taeniaticeps* (Kirschbaum) and *Thamnotettix zelleri* (Kirschbaum) (Deltocephalinae). *Z. nivea* and *R. cruciata*, both collected during the winter on brambles, are firstly recorded from Sicily.

A preliminary trial of chemical control carried out in the “Nero d’Avola” vineyard showed that a single application of flufenoxuron in the middle of July prevented the demographic outbreak of *J. lybica* and, consequently, the development of leaf damage in the treated area.

References

Bergevin (De), E. & Zanon, V. 1922: Danni alla vite in Cirenaica e Tripolitania dovuti ad un nuovo Omottero (*Chlorita lybica* sp. n.). – Agricoltura Coloniale, Firenze 16(2): 58-64.


Monitoring the leafhopper *Scaphoideus titanus* Ball and the planthopper *Hyalesthes obsoletus* Signoret in Northern Italy

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² Servizio Fitosanitario, Regione Lombardia, Milan, Italy

Abstract: Results of a four-year (1999-2002) research project conducted in vineyards in Lombardy and Emilia-Romagna (Northern Italy) are briefly explained. The main objective was the monitoring of the cicadellid *Scaphoideus titanus*, vector of golden flavescence, and the cixiid *Hyalesthes obsoletus*, vector of the ampelopathy called “bois noir”. These hoppers were monitored with yellow sticky traps and with a sweeping net. A distribution map of *S. titanus* in both regions was drawn and in Emilia-Romagna its advance towards the east was noted. Data on the presence of *H. obsoletus* in vine-growing districts were also obtained; for monitoring this species sticky traps in the canopy, even when placed only slightly above ground level, were less successful than a sweeping net passed over weeds (especially *Urtica* and *Convolvulus*) in vineyards and along their borders. A profile of the hopper fauna of the vineyard agrosystem of both regions was also defined. About a hundred species were identified, some of which are known or suspected to be vectors.

Key words: vineyards, hoppers, vectors, sticky traps, insect net, Lombardy, Emilia-Romagna.

Introduction

Following new acute phases and the spread of grapevine yellows in many vinegrowing districts in Northern Italy, during the period 1999-2002 the Institute of Entomology at Piacenza started collaboration with other agencies to carry out a phytosanitary survey of Lombardy and Emilia-Romagna, in order to re-examine and broaden monitoring activity in the main vine-growing areas of both regions to determine the presence, distribution and phenology of the vectors of the phytoplasmas in question. Such research on a more limited scale had already been carried out by the Institute at the end of the eighties.

In recent years the problem of golden flavescence (“Flavescence dorée”, FD), which is transmitted by the leafhopper *S. titanus* Ball (Arzone & Alma, 1997; Belli *et alii*, 2002), has been added to by the spread of another ampelopathy, the so called “Bois noir” (BN), which is transmitted by the planthopper *Hyalesthes obsoletus* Signoret (Maixner, 1994; Sforza *et alii*, 1998) and whose symptoms are very similar to those of FD. BN is now considered a more serious and diffuse ampelopathy than previously believed. The present paper summarizes the monitoring activity conducted and the results obtained over a four-year period.

Material and methods

The monitoring work, carried out from May-June to September-October, involved most provinces of Lombardy and the whole Emilia-Romagna. It was carried out mostly by means of yellow sticky traps placed in the canopies in the rows of vines, and in some vineyards also by sampling with an insect net, sweeping herbaceous plants in vineyards and along the borders and shaking the vine foliage. In the Institute of Entomology in Piacenza 2,854 sticky
traps were examined and several thousand hoppers were identified. Other monitoring data are reported in tab. 1.

Table 1. Data on monitoring of hoppers using sticky traps in vineyards.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>PROVINCES</th>
<th>LOCALITIES</th>
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<td>1999</td>
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<td>59</td>
<td>86</td>
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<td>2002</td>
<td>Mantua, Como, Sondrio</td>
<td>24</td>
<td>29</td>
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<td><strong>EMILIA-ROMAGNA</strong></td>
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</tr>
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<td>62</td>
<td>1,074</td>
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<td>2002</td>
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<td>11</td>
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</table>

Results and discussion

This work allowed the authors to study the presence, spread and phenology of the two most important hoppers of phytopathologic interest in vinegrowing districts of both the regions; to obtain specimens for molecular analyses in order to detect phytoplasmas; and also to draw the profile of the hopper fauna in the vineyard agrosystem.

*S. titanus*

The distribution map of this cicadellid in Lombardy and Emilia-Romagna in 2000 is reported in fig. 1. In Lombardy, especially the widespread monitoring activity conducted during 2000 indicated the presence of this cicadellid in most vinegrowing areas. A comparable situation was observed in the Oltrepò Pavese (Pavia) where its presence had been recorded over a long period previous to this study, and in others provinces (Milan, Lodi, Bergamo and Mantua). In Valtellina (Sondrio) this leafhopper was previously unknown. In 2001 *S. titanus* was also identified in the province of Como, along the northern part of the Lake Como. Its absence from some vineyards in the province of Brescia (in Valtenesi and Franciacorta) during 2000 may be the result of effective insect control, because it had been found in these areas in the eighties. In the Oltrepò Pavese area too, our research during this year demonstrated the absence of populations in several vineyards following insecticide treatment, while in 1999 the presence of the pest was found in nearly all the vineyards monitored (Cravedi & Nicoli Aldini, 2000).

In Emilia-Romagna *S. titanus* was recorded about twelve years ago, but only in the western part of the region (province of Piacenza); in 2000 a remarkable spread of this leafhopper eastwards was noted. At present its advancing front is located in the eastern part of the province of Bologna and in the province of Ferrara.
Moreover this insect has also been found recently in some central and southern areas of the Italian peninsula (Santinelli et alii, 2003).

Sticky traps placed in the canopy are almost exclusively effective for monitoring adults, while immature stages of *S. titanus* are generally captured rarely, or there are remarkable divergences between one trap and another. These traps are useful for monitoring nymphs only if placed close the base shoots or other foliage where juvenile stages live. Otherwise visual inspection of the lower surface of the leaves is better for determining their presence.

![Map of Italy showing the distribution of S. titanus](image)

Fig. 1. *S. titanus* in the main grapevine growing areas of Lombardy and Emilia-Romagna in 2000.

**H. obsoletus**

In the period 1999-2001 our investigations allowed us to obtain some data on the regional distribution of this cixiid in vineyards. Its presence, always in low density, in Lombardy (provinces of Pavia, Bergamo and Sondrio) and Emilia-Romagna (provinces of Piacenza, Modena, Bologna, Ravenna and Rimini) was evidenced during 2000 by sticky traps placed at a certain height on the rows.

During 2002, following the spread and rather serious symptoms of BN, a monitoring activity specifically directed at *H. obsoletus* was carried out in the province of Modena. The planthopper was found in 9 out of 10 vineyards investigated, in some of these being very common between the end of June and the end of August, with a peak around the second half of July. A correlation between its abundance and the presence of its main herbaceous hosts (*Urtica, Convolvulus*) in vineyards or along their borders was recognized. Almost all of the specimens (298 out of 315) were collected by sweeping net on weeds.

The importance of regular sweeping with an entomological net during the period of the adult’s activity in order to monitoring this planthopper must be underlined. This method provided much better results than the use of sticky traps, even when these are placed near ground level (50 cm or less from the ground), as suggested by some authors (Weber & Maixner, 1998; Braccini & Pavan, 2000).
Other hoppers
By studying all the material collected in vineyards, about a hundred species of hoppers were identified, belonging to the families Cixiidae, Delphacidae, Dictyopharidae, Tettigometridae, Issidae, Flatidae, Cercopidae, Membracidae, Cicadellidae. Some species (Metcalfa, Empoasca, Zyaina) are important because of direct damage to the grapevine, while other common species (tab. 2) are known or suspected to be vectors (Arzone & Alma, 1997; Braccini & Pavan, 2000; Nicoli Aldini, 2001); their role in the epidemiology of grapevine yellows deserves more investigation.

Table 2. Common species of hoppers known or suspected to be vectors, present in vineyards in Lombardy and Emilia-Romagna.

<table>
<thead>
<tr>
<th>CIXIIDAE</th>
<th>CICADELLIDAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyalesthes obsoletus Signoret</td>
<td>Neoaliturus fenestratus (Herrich-Schäffer)</td>
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<tr>
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<tr>
<td>Laodelphax striatellus (Fallén)</td>
<td>Macrosteles spp.</td>
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<tr>
<td>Flatidae</td>
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<tr>
<td>Metcalfa pruinosa (Say)</td>
<td>Scaphoideus titerus Ball</td>
</tr>
<tr>
<td>Cercopidae</td>
<td></td>
</tr>
<tr>
<td>Philaenus spumarius (Linné)</td>
<td>Anoplotettix fuscovenosus (Ferrari)</td>
</tr>
<tr>
<td></td>
<td>Euscelidius variegatus (Kirschbaum)</td>
</tr>
</tbody>
</table>

References
Assessment of a two years study of the natural enemy fauna of *Scaphoideus titanus* Ball in its North American native area

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**Abstract:** During 2001 and 2002 seasons was conducted a research program to study the natural enemies of the leafhopper *Scaphoideus titanus* in its native area. Currently, the knowledge of the ecology of *S. titanus* and close species of leafhopper in the North-Eastern USA is very scarce and a general revision of the *Scaphoideus* genus taxonomy seems necessary.

After collection and identification of *Scaphoideus* sp. natural enemies, this study is aimed at introducing into France the best candidates for biological control and to keep live stocks of these species. Several species of insect parasitoids have been reared and isolated from the abundant leafhoppers collected in the region of the Finger Lakes (NY).

Studies are currently underway in quarantine laboratory to keep strains and to produce enough material to study their biological efficacy and safety characteristics prior to release in field experiments.

**Key words:** *Scaphoideus titanus*, entomophagous insect, classical biological control

**Introduction**

In Western Europe, *Scaphoideus titanus* Ball is an alien species first found in France more than fifty years ago (Bonfils et Schvester, 1960). This accidentally introduced pest is strictly ampelophagous and it is the vector of the Flavescence Dorée, a severe phytoplasma disease in grapevine. In order to decrease the infestation and the dissemination of this disease, a biological control program of the vector as part of an overall integrated pest management strategy is considered promising.

Studies on the french native antagonists revealed that they cannot control the populations of *S. titanus* (Bernadette et al., 1996; Martinez et al., pers. comm.). Under these conditions, an exploration for natural enemies of the cicadellid in North America is the first step to consider. Such a study has already been suggested (Delucchi, 1994; Ferron, 1996) but no concrete studies have been initiated although a relatively old introduction into France.

*Scaphoideus titanus* originates from the Great Lakes region in the United States and Canada. The species is not very abundant in this region (Vidano, 1966) and this may be due to the impact of the endemic entomophagous fauna. Until now, very little information were available on antagonists of this species in North America. Preliminary observations have been done on adults and nymphs of *S. titanus* parasitized by Dryinidae and Pipunculidae (Barnett, 1976; Maixner, pers. com.). Same observations were renewed during a short stay in the same area during the summer of 1999. However, the very scarce material collected in the field as immature stages doesn’t allow an identification of the species.

A new research program has been conducted during 2001 and 2002 to study the natural enemies of this leafhopper in its native area. The aims of this research was to collect, to identify and to evaluate the impact of native natural enemies of *S. titanus* in US and to
introduce into France strains of the main interesting parasitoids to study and select the best candidates for biological control releases.

**Material and methods**

*Field collections*
Observations and collections of leafhoppers were made throughout June to October 2001 and 2002 in upstate New York, most of the time in the Fingers Lakes area and especially in the vicinity of the Seneca Lake. Most of the specimens were collected in the sites of Geneva, Dresden and Valois. A special attention is given to the species living on wild or cultivated *Vitis* species and the surroundings of vineyards. Nymphs and adults of cicadellids are swept from woody and herbaceous vegetation using a sweeping net or occasionally a 2-cycle aspirator (J.W. Hock model) and kept in boxes or cages for study or rearing in the laboratory. All individuals for identification are kept in 70% alcohol or dessicated.

*Laboratory rearing*
Parasitized leafhoppers are provided with the vegetation on which they are swept from in glass vials and maintained in the laboratory until obtention of the cocoon. Parasitism by Dryinids is betrayed by the presence of a kystic bag constituted by the successive exuviae of the dryinid ectoparasitoid larva on the body of the Cicadellid. In the last larval stages, this bag is black and obviously rounded and so easily noticed. After at most two weeks of rearing the host, a typical hymenopteriform larva slit this bag as the host dies and soon spins a cocoon, sometimes incorporating some materials (vegetation or sand). Great attention must be paid to avoid excessive humidity. Depending on the phenology of species and the period of the year, an adult dryinid can emerge within a few weeks or the following year.

All the leafhoppers collected were kept in laboratory conditions during at least two weeks to detect possible parasitism. This period of time is generally sufficient to reveal parasitization by dryinid wasps or to obtain the pupation of pipunculids flies.

*Sentinel eggs experiment*
Numerous adults of *S. titanus* are collected in the field and kept in laboratory for egg-laying on vine shoots during the month of July and August. The eggs obtained are exposed to potential parasitism in the field on vine vegetation from September to October. The exposed shoots are brought back to the laboratory for study.

*Introduction in France*
The biological material obtained is sent to France by express air mail during the field season or eventually directly brought back in the French quarantine at the end of each season. Introductions were made in the quarantine laboratory of INRA-Antibes where the insects are reared or kept at different temperature according to the life stages.

**Results and discussion**

*Field collection and identification of leafhoppers*
Many cicadellids have been collected during the field collection seasons and more than 20 species have been identified on vine and on surrounding areas. These species occur on wild and cultivated *Vitis* sp. The species close to *S. titanus* are sometimes very difficult to identify in the field especially at the nymphaal stages. Five species of the genus *Scaphoideus* have been found: *S. titanus*, *S. elongatus*, *S. major*, *S. amplus* and *S. melanotus*. The knowledge of the ecology of *S. titanus* and close species of leafhopper in the North-Eastern USA is very scarce and a general taxonomic revision of the genus *Scaphoideus* is necessary.
**Insect parasitoids**

Several species of insect parasitoids have been isolated from the abundant leafhoppers collected in this region. Three species of the genus *Scaphoideus* (*S. titanus*, *S. amplus* and *S. intricatus*) and one close species, *Osbornellus limosus* have been found to be parasitized. Nymphs and adults of these species are mainly parasitized by Dryinidae (Hymenoptera) and Pipunculidae (Diptera).

**Hymenoptera: Dryinidae**

Five new relationships between Dryinidae and *Scaphoideus* sp. have been discovered. The dryinid species involved are: *Lonchodryinus flavus* Olmi, *Anteon masoni* Olmi, *Gonatopus peculiaris* Brues, *Esagonatopus perdebilis* (R. Perkins) and *Esagonatopus niger* (Fenton).

One of them, *L. flavus* belonging to the subfamily of Anteoninae seems to be very closely adapted to the *Scaphoideus* genus on wild grapevine. This species can be considered as a good potential candidate for biological control of the pest and first introduction into France was made in 2001 to try to rear and to keep it in the laboratory. 61 cocoons obtained from parasitized leafhoppers collected during the first season 2001 have been introduced in France but no adult emerged from them after exposure at cold conditions during several months. During 2002, 114 cocoons of *L. flavus* obtained in the same conditions gave more opportunity to try to get emergence of adults by trying more cold condition exposure. Now, these cocoons are exposed at 22°C under laboratory conditions for reactivation but results are not yet available.

At the end of the larval development, mature nymphs of *L. flavus* generally drop on the soil and pupate in the substratum in a cocoon made with available material. The optimal conditions of pupation are not known and many difficulties occur in laboratory conditions to obtain cocoons and to keep them alive. To avoid such difficulties and to try to insure live stocks of this species, introductions of adults have been organized directly from the US in semi-field cages in South of France to offer natural conditions. Each cage contains an adult vine directly on the soil with many individuals of *S. titanus* as hosts. In addition, parasitized nymphs and adults of *S. titanus* obtained in the quarantine laboratory in France have been released in such cages to try to obtain a better pupation in semi-natural conditions. A total of 60 individuals of *L. flavus* have been put in such cages and results of overwintering will be known during next summer (2003).

Another species of dryinid belonging to another subfamily (Gonatopodinae) have also been obtained from collected leafhoppers. Six adults of *E. perdibilis* emerged from the 11 cocoons introduced in quarantine laboratory and gives a second generation without exposure at cold conditions. We have now reached the third generation in the lab and this first permanent rearing would allow the first biological study of this species in the laboratory.

**Diptera: Pipunculidae**

Pipunculid species were found to parasite nymphs and adults of *Scaphoideus* spp.: 41 and 78 pupae have been respectively obtained in 2001 and 2002 from rearing of collected leafhoppers in the US. They have been introduced in France and reared in the quarantine laboratory to get adult emergence. A very small number of adults have emerged before or after exposure at cold conditions but without any synchronisation. Their maximum longevity of 1 or 2 days does not allow strains to be kept and only adults obtained will allow us to identify the species.

**Hymenopteran egg parasitoids**

Egg parasitoids (Hymenoptera, Mymaridae) have been obtained from ‘sentinel egg’ trials in 2001. Individual observations of *S. titanus* parasitized eggs will be necessary to confirm the
direct relation with the emerged parasitoids. From the first ‘sentinel egg’ trials in 2001, 20 individuals have been obtained in the following spring and results of the 2002 trials are not yet available and will be known at the end of spring 2003.

The species of these last two groups are not yet identified because of a too low number of adults obtained and kept alive for rearing.

Conclusions

Field collections in North America show that the insect antagonists of Scaphoideus species belong to the same 3 taxonomic groups as those identified on leafhoppers in our European regions. After the more precise identification of the different species detected during this first step of research, studies will continue to keep strains and produce enough material to study biological, efficacy and safety characteristics prior to release in field experiments. Next seasons rearing and results on the production of sufficient material will be determinant for the issue of this biological control project.

Acknowledgements

We thank Prof. M. Olmi (Viterbo, Italy) for the identification of the Dryinids and for all the ecological information. We are also very grateful to Dr. G. English-Loeb (Geneva, New York) who provided us facilities for our research in the US. This project has been funded by ONIVINS (France).

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Leafhopper species, its behaviour and its risk assessment in Portuguese vineyards from 1997 to 1999

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Abstract: The aim of this study was to increase knowledge on which factors could affect the leafhoppers behaviour concerning its risk assessment. The field observations were done by an extensive team working on three projects from 1997 to 1999. During these three years adult leafhoppers were, periodically, captured in four Portuguese regions: Algarve, Alentejo, Setúbal and Dão, and, occasionally, in three other regions: Vinhos Verdes, Douro and Trás-os-Montes. Samples from these adults were taken for a more precise identification, later on. At the same time, leafhopper nymphs were observed in several vineyards, in order to find out how factors like vineyard varieties, training systems, leaf age and location could affect their number.

The results are discussed in order to relate leafhopper species with the behaviour of adults and nymphs and the studied factors.

Key-words: leafhopper; species, risk assessment

Introduction

Leafhopper importance has been increasing in Portuguese vineyards for the past ten years. Although the attack registered in the superior Douro in 1998 (Freitas & Amaro, 2001) did not repeat in any other Portuguese region, the reference to the pest at superior levels to the economic threshold of 50 nymphs in 100 leaves (Gonçalves & Cavaco, 1997) has been frequent.

Concerning leafhopper risk assessment some results have already shown differences between the preferred canopy vineyard localisation of leafhopper in Alentejo (Raposo et al., 1999), in the South of Portugal and in Dão (Raposo et al., 2001), in the North of Portugal. Admitting that this may be related with leafhopper species, it was decided to study the leafhopper species, continuing the work of Rebelo (1993) and the effects of canopy leaf localisation, leaf age, training systems and vineyard varieties on nymphs number in different Portuguese regions.

Material and methods

The identified adult leafhoppers were captured in five vitivinicultural regions: the Algarve, Alentejo and Setúbal, in the South of Portugal, and, in Dão, Douro and Trás-os-Montes, in the North. Captures were carried out using sticky yellow panels of 15 x 20 cm or 20 x 22 cm which were tied to the upper vegetation support wire located from 0,8m to 1,5 m high.

Captures periodicity was casual in Douro; weekly in Dão, in 1998 and 1999; and every other week, in 1997 and in the other three regions.
Concerning infestation, the nymphs number was counted each week in the inferior leaves page during vegetative growth period, for three years and in five regions: the Algarve, Alentejo, Setúbal, Dão and Vinhos Verdes.

In the Algarve and Vinhos Verdes, the leaves from the first level (L1) were observed until August and from the medium level (L2) after August (Fig. 1). In Alentejo, Setúbal and Dão three canopy levels were observed in all observation dates. In Setúbal and Alentejo, principal and lateral leaves were considered in each level.

Concerning varieties effects on leafhopper number, the varieties number studied varied according to the regions: four in the Algarve; three and seven in Alentejo, according to nymph assessment or adults captures studies, respectively; two in Dão and four in Douro.

In each vineyard, defined by the region, variety, training system, canopy level and leaf age, 100 leaves were observed almost every week.

In Dão, 50 leaves were observed but the results were estimated to 100 leaves.

In Dão, two training systems were also considered: Royat and Guyot.

For results analysis Statistica 5.0 was used. Independent variables were compared using ManWhitney test.

![Fig. 1. Canopy scheme.](image)


**Results and discussion**

**Species**

The 8062 adult males identified from five Portuguese regions showed the presence of six leafhopper species: *Jacobiasca lybica*, *Empoasca vitis*, *Empoasca solani*, *Empoasca decipiens*, *Euscelidius variegatus* and *Psamotetrix striatus*.

![Fig. 2. Leafhopper species registered in vineyards from three Portuguese regions, from 1997 to 1999](image)

The analysis of each region, separately, shows in Alentejo and Setúbal the dominance of *J. lybica* with 93,0% in Alentejo, followed by *E. solani* (4,5%), by *E. decipiens* (1,8%) and by
E. vitis (0.7%); and with 99.4% in Setúbal, followed by E. solani (0.4%) and by E. decipiens and E. vitis (0.1% each) (Fig. 2). In the Algarve, the J. lybica percentage identified by the Algarve University team was less than what was found in Alentejo and Setúbal but, even so, J. lybica dominated leafhopper population with 71.7%, followed by E. vitis (21.9%); E. decipiens (4.8%) and E. solani (1.6%) (Fig. 2).

In Dão, the dominating species was E. vitis (87.5%) followed by E. solani (11.5%) and with the same percentage E. decipiens and J. lybica: 0.5% each (Fig. 3). In Douro and in Trás-os-Montes the dominating species was also E. vitis (74.6%), but in this case, it was followed by J. lybica (18.3%), E. variegatus (5%), E. solani (1.4%) and P. striatus (0.7%) (Fig. 3).

![Fig. 3. Leafhopper species registered in vineyards from two Portuguese regions, from 1997 to 1999](image)

Table 1. Average of leafhopper nymph number in three canopy levels from 1997 to 1999 in three vineyards and six varieties.

<table>
<thead>
<tr>
<th>Region</th>
<th>Variety</th>
<th>Year</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
<th>Region</th>
<th>Variety</th>
<th>Year</th>
<th>L1</th>
<th>L2</th>
<th>L3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alentejo</td>
<td>Manteúdo</td>
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<td>46.9a</td>
<td>36.4a</td>
<td>39.5a</td>
<td>Setúbal</td>
<td>F. Pires</td>
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<td>10.7c</td>
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<td></td>
<td>99</td>
<td>0.8a</td>
<td>0.7a</td>
<td>0.8a</td>
<td></td>
<td></td>
<td>99</td>
<td>1.2a</td>
<td>1.4ab</td>
<td>3.7b</td>
</tr>
<tr>
<td></td>
<td>Roupeiro</td>
<td>97</td>
<td>0.9a</td>
<td>1.9a</td>
<td>20.4b</td>
<td>Dão</td>
<td>Rz, T</td>
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<td>49.4a</td>
<td>78.0a</td>
<td>40.1b</td>
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</tr>
</tbody>
</table>

In each year numbers followed by the same letter are not statistically different for a p value <0.05 with Man-Whitney test. F. Pires – Fernão Pires; Rz – Tinta Roriz; T – Touriga Nacional.

**Nymphs number**

The canopy level effect on nymph number was registered, in Alentejo, in 1997, in two cultivars, and in 1998 in the three studied cultivars, with more nymphs in level three (L3) when compared with levels one (L1) and two (L2), in 1997, and with level one (L1), in 1998 (Table 1). In 1999 the same trend was registered in two varieties: Roupeiro and Rabo de ovelha (Table 1). In 1997, in Manteúdo, no difference was found among levels, not even the referred trend (Table 1) as a result of the lack of an adequate treatment in this variety. Because of this, the attack grew in such a way that after August there were no green leaves in the upper level (L3), which induced the attack in the first (L1) and medium (L2) level.
increase. In Setúbal, where \textit{J. lybica} also dominated, more nymphs were registered in level three (L3), mainly in 1997, comparing to level one (L1) and two (L2), and in 1999, comparing with level one (L1) (Table 1). Different results were observed in Sardinia, Italy, where a preference by \textit{J. lybica} for mid shoots leaves was registered (Delrio et al., 2001). 

In Dão, where \textit{E. vitis} dominated, considering both vineyard varieties, the nymph number results were different from the ones in the South: more nymphs in level two (L2), in the three years (Table 1). However, in Palatinate, Germany, a preference for the upper level was registered during the second generation of this species (Lehmann et al., 2001).

The leaf age effect on nymph number was registered in 1998, in Alentejo, in the three varieties, expressed by a preference for younger leaves (Table 2). The same trend was registered in 1997 in two varieties (Table 2). In Manteúdo, it did not happen, probably because of the same reason why the level three was not preferred: after August most of lateral leaves were brown. In Setúbal the same preference was noticed in 1998, in 1997 for a p value of 0.07, and the same trend in 1999 (Table 2). However, this preference for younger leaves was not registered in Sardinia (Delrio et al., 2001).

Table 2. Average of leafhopper nymph number in two leaf ages from 1997 to 1999 in two vineyards and four varieties.

<table>
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<th>Principal</th>
<th>Laterals</th>
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<td>0,8a</td>
</tr>
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<td></td>
<td>99</td>
<td>5,0a</td>
<td>4,2a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>3,6a</td>
<td>6,7a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>1,6a</td>
<td>2,6a</td>
<td></td>
</tr>
</tbody>
</table>

In each year numbers followed by the same letter are not statistically different for a p value <0.05 with Man-Whitney test. R.ovelha-Rabo de ovelha; F. Pires – Fernão Pires; (1) – different for a p value = 0.07

Concerning training system effect on nymph number a preference for training system Guyot (Table 3) was registered, which was not found in the 2001 analysis (Raposo et al., 2001). This happened because in 2003 the analysis considered only date observations with, at least, one nymph registered, while in the 2001 analysis, all date observations were considered.

Table 3. Average of leafhopper nymph number in two training systems from 1997 to 1998 in Dão region.

<table>
<thead>
<tr>
<th>Training system</th>
<th>1997</th>
<th>1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guyot</td>
<td>93,3a</td>
<td>25,6a</td>
</tr>
<tr>
<td>Royat</td>
<td>70,5b</td>
<td>16,5b</td>
</tr>
</tbody>
</table>

In each year numbers followed by the same letter are not statistically different for a p value <0.05 with Man-Whitney test.

Concerning vineyard varieties some differences were found: in the Algarve more nymphs were found in Itália and Moscatel comparing with Cardinal and D. Maria; in Alentejo, more
nymphs were found in Roupeiro and Rabo de ovelha, comparing with Manteúdo; in Setúbal a preference for Esgana Cão and Trincadeira was registered, comparing with Aragonez; and in Dão, more nymphs were registered in Tinta Roriz than in Touriga Nacional (Table 4). Similar results were found in the Algarve and Alentejo when the variety effect on adult number (Table 5) was analysed. In Setúbal more adults were registered in Fernão Pires than in Periquita (Table 5).

Table 4. Average of leafhopper nymph number in four varieties in the Algarve, three varieties in Alentejo and in Setúbal and two varieties in Dão, from 1997 to 1999.

<table>
<thead>
<tr>
<th>Region</th>
<th>Variety</th>
<th>1997</th>
<th>1998</th>
<th>1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algarve</td>
<td>Cardinal</td>
<td>6.2a</td>
<td>23.1a</td>
<td>6.8a</td>
</tr>
<tr>
<td></td>
<td>D, Maria</td>
<td>18.3a</td>
<td>23.0a</td>
<td>15.3ab</td>
</tr>
<tr>
<td></td>
<td>Itália</td>
<td>12.4a</td>
<td>47.8a</td>
<td>34.3b</td>
</tr>
<tr>
<td></td>
<td>Moscatel</td>
<td>13.5a</td>
<td>40.8a</td>
<td>35.1b</td>
</tr>
<tr>
<td>Alentejo</td>
<td>Rabo de ovelha</td>
<td>10.0a</td>
<td>2.8a</td>
<td>3.7b</td>
</tr>
<tr>
<td></td>
<td>Manteúdo</td>
<td>–</td>
<td>2.5a</td>
<td>0.8a</td>
</tr>
<tr>
<td></td>
<td>Roupeiro</td>
<td>3.1b</td>
<td>2.0a</td>
<td>4.6b</td>
</tr>
<tr>
<td>Setúbal</td>
<td>Aragonez</td>
<td>–</td>
<td>–</td>
<td>10.9a</td>
</tr>
<tr>
<td></td>
<td>Esgana cão</td>
<td>–</td>
<td>–</td>
<td>33.8b</td>
</tr>
<tr>
<td></td>
<td>Trincadeira</td>
<td>–</td>
<td>–</td>
<td>32.4b</td>
</tr>
<tr>
<td>Dão</td>
<td>Tinta Roriz</td>
<td>88.5a</td>
<td>31.4a</td>
<td>1.7a</td>
</tr>
<tr>
<td></td>
<td>Touriga Nacional</td>
<td>23.1b</td>
<td>8.1b</td>
<td>0.4b</td>
</tr>
</tbody>
</table>

In each year numbers followed by the same letter are not statistically different for a p value <0.05 with Man-Whitney test. R. ovelha: Rabo de ovelha; F. Pires: Fernão Pires; (1) different for a p value = 0.07

Table 5. Average of leafhopper adult number in four varieties in the Algarve, two in Setúbal, in 1997 and 1998, and seven in Alentejo from 1997 to 1999.

<table>
<thead>
<tr>
<th>Region</th>
<th>Variety</th>
<th>1997</th>
<th>1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algarve</td>
<td>Cardinal</td>
<td>41.6a</td>
<td>46.9a</td>
</tr>
<tr>
<td></td>
<td>D, Maria</td>
<td>86.0b</td>
<td>60.6b</td>
</tr>
<tr>
<td></td>
<td>Itália</td>
<td>188.0c</td>
<td>118.7c</td>
</tr>
<tr>
<td></td>
<td>Moscatel</td>
<td>201.7c</td>
<td>66.6b</td>
</tr>
<tr>
<td>Setúbal</td>
<td>Fernão Pires</td>
<td>164.2a</td>
<td>104.2a</td>
</tr>
<tr>
<td></td>
<td>Periquita</td>
<td>61.2b</td>
<td>69.0b</td>
</tr>
<tr>
<td>Alentejo</td>
<td>Rabo de ovelha</td>
<td>288.9e</td>
<td>193.4d</td>
</tr>
<tr>
<td></td>
<td>Manteúdo</td>
<td>269.3de</td>
<td>195.8cd</td>
</tr>
<tr>
<td></td>
<td>Roupeiro</td>
<td>197.5c</td>
<td>210.7d</td>
</tr>
<tr>
<td></td>
<td>Aragonez</td>
<td>204.3bcd</td>
<td>125.4c</td>
</tr>
<tr>
<td></td>
<td>Periquita</td>
<td>181.2abc</td>
<td>150.3ed</td>
</tr>
<tr>
<td></td>
<td>Trincadeira</td>
<td>136.0ab</td>
<td>97.9b</td>
</tr>
<tr>
<td></td>
<td>Moreto</td>
<td>102.1a</td>
<td>47.2a</td>
</tr>
</tbody>
</table>

In each year numbers followed by the same letter are not statistically different for a p value <0.05 with Man-Whitney test.

**Conclusion**

In Alentejo and Setúbal, where the dominant species was *J. lybica*, the highest nymph number was registered in level three (L3). In Dão, where the dominant species was *E. vitis*, level two (L2) was the preferred canopy level. So, according to Portuguese results and admitting that canopy level may be related with leafhopper species, in the Algarve, where *J. lybica*, dominated, the nymph number should be assessed in level three (L3), and in Trás-os-Montes, where *E. vitis* dominated, nymphs should be assessed in level two (L2). However, Italian and German results do not allow us to reach the same conclusions.
A preference for lateral leaves was registered in the South of Portugal, which was not observed in Italy, and a preference for the Guyot training system, in Dão, was also registered. The varieties preferred by the leafhopper were Itália and Moscatel, in the Algarve; Roupeiro and Rabo de ovelha, in Alentejo; Esgana Cão and Trincadeira, in Setúbal; and Tinta Roriz in Dão.

References


Etude des Cochenilles et des antagonistes qui leur sont associés dans des vignobles en Bourgogne et en Alsace de 2000 à 2002

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¹ 6 rue du 16ème Chasseurs, 21200 Beaune, France
² 28 rue de Herrlisheim, 68000 Colmar, France

De par leur niveau d’infestation et de dommages directs généralement faibles, les cochenilles peuvent être considérées comme des ravageurs secondaires dans les régions viticoles productrices ci dessus.

Néanmoins, des études récentes sur le rôle possible de certaines espèces de cochenilles en tant que vecteurs de virus de l’enroulement, sont susceptibles de remettre cette affirmation en cause; à cela on peut ajouter le risque d’une augmentation des niveaux d’infestation, conséquence entre autres, de l’usage réduit d’insecticides à large spectre en Protection Intégrée, au profit d’insecticides plus spécifiques.

Ainsi il nous a semblé intéressant d’avoir une idée plus précise des différents antagonistes rencontrés sur cochenilles en vigne, et de leur niveau d’efficacité, étude préalable à la mise en œuvre d’une lutte biologique au vignoble.

Nous avons étudié Parthenolecanium corni (Bouché) et Pulvinaria vitis (Linné) en Alsace et en Bourgogne pour les coccides, ainsi que Phenacoccus aceris (Signoret) en Alsace et Heliococcus bohemicus (Sulc) en Bourgogne pour les pseudococcides, toutes trouvées à des niveaux d’infestation variables dans les parcelles suivies durant ces trois années.

Tableau 1. Espèces de parasitoides collectées.

<table>
<thead>
<tr>
<th>Famille</th>
<th>Espèce</th>
<th>Cochenille hôte</th>
<th>Cochenille hôte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>espèce</td>
<td>stade concerné</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(par région et</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>chronologiquement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>dans la saison)</td>
</tr>
<tr>
<td>Encyrtidae</td>
<td>Metaphycus insidiosus (Mercet)</td>
<td>P. corni</td>
<td>L2, ♀, L2, ♀, L2</td>
</tr>
<tr>
<td></td>
<td>Metaphycus punctipes (Dalman)</td>
<td>P. corni</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td>Blastothrix longipennis (Howard)</td>
<td>P. corni</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td>Blastothrix sp.</td>
<td>P. corni</td>
<td>L2</td>
</tr>
<tr>
<td></td>
<td>Blastothrix britannica (Girault)</td>
<td>P. corni</td>
<td>L2, ♀</td>
</tr>
<tr>
<td></td>
<td>Anagyrus schoenherri (Westwood)</td>
<td>P. aceris</td>
<td>L2, ♀</td>
</tr>
<tr>
<td></td>
<td>Microterys chalcotomus (Dalman)</td>
<td>P. aceris</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td>Ericydus sipylus (Walker)</td>
<td>H. bohemicus</td>
<td>L2, ♀, L2</td>
</tr>
<tr>
<td></td>
<td>Anagyrus szödensis (Erdős)</td>
<td>H. bohemicus</td>
<td>L2, ♀</td>
</tr>
<tr>
<td></td>
<td>Leptomastidea bifasciata (Mayr)</td>
<td>H. bohemicus</td>
<td>L2, ♀, L2</td>
</tr>
<tr>
<td></td>
<td>Aphycus apicalis (Dalman)</td>
<td>H. bohemicus</td>
<td>L2 (hiver)</td>
</tr>
<tr>
<td></td>
<td>Prochiloneurus bolivari (Mercet)</td>
<td>H. bohemicus</td>
<td>♀, L2</td>
</tr>
<tr>
<td></td>
<td>Cheiloneurus claviger (Thomson)</td>
<td>P. corni</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td>Eusemion cornigerum (Walker)</td>
<td>P. corni</td>
<td>L2 (automne)</td>
</tr>
</tbody>
</table>


23 espèces de parasitoïdes primaires et d’hyperparasites (soulignés dans le tableau), principalement des encyrtidae, ont été collectées durant ces trois années, sur les différentes espèces et à divers stades de l’hôte comme le montre le tableau 1.

Pour *H. bohemicus*, *P. aceris* et *P. corni*, les observations réalisées ont permis de déterminer le taux de régulation pour chaque espèce et aussi de préciser le parasitoïde principal.

**H. bohemicus: taux de parasitisme et parasitoïdes rencontrés**

Ainsi pour *H. bohemicus*, au printemps, *Leptomastidea bifasciata*, *Anagyrus szodensis* et surtout *Ecerydnus sipylos* sont les parasitoïdes majeurs en 2001 (tableau 2) que ce soit sur larves (1 seul individu par hôte) ou femelles (1 seul individu par hôte). En été, sur femelles et larves L2, c’est *Ecerydnus sipylos* qui domine. On arrive ainsi, en fin d’été, à des parasitismes sur L2 allant de 4 à plus de 10% principalement dus à *E. sipylos*.

En 2002 au printemps (tableau 3), curieusement, ce n’est pas cette espèce qui domine sauf pour le site de Corgoloin, alors que *Anagyrus szodensis* représente une bonne part des émergences. Sur femelles en été, il y a beaucoup plus d’hyperparasites (*Pachyneuron muscarum*, *Prochiloneurus bolivari* et Megaspilidae) que en 2001. Par contre sur larves L2 en fin d’été, on retouve *E. sipylos* comme parasitoïde majeur.

*Ecerydnus sipylos* peut donc être considéré comme le parasitoïde majeur sur *H. bohemicus* en Bourgogne, avec cependant des variations selon les sites et les années.

Les taux globaux de régulation sont assez élevés pour *H. bohemicus*, allant de 5 à 60% pour larves et femelles sous écorces et femelles avant oviposition, de 17% à 70% pour les femelles jusqu’à l’oviposition, et enfin de 1% à 17% pour les L2 estivales.

Si on prend l’exemple de Corgoloin en 2001/2002, le taux de régulation globale dépasse les 90%: de 100 larves L2 estivales en 2001, seulement 88 vont arriver à passer l’hiver; de ces 88 larves seules 27 vont arriver à maturité sexuelle mi mai 2002; enfin, le taux de parasitisme sur femelles étant de 72% en 2002, seules de 7 à 8 femelles vont arriver au stade de l’oviposition sans être parasitées.

<table>
<thead>
<tr>
<th>Famille</th>
<th>Espèce</th>
<th>Hôte</th>
<th>Stade</th>
<th>Mode de vie</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphelinidae</td>
<td><em>Coccophagus semicircularis</em></td>
<td><em>P. corni</em></td>
<td>L2 (hiver)</td>
<td>B:A:B</td>
</tr>
<tr>
<td></td>
<td>(Förster)</td>
<td><em>P. vitis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Coccophagus lycomnia</em> (Walker)</td>
<td><em>P. corni</em></td>
<td>L2,♀;L2</td>
<td>A:B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(hiv.,automne)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Encarsia lutea</em> (Masi)</td>
<td><em>P. corni</em></td>
<td>L2 (automne)</td>
<td>B</td>
</tr>
<tr>
<td>Megaspilidae</td>
<td><em>Conostigmus fasciatipennis</em></td>
<td><em>H. bohemicus</em></td>
<td>♀</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>(Kieffer)</td>
<td></td>
<td>L2,♀</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Conostigmus sp.</em> (Dahlbom)</td>
<td><em>H. bohemicus</em></td>
<td>L2,♀,♀,♀,♀,L2</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td><em>Dendrocerus sp.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platigastridae</td>
<td><em>Allotropa mecrata</em></td>
<td><em>H. bohemicus</em></td>
<td>L2</td>
<td>B</td>
</tr>
<tr>
<td>Pteromalidae</td>
<td><em>Pachyneuron muscarum</em> (Linnaeus)</td>
<td><em>P. corni</em></td>
<td>♀</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>H. bohemicus</em></td>
<td>♀</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P. aceris</em></td>
<td>♀</td>
<td>A</td>
</tr>
<tr>
<td>Signiphoridae</td>
<td><em>Chartocerus subaenus</em> (Förster)</td>
<td><em>H. bohemicus</em></td>
<td>♀</td>
<td>B</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Nantoux</th>
<th>Corgoloin</th>
<th>Pernand – V.</th>
<th>Echevronne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larves et femelles sous écorces (début mai)</td>
<td>34,5% <em>L. bifasciata</em> (37%) <em>A. szodensis</em>(37%) <em>A. apicalis</em> Conostigmus sp.</td>
<td>5% <em>L. bifasciata</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>13,2% E. sipylus (85%) <em>A. szodensis</em></td>
<td>9% <em>E. sipylus</em> (50%) <em>A. szodensis</em> <em>L. bifasciata</em></td>
<td>15% <em>E. sipylus</em> (75%) <em>A. szodensis</em></td>
<td>14,4% <em>E. sipylus</em> (75%) <em>A. szodensis</em> <em>P. bolivari</em></td>
</tr>
<tr>
<td>Larves et femelles sur rameaux (mi mai)</td>
<td>34,5% <em>L. bifasciata</em> (37%) <em>A. szodensis</em></td>
<td>13,2% E. sipylus (85%) <em>A. szodensis</em></td>
<td>35,9% <em>E. sipylus</em> (94%) <em>P. bolivari</em> <em>C. subaenus</em> <em>P. muscarum</em> Conostigmus sp.</td>
<td>40,7% <em>E. sipylus</em> (88%) Conostigmus sp. <em>C. fasciatipennis</em> <em>A. szodensis</em></td>
</tr>
<tr>
<td>Femelles sous écorce (fin juin)</td>
<td>38,2% E. sipylus (87%) <em>A. szodensis</em> <em>P. bolivari</em></td>
<td>52,8% E. sipylus (85%) <em>P. bolivari</em> <em>C. subaenus</em> <em>P. muscarum</em> Conostigmus sp.</td>
<td>35,9% <em>E. sipylus</em> (94%) <em>P. bolivari</em> <em>C. subaenus</em> <em>P. muscarum</em> Conostigmus sp.</td>
<td>40,7% <em>E. sipylus</em> (88%) Conostigmus sp. <em>C. fasciatipennis</em> <em>A. szodensis</em></td>
</tr>
<tr>
<td>L2 sur feuilles (fin aout)</td>
<td>3,8% E. sipylus</td>
<td>12,6% E. sipylus (81%) <em>P. bolivari</em> <em>A. mecrida</em> <em>L. bifasciata</em></td>
<td>14,8% E. sipylus</td>
<td>13,6% E. sipylus</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th></th>
<th>Nantoux</th>
<th>Corgoloin</th>
<th>Pernand</th>
<th>Echevronne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larve et femelle sous écorce</td>
<td>39% E. sipylus 5 % <em>A. szodensis</em> 83% <em>L. bifasciata</em> 10 % Megastilidae 2 %</td>
<td>59.3% E. sipylus 85 % <em>A. szodensis</em> 11% <em>L. bifasciata</em> 3 % Megastilidae 1 %</td>
<td>28.8% E. sipylus 6.5 % <em>A. szodensis</em> 84% <em>L. bifasciata</em> 6.5 % Megastilidae 3 %</td>
<td>35.5% E. sipylus 32 % <em>A. szodensis</em> 43% <em>L. bifasciata</em> 25 %</td>
</tr>
<tr>
<td>Larve et femelle sur rameau</td>
<td>31.9% E. sipylus 16 % <em>A. szodensis</em> 84%</td>
<td>24.6 % E. sipylus 93 % <em>A. szodensis</em> 7 %</td>
<td>23.3 % E. sipylus 11 % <em>A. szodensis</em> 89 %</td>
<td>37 % E. sipylus 69 % <em>A. szodensis</em> 31%</td>
</tr>
<tr>
<td>Femelle sous écorce</td>
<td>49.1 % E. sipylus 84 % Megastilidae 16%</td>
<td>72 % E. sipylus 11 % Megastilidae 52% <em>P. bolivari</em> 32 % <em>P. muscarum</em> 5 %</td>
<td>43 % E. sipylus 59 % Megastilidae 18% <em>P. bolivari</em> 20 % <em>Chartocerus subaenus</em> 3 %</td>
<td>59.5 % E. sipylus 60 % Megastilidae 38% <em>P. bolivari</em> 2 %</td>
</tr>
<tr>
<td>Larve sur feuille (Août)</td>
<td>0.8% E. sipylus 100 %</td>
<td>8.5 % E. sipylus 88 % <em>A. szodensis</em> 12%</td>
<td>14.8 % E. sipylus 50 % <em>A. szodensis</em> 50%</td>
<td>16.9 % E. sipylus 90 % <em>A. szodensis</em> 5% Megastilidae 5 %</td>
</tr>
</tbody>
</table>

*P. aceris: taux de parasitisme et parasitoïdes rencontrés*  
Pour *P. aceris* le parasitoïde majeur est *Anagyrus schoenherri* (tableau 4). Les taux de parasitisme sont de 10% environ sur L2 hivernantes (1 seul individu par hôte) à environ 50% pour les femelles (de 4 à 4.5 individus par hôte, avec des maxima de 10). Les taux de parasitisme des femelles sous les écorces sont beaucoup plus faibles que ceux des femelles se trouvant sur les rameaux ou sous les feuilles.
Tableau 4. Taux de parasitisme et taux d’émergence par espèce pour *P. aceris*.

<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 sous écorces</td>
<td>9.9% (A. schoenherri)</td>
<td>13% (A. schoenherri)</td>
</tr>
<tr>
<td>♀ sous écorces et sur bois 2ans</td>
<td>{ } 61%</td>
<td>28%</td>
</tr>
<tr>
<td>♀ sur bois 1 an et rameaux</td>
<td>{ } 58%</td>
<td>64%</td>
</tr>
<tr>
<td>♀ sous feuilles</td>
<td>59%</td>
<td>71%</td>
</tr>
<tr>
<td>♀ global</td>
<td><strong>♀</strong> A. schoenherri 98% M. chalcostomus</td>
<td>43%</td>
</tr>
</tbody>
</table>

Tableau 5. Taux de parasitisme et taux d’émergence par espèce pour *P. corni* en Bourgogne.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Vosne - Romanée</th>
<th>Magny l. V.</th>
<th>Pommard</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 spring</td>
<td>28% C. lycimnia</td>
<td>28% C. lycimnia</td>
<td>43% B. britannica</td>
</tr>
<tr>
<td></td>
<td>64% M. insidiosus</td>
<td>23% M. insidiosus</td>
<td>37% B. sp.</td>
</tr>
<tr>
<td></td>
<td>28% Blastothrix sp.</td>
<td>13% M. insidiosus</td>
<td>37% ♀</td>
</tr>
<tr>
<td></td>
<td>♀♂ Blastothrix sp.</td>
<td>43% Blastothrix sp.</td>
<td>♀</td>
</tr>
<tr>
<td>♀</td>
<td>2% M. insidiosus</td>
<td>0% n.e.</td>
<td>86% Blastothrix sp.</td>
</tr>
<tr>
<td></td>
<td>25% M. insidiosus</td>
<td>♀</td>
<td>4% M. insidiosus</td>
</tr>
<tr>
<td></td>
<td>71% B. longipennis</td>
<td>3% B. sp.</td>
<td>2% M. insidiosus</td>
</tr>
<tr>
<td></td>
<td>♀♂ 24% Coccophagus sp.</td>
<td>24%</td>
<td>♀♂ 3% B. sp.</td>
</tr>
<tr>
<td>L2 summer autumn</td>
<td>15% M. insidiosus</td>
<td>2% M. insidiosus</td>
<td>4.3% M. insidiosus</td>
</tr>
<tr>
<td></td>
<td>78% C. lycimnia</td>
<td>2% C. lycimnia</td>
<td>n.e.</td>
</tr>
<tr>
<td></td>
<td>13% Encarsia lutea</td>
<td>n.e.</td>
<td>♀</td>
</tr>
<tr>
<td></td>
<td>E. cornigerum</td>
<td>n.e.</td>
<td>♀</td>
</tr>
</tbody>
</table>

**P. corni: taux de parasitisme et parasitoïdes rencontrés**

En Bourgogne (tableau 5), sur L2 au printemps, *Metaphycus insidiosus, Coccophagus lycimnia* et *Blastothrix* spp. sont les parasitoïdes majeurs selon le site et l’année. Sur femelles, il s’agit de *Metaphycus insidiosus* et *Blastothrix* spp. selon le site. Enfin on retrouve *Coccophagus lycimnia* associé à *Metaphycus insidiosus* comme parasitoïdes majeurs sur L2 en automne selon le site et l’année.
Sur larves il n’y a qu’un seul parasitoïde par hôte, alors que sur femelles on compte plusieurs émergences par hôte, en moyenne 2 pour l’Alsace, où le rôle de parasitoïdes majeurs est tenu par différentes espèces de Blastothrix (*Blastothrix* sp., *B. britannica* et *B. longipennis*), aussi bien sur larves au printemps que sur femelles en été (voir tableau 6). Le parasitisme sur larves L2 automnales a été constaté en 2002, mais n’a pu être évalué en raison de son faible niveau d’expression. En Alsace les taux de parasitisme étaient de 45% sur larves en 2001 et de 70% à 80% sur femelles pour les deux années. En 2002 des hyperparasites ont été collectés sur femelles.


<table>
<thead>
<tr>
<th></th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>L2 printemps</td>
<td>n.e.</td>
<td>45%</td>
</tr>
<tr>
<td></td>
<td><em>B. sp.</em>♀ 28.8%</td>
<td><em>B. sp.</em>♀ 39.4%</td>
</tr>
<tr>
<td></td>
<td><em>B. britannica</em>♀♂ 6%</td>
<td><em>B. britannica</em>♀♂ 11.8%</td>
</tr>
<tr>
<td></td>
<td><em>Blastothrix</em>♀♂ 61.2%</td>
<td><em>Blastothrix</em>♀♂ 48.1%</td>
</tr>
<tr>
<td></td>
<td><em>M. insidiosus</em>♀♂ 4%</td>
<td><em>C. lycimnia</em>♀♂ 0.7%</td>
</tr>
<tr>
<td>♀</td>
<td>68%</td>
<td>77% (78.8% au champ)</td>
</tr>
<tr>
<td></td>
<td><em>B. longipennis</em>♀♂ 43.3%</td>
<td><em>B. longipennis</em>♀♂ 31%</td>
</tr>
<tr>
<td></td>
<td><em>B. britannica</em>♀♂ 18%</td>
<td><em>B. britannica</em>♀♂ 20%</td>
</tr>
<tr>
<td></td>
<td><em>Blastothrix</em>♀♂ 38.4%</td>
<td><em>Blastothrix</em>♀♂ 41.5%</td>
</tr>
<tr>
<td></td>
<td><em>C. lycimnia</em>♀♂ 0.3%</td>
<td><em>Coccophagus</em>♀♂ 0.3%</td>
</tr>
<tr>
<td></td>
<td>45%</td>
<td>77% (78.8% au champ)</td>
</tr>
<tr>
<td></td>
<td><em>B. sp.</em>♀ 39.4%</td>
<td><em>B. longipennis</em>♀♂ 31%</td>
</tr>
<tr>
<td></td>
<td><em>B. britannica</em>♀♂ 11.8%</td>
<td><em>B. britannica</em>♀♂ 20%</td>
</tr>
<tr>
<td></td>
<td><em>Blastothrix</em>♀♂ 48.1%</td>
<td><em>Blastothrix</em>♀♂ 41.5%</td>
</tr>
<tr>
<td></td>
<td><em>C. lycimnia</em>♀♂ 0.7%</td>
<td><em>Coccophagus</em>♀♂ 0.3%</td>
</tr>
<tr>
<td></td>
<td>45%</td>
<td>77% (78.8% au champ)</td>
</tr>
<tr>
<td></td>
<td><em>B. sp.</em>♀ 39.4%</td>
<td><em>B. longipennis</em>♀♂ 31%</td>
</tr>
<tr>
<td></td>
<td><em>B. britannica</em>♀♂ 11.8%</td>
<td><em>B. britannica</em>♀♂ 20%</td>
</tr>
<tr>
<td></td>
<td><em>Blastothrix</em>♀♂ 48.1%</td>
<td><em>Blastothrix</em>♀♂ 41.5%</td>
</tr>
<tr>
<td></td>
<td><em>C. lycimnia</em>♀♂ 0.7%</td>
<td><em>Coccophagus</em>♀♂ 0.3%</td>
</tr>
</tbody>
</table>

*Cochenilles et prédateurs généralistes*

Des prédateurs généralistes comme les chrysopes *Chrysoperla lucasina* Lacroix et *Chrysoperla kolthoffi* Navâs et comme les coccinelles *Scymnus frontalis* (Fabricius) et *Nephus bisignatus* ont montré leur efficacité sur *H. bohemicus* en conditions de non choix (tableau 7).

Tableau 7.

<table>
<thead>
<tr>
<th>proie</th>
<th><em>Chrysoperla</em> spp.</th>
<th><em>Chrysoperla lucasina</em></th>
<th><em>Chrysoperla kolthoffi</em></th>
<th><em>Scymnus frontalis</em></th>
<th><em>Nephus bisignatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. corni</em> larves</td>
<td>de 25 à 45 larves</td>
<td>jeunes larves</td>
<td>larves agées</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>consommées en 2 semaines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. bohemicus</em> larves</td>
<td>30 larves</td>
<td>224 larves</td>
<td>224 larves</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>consommées en 2 semaines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. bohemicus</em> femelles</td>
<td></td>
<td></td>
<td></td>
<td>observation sans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>évaluation de</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>l’efficacité</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

Cette étude laisse espérer des possibilités réellement intéressantes de lutte biologique contre les cochenilles, en particulier par le biais de parasitoïdes, même si les prédateurs ont montré une efficacité complémentaire sur des stades larvaires non touchés par les parasitoïdes. Encore faudrait-il étoffer nos connaissances dans le domaine des relations entre parasitoïdes et hyperparasitoïdes, et surtout mieux connaître leur biologie et cycles annuels, afin de favoriser leur présence dans les vignobles par le biais de mesures de conservation.
The scale *Parthenolecanium persicae* (Fabricius) on grapes in Greece

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E-mail: georgestathas@hotmail.com

**Abstract**: The phenology of the scale insect *Parthenolecanium persicae* (Fabricius) (Hemiptera: Coccidae) was studied in vineyards in Southern Greece, during the years 2001-2002. The experimental procedure comprised samplings from infested vines, field assessments and examination of yellow sticky traps. *Parthenolecanium persicae* was found to be univoltine in Greece. It overwinters as a 2nd instar nymph. Adults begin to occur in April, while oviposition takes place from early May till late June. Crawlers hatched in May and during the rest of the summer period the scale is present as 1st and 2nd instar nymphs. From late September till the following spring the whole population of *P. persicae* consisted of 2nd instar nymphs. Two *Metaphycus* (Hymenoptera: Encyrtidae) species were found to parasitise the scale and the parasitism level reached up to 52%. The predator *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae) was also recorded to act as a natural enemy of the scale.

**Key words**: *Metaphycus*, grapes, Greece, *Parthenolecanium persicae*, Peloponnese, phenology.

**Introduction**

The scale insect *Parthenolecanium persicae* (Fabricius) (Hemiptera: Coccidae) has been reported in the past with 43 synonyms that were ascribed to genus *Coccus*, *Chermes*, *Eulecanium*, *Lecanium*, *Palaeolecanium* and *Parthenolecanium*. It is a polyphagous species and has been recorded infesting 74 plant taxa belonging to 30 families (Ben-Dov et al., 1991; Ben-Dov, 1993; Ben-Dov et al., 1997; Gill, 1988; Kosztarab & Kozár, 1988; Stathas, 2003). It is a worldwide spread species. Kozár (1985) reported it for the first time in Greece on *Morus* sp in Epanomi (July 1983). It has also been found in adjacent countries such as Italy, Yugoslavia and Bulgaria (Kosztarab & Kozár, 1988; Kozár, 1983; Marotta, 1987). *Parthenolecanium persicae* was recorded on *Viburnum tinus* in Central Greece (Athens) in June 2000, as well as on *Vitis vinifera* in Southern Greece (Co. Messinia) in October of the same year (Stathas, 2003). Although infestation level wasn’t high on the plants where the scale was found, *P. persicae* has been reported as a serious pest of vines and plums in other countries (Bartlett, 1978). Furthermore, *P. persicae* hadn’t been studied in Greece, which prompted the study of its phenology in vines in Southern Greece.

**Materials and methods**

The fieldwork was conducted in Arfara, Messinia, Peloponnese, Southern Greece (February 2001 - December 2002) in a vineyard containing the variety Rodites. Monitoring of population of *P. persicae* was achieved by sampling of vine-shoots, from 20 infested vine stocks. Twenty-five vine-shoots of 20 - 40 cm were cut from each vine-stock. Each vine-shoot was examined in the laboratory, under stereoscope, and the number and developmental stages of live individuals of *P. persicae* were recorded, as well as those that were dead and parasitised. Samples were taken every 15 days during the warm period of the year (April -
September) and monthly during the rest period (October - March). Monitoring of parasitoid flights was made by the examination of 4 yellow sticky traps (sized 20 x 20 cm²), which were replaced on sampling dates. Crawlers were monitored by examining 12 double-sided sticky traps that were placed on the root of 12 vine-shoots. Those traps were installed during April-September, replaced on each sampling date and examined at the laboratory in order to count crawler individuals and estimate crawlers’ density per surface unit.

Fig. 1. Developmental stages of *Parthenolecanium persicae* on grapes and monthly average temperatures from February 2001 to December 2002, in Southern Greece.
Results and discussion

*Parthenolecanium persicae* completes one generation per year in Southern Greece (Fig. 1). Adults emerge by mid April. Oviposition takes place from early May till late June. Crawlers appeared by the middle of May until the end of June (Fig. 1, 2A). Nymphs of 1st and 2nd instar were observed from middle of June. From late September till the following spring the whole population of *P. persicae* consisted of 2nd instar nymphs.

![Graph A: Tape traps](image)

![Graph B: Parasitoid records](image)

Fig. 2. A: Number of crawlers recorded on tape traps.
B: Number of adult parasitoids (Metaphycus) recorded on yellow traps and percentage (%) of parasitized adults of *Parthenolecanium persicae*.

Adult females of *P. persicae* were parasitised by two parasitoid species of the genus *Metaphycus* and the level of parasitism reached up to 34.5 % (Fig. 2B). All parasitised individuals and individuals bearing an exit hole were found to be adults. During the examination of the samples at the laboratory, small numbers of individuals of *Chilocorus bipustulatus* L. (Coleoptera: Coccinellidae) were found (9 larvae and 12 adults during the 1st year and 12 larvae and 5 adults during the 2nd year of the present study). The percentage of 1st
and 2nd instar nymphs attacked by predators reached the percentage of 2.1% during the first year and 1.4% of the total population of the scale during the 2nd year of the study.

As far as the phenology of *P. persicae* is concerned, it is cited in literature that it completes one generation per year in many other geographical regions, such as Central Europe (Hoffman & Schmutterer, 1999; Kosztarab & Kozár, 1988), Chile (Gonzalez, 1989) and Israel (Ben-Dov et al., 1991). However, Borchsenius (1957) reported two generations per year in Central Asia. During the present study, examination of samples and traps didn’t reveal the presence of male adults of the scale in Southern Greece. The fact that the scale reproduces parthenogenetically is also mentioned in other reports in Chile (Gonzalez, 1989) and in Israel (Ben-Dov et al., 1991), although males are known (Williams & Kosztarab, 1972).

Several natural enemies of *P. persicae* have been reported, namely the hymenopteran parasitoids *Aphycus timberlakei*, *Blastothrix hungarica*, *Cheiloneurus formosus*, *Cocchophagus lycimnia*, *C. pulchellus*, *C. scutellaris*, *Enchyrtus swederi*, *Eunotus cretaceus*, *E. obscurus*, *Homalotylus quaylei*, *Metaphycus dispar*, *M. parvus*, *M. punctipes*, *M. maculipennis*, *Microtorys sylvius*, *M. temporarvis*, *M. xanthopsis*, *Muscidopsis lecanii*, *Phaenodiscus aeneus*, *Trichomastus albimans* (Hoffman & Schmutterer, 1999; Trijapizin, 1978) and the predatory species *Chilocorus bipustulatus* (Coleoptera), *Leucopis alticeps* (Diptera) (Babaev, 1970) and *Holococera iceryaella* (Lepidoptera) (Borchsenius, 1957). The two different *Metaphycus* species found during the present study could not be identified beyond genus level in order to ascertain if they belonged in any of the aforementioned *Metaphycus* species. By the relatively high level of parasitism (43.2%), it can be concluded that they contribute notably to the constraint of vine infestation by *P. persicae* in Southern Greece. As shown in Fig. 2B, greater numbers of parasitoids were captured during May-July. This can be explained by the fact that parasitoids emerge by adult scales, that are present during that period. A similar trend in population increase has been reported for *Blastothrix hungarica*, another parasitoid of *P. persicae* by Hoffman & Schmutterer (1999). This species overwinters at the egg stage, inside 2nd instar larvae of *P. persicae* and emerges from adult scales in spring, increasing in numbers until June.

The predatory coccinellid *Ch. bipustulatus* wasn’t found in great numbers during the present study and predated individuals of *P. persicae* consisted a low percentage of its population (1.4 - 2.1%). Although population decreases of *Ch. bipustulatus* larvae due to their parasitism by the hymenopterous parasitoids *Homalotylus flaminius* (Encyrtidae) and *Tetrastichus coccinellae* (Eulophidae) have been recorded in Greece (Stathas, 2001), this predator is regarded as one of the most important natural enemies of scale insects nationwide. The fact that it was found in small numbers during the present study should be attributed to the low infestation level of the vines by the scale.

**References**


Marotta, S. 1987: Coccids (Homoptera: Coccoidea: Coccidae) found in Italy, with bibliographic references on taxonomy, geographic range, biology and host plants. (In Italian; Summary in English). – Bollettino del Laboratorio di Entomologia Agraria Filippo Silvestri 44: 97-119.


A novel scarring symptom on seedless grapes in the Corinth region (Peloponnese, southern Greece) caused by the western flower thrips, *Frankliniella occidentalis*, and pest control tests

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² Institute of Plant Protection, NAGREF, 711 10, Heracleion Crete, Greece
³ ZENECA HELLAS, Athens, Greece
⁴ Plant Protection Institute, Budapest, Hungary

**Abstract:** A study was carried out in vineyards of seedless grape varieties in the Corinth region, southern Greece in order to investigate the nature of a novel scarring symptom on the grape berries, initially attributed to phytotoxicity. Examination of the grape epidermis under the dissecting microscope showed that the symptom was caused by the feeding activity of piercing-sucking insects. During the years 1997-1998 experiments, in both field and laboratory, revealed that *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) was the causing agent of the symptom. In 1997 a network of color sticky traps was set up to monitor thrips species and surveys were made for the presence of thrips on weeds and grapes. The western flower thrips, *F. occidentalis*, was the prevailing thrips species. In 1998 thrips population monitoring was done and artificial infestation of grapes with the thrips, deriving from a lab culture, was also performed in both field and in the lab. In 1999, the efficacy of several insecticides against thrips was evaluated in an experimental vineyard in Corinth. The thrips population was monitored by blue sticky traps and final infestation of the grapes was evaluated at harvest. Totally, 280 vines were marked and 32 traps were set up. The results revealed differences among the treatments. The number of captured thrips per trap was higher in the untreated control and in plots treated with methamidophos and spinosad than in plots treated with chlorfenapyr, mass trapping and mass trapping + *Beauveria bassiana*. Regarding the grapes protection, measured by thrips presence on grapes, methamidophos, methiocarb (liquid concentrates) and chlorfenapyr proved the most efficient.

**Key words:** scarring symptom, seedless grapes, *Frankliniella occidentalis*, thrips control.

**Introduction**

Viticulture is a dynamic sector of Greek economy. Particularly, the production of seedless grapes in the Corinth region (Peloponnese, southern Greece) is considered of major importance. The seedless grape variety “Soultanina” is cultivated in the latter region for decades.

In 1996 a problem arose in the local vineyards with a newly appeared scarring symptom on the grape berries. In Figure 1 two symptoms caused by phytotoxicity and the ‘new’ symptom are shown. The symptom looked like the one caused by phytotoxicity from pesticides. Extensive scarring was observed and in areas, corresponding to the surfaces of contact between berries, ring like formations appeared. The first examination revealed that the symptom appeared in the upper areas of the grape and the berries. Conversely, phytotoxicity symptoms appear in the lower part of the grape and the berries where the spraying liquid
concentrates due to gravity where condensation of the pesticide occurs. Additionally, the nature of the symptom differs. Phytotoxicity ‘burns’ epidermis, while the symptom under consideration showed an appearance of damaged cells from the feeding activity of piercing-sucking insects (Tsitsipis et al., 1997). Examination under a dissecting scope made the difference more clear. The western flower thrips was known to infest the grape, early in the development of the berries, and to cause a different symptom: a central damaged area surrounded by an halo. The symptom observed in our case was different. The infested area was extended and it appeared much later just before the berries matured. It was deemed necessary, therefore to investigate what the causal agent of the symptom was, and if it were an insect to try and test materials for its control.

Fig. 1. The novel scarring symptom (a, b) and phytotoxicity symptom (c) observed on grape berries (Corinth, Greece, 1996).

Materials and methods

During the years 1997-1998 experiments were carried out, in vineyards in the region of Corinth, southern Greece and in the laboratory, to detect the causing agent of the symptom. The western flower thrips, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), was suspected to cause the symptom.

During 1997-98 a network of blue sticky traps was set up in six vineyards to monitor thrips species and surveys were made for the presence of thrips on weeds and grapes. Of paramount importance was considered the need for the reproduction of symptoms under controlled conditions. In 1998 grapes in the field were artificially infected with individuals of *F. occidentalis* derived from a laboratory culture and enclosed in plastic bags with openings covered with a fine cloth not allowing the entrance of thrips. In the laboratory, grape berries were exposed to thrips for the reproduction of symptoms.

In 1999, the efficacy of different insecticides was evaluated in a field experiment. The experimental design was randomised complete block with four replications. The various treatments are shown in Table 1. For mass trapping four blue sticky traps in four replications were set up in the lower parts of the vines near to the soil.

Results and discussion

*Thrips population monitoring and scarring symptom reproduction*

The thrips population monitoring during the years 1997-98 and surveys on weeds revealed that the prevailing thrips species was the western flower thrips *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae). In the vicinity of vineyards 12 weed taxa were found to harbour *F. occidentalis*. The highest number of thrips was observed on *Amaranthus* sp. (Figure 2). In both field and laboratory artificial infestations the scarring symptom was reproduced showing that the responsible agent was the western flower thrips (Table 2).
Table 1. Insecticides used against thrips in an experimental vineyard in southern Greece during 1999.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>methiocarb WP</td>
<td>200 gr /100 lit</td>
</tr>
<tr>
<td>methiocarb</td>
<td>200 gr /100 lit</td>
</tr>
<tr>
<td>spinosad</td>
<td>25 gr /100 lit</td>
</tr>
<tr>
<td>chlorfenapyr</td>
<td>50 gr /100 lit</td>
</tr>
<tr>
<td>methamidophos</td>
<td>120 gr /100 lit</td>
</tr>
<tr>
<td>Beauveria bassiana</td>
<td>250 gr /100 lit</td>
</tr>
<tr>
<td>Beauveria bassiana + mass trapping</td>
<td>250 gr /100 lit + 16 traps</td>
</tr>
<tr>
<td>Mass trapping</td>
<td>16 traps</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

Insecticides evaluation

The results regarding the evaluation of insecticides for thrips control revealed differences among the treatments. The numbers of captured thrips per trap was higher in the untreated control and in plots treated with methamidophos and spinosad than in plots treated with chlorfenapyr, mass trapping and mass trapping + Beauveria bassiana (Figure 3a). Regarding the grapes protection as measured by the thrips presence on grapes, methamidophos, methiocarb (liquid concentrates) and chlorfenapyr proved the most efficient treatments (Figure 3b).

Table 2. Results of thrips artificial infestation in laboratory and field.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Grapes treated</th>
<th>Infestation level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Examined berries</td>
</tr>
<tr>
<td></td>
<td></td>
<td>no.</td>
</tr>
<tr>
<td>Field</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial infestation</td>
<td>16</td>
<td>2634</td>
</tr>
<tr>
<td>Untreated</td>
<td>8</td>
<td>1680</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial infestation</td>
<td>2</td>
<td>102</td>
</tr>
<tr>
<td>Untreated</td>
<td>2</td>
<td>110</td>
</tr>
</tbody>
</table>

Conclusively, during the first two years of experimentation F. occidentalis was identified as the causing agent of the scarring symptoms (Tsitsipis et al., 1997). The symptoms were successfully reproduced in the field and in the laboratory by artificial infestations. Insecticide applications led to the reduction of scarring symptoms and new products (e.g. chlorfenapyr) provided satisfactory protection. The grapes quality was improved and consequently the farmer’s income was increased. Furthermore, weed management, irrigation regulation to avoid high relative humidity, which improves conditions for thrips population increase, better aeration of the plant by removing leaves, which also reduce relative humidity, greatly contribute to the reduction of thrips infestation of the grapes.

In general, a sampling schedule, population monitoring, cultural methods and chemical control using the above mentioned insecticides should be considered as an adequate control
strategy against *F. occidentalis* and the reduction of the novel scarring symptom produced by its feeding activity.

![Figure 2](image_url)


![Figure 3](image_url)

**Fig. 3.** Mean number of thrips captured per sticky trap (a) and percent grapes infested by thrips (b) in the experimental vineyard in Corinth during 1999. A: methamidophos, B: methiocarb (WP), C: methiocarb (liq), D: spinosad, E: chlorfenapyr, F: *Beauveria bassiana*, G: mass trapping, H: *B. bassiana* + mass trapping, and I: untreated control.
References


The use of *Phacelia tanacetifolia* (Muntz, 1973) (Solanales: Hydrophyllaceae) to control *Frankliniella occidentalis* (Pergande) on table grapes

T. Moleas  
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**Abstract:** One of the cultivation techniques used in Integrated Pest Management is the association of plants. An interesting association is represented by the simultaneous flowering of plants of different species, which both attract a specific phytophagus. In this way, the ensuing economic damage caused by the insect can be reduced in one of the crops, as the pest prefers the other, less valuable plant.

This work reports on a research carried out for several years to test the effect of the association of vine with *Phacelia tanacetifolia*, on *Frankliniella occidentalis*, which is currently one of the most dangerous pests of table grapes in Mediterranean areas. *P. tanacetifolia* is a herbaceous plant with blue flowers (a colour notoriously preferred by the thrip), which blooms from mid-May until about the 20th of June.

This is the same period in which vine flowers. In Southern Italy, this period lasts for one or two weeks, on average, during the last ten days of May.

Trials were run during the period 2000-2002, on an experimental vineyard belonging to the University of Bari in an area close to the town of Valenzano (Bari, Italy). In mid-March *P. tanacetifolia* was sown between the rows of vines, grown under an awning. During the trial period, the experimental field was not treated with any insecticide. Trials in the first year (2000) concerned:

1. the number of adult *Frankliniella*/cluster at the time of maximum flowering of vine in the plots associated with *Phacelia* and *Frankliniella*/cluster in the plots not associated with *Phacelia*;
2. the number of "halo spots"/cluster after fruit formation in the associated and non-associated plots;
3. the number of damaged grapes/cluster in the two types of plot.

In the following years (2001 and 2002) only parameters 1 and 3 were monitored with a view to test the actual damage caused by the insect (depreciation of grape clusters due to "halo spot" and damaged grapes/cluster).

The following results were observed over the three years: in the 1st year 0.32 adult *Frankliniella*/cluster were observed in the plots associated with *Phacelia* and 2.18 *Frankliniella* in the non-associated plots. As to the damage, there were: a) 9.76 "halo spots"/cluster in the associated plots, and 13.12 in the control; b) 4.97 damaged grapes/cluster in the associated plots and 6.84 in the control.

In the 2nd year the following was found: a) 2.3 "halo spots"/cluster in the associated plots, and 7.1 in the control; b) 1.4 damaged grapes/cluster in the associated plots, and 3.7 in the control.

In the 3rd year a) 3.57 "halo spots"/cluster were observed in the associated plots, and 9.21 in the control, while there were 2.74 damaged grapes/cluster in the experimental plots, and 5.61 in the control.

*Phacelia* contributes to substantially reduce the insect population and the subsequent damage, especially in the years when there is low or medium presence of *Frankliniella*, thus giving the possibility of avoiding chemical treatments.

**Introduction**

In Apulian (Southern Italy) vineyards, plant protection from pests is mostly aimed to prevent problems primarily due to the berry moth species (*Lobesia botrana* (Den and Schiff), a key insect of table grapevine), and to Western Flower Thrips (WFT) (*Frankliniella occidentalis*). Observations on WFT (Moleas T. & Addante, 1992) showed that WFT over-wintered on wild
and cultivated blooming plants, growing around or in vineyard. In May, the population increased on blooming citrus, olive plants and greenhouse crops, and then migrated into neighbouring vineyards (Moleas & Addante, 2000). On grapevine WFT female appeared firstly soon after sprouting until the flowers were ready to bloom, usually around 10-25 May in Apulia and peaking at fruit setting. WFT damage on grapevines mainly consists in "halo spotting" that can make the fruit of some white varieties unsightly and unmarketable. Halo spots are the result of ovipositing. Halo spotting is lighter with white skin table varieties like "Italia", "Matilda" and "Victoria". The critical time of halo spotting on table grapevine occurs from blooming up to fruit setting.

*Phacelia tanacetifolia* (Order Solanales; Family Hydrophyllaceae) is an annual plant native to California. This plant blooms at the same time as vine, and its blue flowers (Moleas & Sportelli, 2000) are highly attractive to WFT. It is important to remark that when "Italia" cultivar has reached 5% blooming, Phacelia is approximately at 75% bloom and its flowering persists just 15 days after vine.

The aim of this study is to test whether *P. tanacetifolia*, as trap crop intercropped in vine rows, can help to dilute the population of the insect on vine blossoms.

**Materials and methods**

This study was conducted on a vineyard in an experimental field of Bari University during the years 2000-2002. The vineyard (covering an area of about 1800 m²) was sited in Valenzano (BA), in a typical table grapevine-growing region. The vineyard "Italia" block was grafted onto 140 Ru rootstock and rows were exposed North-South, with 2x2 m planting density. The “tendone” trained system was applied and the dormant pruning was effected in late winter, followed by green pruning in late spring. Prior to the test and through its conduct, no insecticide treatment was applied in the experimental field.

Six plots of "Italia" cultivars were selected and marked in the field; each plot consisted of a row of 10-vine plants sequence, (20m in length by 2m width).

For the determination of *Phacelia* attractiveness: a - 3 plots were left free from *Phacelia* and 3 plots were intercropped with *Phacelia*. In the three years until 15 March, the 3 first plots (without *Phacelia*) were kept free of ground cover vegetation by one spring disking (monoculture vineyard) and the 3 other blocks (cover-cropped vineyard) were hand under-sown with *Phacelia* seeds at the density of 1Kg/100m, and irrigated by a minisprinkler system.

One month later, *Phacelia* showed good emergence, and plant density is an important issue for *Phacelia* growing and health. On 10th, 15th and 20th May *Phacelia* showed good flowering rates, which were respectively 5, 40 and 95% blooming.

During vineyard blooming, 100 clusters were collected randomly from the three plots covered with *Phacelia*, and 100 clusters from the three-monoculture plots.

All these clusters were placed in Berlese traps to collect *Frankliniella* adults to check whether the presence of *Phacelia* was sufficient to reduce the number of adults.

After vineyard fruit setting, 100 clusters were collected randomly from the three plots covered with *Phacelia*, and 100 clusters from the three-monoculture plots. The number of berries attacked in each cluster was assessed, and the level of damaged berries per cluster was compared in the two different plots (monoculture vineyard, and *Phacelia* cover cropped vineyard) to test whether the presence of *Phacelia* was sufficient to reduce the infestation.
Results

The following results were obtained over the three years: A – in the 1st year (2000), 0.32 adult *Frankliniella* cluster were observed in the plots associated with *Phacelia*, and 2.18 *Frankliniella* in non-associated plots (Table 1). As to damage, there were: a) 9.76 "halo spots"/ cluster in the associated plots and 13.12 in the control (Table 2), b) 4.97 damaged grapes/cluster in the associated plots and 6.48 in the control (Table 3).

Table 1. Number adults of *F. occidentalis* per cluster.

<table>
<thead>
<tr>
<th></th>
<th>N° of Clusters</th>
<th>N° of adults</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with <em>Phacelia</em></td>
<td>100</td>
<td>0.32</td>
</tr>
<tr>
<td>Plots without <em>Phacelia</em></td>
<td>100</td>
<td>2.18</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Number "halo spots" per cluster.

<table>
<thead>
<tr>
<th></th>
<th>Number of Clusters</th>
<th>&quot;halo spots&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with <em>Phacelia</em></td>
<td>100</td>
<td>9.76</td>
</tr>
<tr>
<td>Plots without <em>Phacelia</em></td>
<td>100</td>
<td>13.12</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 3. Number of damaged grapes per cluster.

<table>
<thead>
<tr>
<th></th>
<th>Number of Clusters</th>
<th>Grapes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with <em>Phacelia</em></td>
<td>100</td>
<td>4.97</td>
</tr>
<tr>
<td>Plots without <em>Phacelia</em></td>
<td>100</td>
<td>6.84</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

Table 4. Number "halo spots" per cluster.

<table>
<thead>
<tr>
<th></th>
<th>Number of Clusters</th>
<th>Halo spots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with <em>Phacelia</em></td>
<td>100</td>
<td>2.26</td>
</tr>
<tr>
<td>Plots without <em>Phacelia</em></td>
<td>100</td>
<td>7.06</td>
</tr>
<tr>
<td>Significance</td>
<td></td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

B – In the 2nd year (2001) the following was observed: a) 2.3 "halo spots"/cluster in the associated plots, and 7.1 in the control. (Table 4). 1.4 damaged grapes/cluster in the associated plots, and 3.7 in the control (Table 5).
Table 5. Damaged grapes per cluster.

<table>
<thead>
<tr>
<th>Number of Clusters</th>
<th>Grapes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with Phacelia</td>
<td>100</td>
</tr>
<tr>
<td>Plots without Phacelia</td>
<td>100</td>
</tr>
<tr>
<td>Significance</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

C – In the 3rd year (2002), a) 3.57 "halo spots"/cluster were found in the associated plots, and 9.21 in the control (Table 6). There were 2.55 damaged grapes/cluster in the experimental plots, and 5.61 in the control (Table 7).

Table 6. Number of halo spots per cluster.

<table>
<thead>
<tr>
<th>Number of Clusters</th>
<th>Halo spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with Phacelia</td>
<td>90</td>
</tr>
<tr>
<td>Plots without Phacelia</td>
<td>90</td>
</tr>
<tr>
<td>Significance</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 7. Number of damaged grapes per cluster.

<table>
<thead>
<tr>
<th>Number of Clusters</th>
<th>Grapes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Mean</td>
</tr>
<tr>
<td>Plots with Phacelia</td>
<td>90</td>
</tr>
<tr>
<td>Plots without Phacelia</td>
<td>90</td>
</tr>
<tr>
<td>Significance</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion

Phacelia contributes to substantially reduce the insect population and the subsequent damage, especially in the years when there is low or medium presence of Frankliniella, thus giving the possibility of avoiding chemical treatments.

References

Survey on aerial dispersal of Phytoseiids (Acarina: Phytoseiidae) in a vineyard in Northern Italy

I. E. Rigamonti and S. Rena
University of Milan, Institute of Agricultural Entomology, Via Celoria 2, 20133, Italy

Abstract: In 2002, a survey on aerial dispersal of Phytoseiid mites was carried out in Piedmont. Furthermore, the presence of Phytoseiid mites on natural vegetation and on the vine was studied. The most abundant mites on wild flora were *Euseius finlandicus* (Oud.) and *Phytoseius* gr. *horridus*. The vine population was dominated by *Typhlodromus pyri* Scheuten. The average size of captures with the funnels was about 0.5 specimens/m² day. Almost 90% of specimens were collected within 15 metres of the wood. The most abundant species were *E. finlandicus* and *T. pyri*. The data confirms the importance of aerial dispersal and how it is effective only on short distances. The dominance on the vine of *T. pyri*, a rare species on natural flora, shows how there is a strong selection among the specimens that reach the cultures as well as the importance of the factors that condition the settlement.

Key words: Phytoseiids, aerial dispersal, vineyard, wood, Northern Italy

Introduction

The Phytoseiid mites are among the most important auxiliary arthropods for many agricultural crops, amongst which the vine. They have been studied for decades in order to evaluate their potential and the factors that can improve or limit their activity. More recently, agroecosystems have been considered in their entirety and attention has been placed on natural vegetation as a source for these mites. Many authors believe that the populations of trees and shrubs are an important reservoir from which the Phytoseiid mites, due to passive dispersal, move in order to colonise the new vineyards or plots with no mites (Boller et al., 1988; Lozzia & Rigamonti, 1990; Duso et al., 1993). The results of a survey whose aim was to evaluate the contribution of aerial dispersal in the colonisation of vineyards in Northern Italy are shown in this work.

Material and methods

The observations were drawn in 2002 in a vineyard in Briona in Piedmont, Northern Italy. The plot was about 2 ha wide and Nebbiolo and Croatina were the main varieties of vine to be found. It had a permanent green cover and it bordered on three sides with other vineyards and on the fourth with a wood made up mainly of trees with hairless leaves (chestnut, cherry and elder) and shrubs with hairy leaves (hazelnut, bramble bush). The vineyard was looked after following normal cultural practices adopted in the area. The pest management is made up of two fungicide treatments. The sampling took place from mid May to the end of October. The intercepting of mites in aerial dispersal was obtained using 16 funnels, with a diameter of 42 cm, filled with water to which formaline and a tensioactive was added. This was done to improve the sedimentation and conservation of the mites. The funnels were placed above the canopy in four rows placed 5, 15, 35 and 75 meters from the border vegetation. The presence of Phytoseiids on the main natural plants and on the vine were followed by weekly sampling.
of 25 and 50 leaves respectively. Furthermore, a visual evaluation was carried out of the incidental percentage of each natural species out of the total of their biomass. Due to the remarkable difference in the size of the leaves of the species studied, the density was expressed as mites per square decimeter too. For this reason the area of 100 leaves of every species was calculated using the software programme Sigma Scan Pro®. As the data thus obtained was approximate, it was preferable to express it as "classes of density" (table 1), rather than with the numerical value really obtained.

Table 1. Classes of densities per unit of area.

<table>
<thead>
<tr>
<th>Classes</th>
<th>0</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (Phytoseiids / dm²)</td>
<td>0</td>
<td>0–0.2</td>
<td>0.2–0.6</td>
<td>0.6–1.4</td>
<td>&gt;1.4</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the vine and of natural vegetation and the presence of Phytoseiid mites. The data refers to simple leaves.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>%</th>
<th>Leaf surface cm² ± st. dev.</th>
<th>Total samples / with Phytoseiids</th>
<th>Density Mites / leaf</th>
<th>Mites / dm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bramble bush</td>
<td>15</td>
<td>23.9 ± 9.9</td>
<td>24 / 22</td>
<td>0.40</td>
<td>IV</td>
</tr>
<tr>
<td>Cherry</td>
<td>15</td>
<td>40.5 ± 16.2</td>
<td>24 / 20</td>
<td>0.34</td>
<td>III</td>
</tr>
<tr>
<td>Chestnut</td>
<td>20</td>
<td>75.8 ± 32.7</td>
<td>24 / 19</td>
<td>0.37</td>
<td>II</td>
</tr>
<tr>
<td>Elder</td>
<td>15</td>
<td>28.4 ± 8.8</td>
<td>24 / 17</td>
<td>0.21</td>
<td>III</td>
</tr>
<tr>
<td>Hazelnut</td>
<td>20</td>
<td>53.9 ± 29.0</td>
<td>24 / 21</td>
<td>0.44</td>
<td>III</td>
</tr>
<tr>
<td>Oak</td>
<td>10</td>
<td>32.3 ± 14.5</td>
<td>24 / 6</td>
<td>0.02</td>
<td>I</td>
</tr>
<tr>
<td>Poke</td>
<td>5</td>
<td>49.5 ± 27.5</td>
<td>24 / 8</td>
<td>0.02</td>
<td>I</td>
</tr>
<tr>
<td>Vine</td>
<td></td>
<td>88.7 ± 48.1</td>
<td>24 / 19</td>
<td>0.10</td>
<td>I</td>
</tr>
</tbody>
</table>

% = percentage of natural essences on the total of their biomass
Total samples / with Phytoseiids = total number of samples / samples with presence of Phytoseiids

Results and discussion

The wooded area was almost completely made up of 7 species (table 2). Among these there were 4 types of trees, which were the dominating layer, and two types of shrubs and one grass, which were the dominated layer. Phytoseiid mites were found on all controlled species (table 2). Normally colonisation was stable, except on poke (Phytolacca decandra) and oak. The most consistent populations were found on the bramble bush. The least abundant on chestnut, poke and oak. The most abundant mites on wild flora (table 3) were Euseius finlandicus (Oud.), which was not to be found only on the bramble bush but dominated on trees with glabrous leaves, and a species of Phytoseius of the group horridus, which prevailed on shrubs with hairy leaves and was also present on elder. Kampimodromus sp. is of lesser importance and it is less abundant but it has colonised all the plants. Typhlodromus pyri Scheuten and Amblyseius andersoni (Chant), although rather frequent, are for sure of secondary importance. The annual average of captures with the funnels (table 4) was about 0.5 specimens per square meter per day. The highest levels, over 1 mite/m² day were reached in mid summer, at the end of July and mid August, in relation to the hottest and driest periods. The captures were greatly influenced by the distance from the natural vegetation (table 4). Almost 60% of specimens was collected 5 metres from the wooded area and 30% at 15
metres. In the first row the annual average was 1.2 mites/m² per day with a maximum of between 2 and 2.5 mites/m² per day. In the rows furthest away from the wood Phytoseiid mites were only collected in periods of greatest captures. The species collected are the same as the ones collected on natural vegetation with the addition of *Paraseiulus talbii* (Athias-Henriot) (table 3). On the whole the incidence in percentage of the single species is similar to the one on border vegetation, with more *T. pyri* and fewer *Ph. gr. horridus*. If the single rows are analysed separately, a gradual increase of *Kampimodromus* sp., and especially *T. pyri*, can be seen. The latter at 75 meters made up about half of all captures. Furthermore, *Ph. gr. horridus* was collected only in the first and in the second rows of funnels and *A. andersoni* only in the first one. The colonisation of the vine was stable but very low (table 2), about 0.1 mites/leaf as an annual average with a maximum below 0.3 mites/leaf. It is mainly made up of *T. pyri*. *Kampimodromus* sp. and *P. talbii* are present too but they have a lesser importance.

Table 3. Phytoseiid mites found on the vine, on natural vegetation and in the funnels.

<table>
<thead>
<tr>
<th>Species</th>
<th>Bramble bush</th>
<th>Cherry</th>
<th>Chestnut</th>
<th>Elder</th>
<th>Hazelnut</th>
<th>Oak</th>
<th>Wild flora</th>
<th>Vine</th>
<th>Funnels row I - 5 m</th>
<th>Funnels row II - 15 m</th>
<th>Funnels row III - 35 m</th>
<th>Funnels row IV - 75 m</th>
<th>Funrels total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aa %</td>
<td>-</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>II</td>
<td>IV</td>
<td>I</td>
<td>II</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ef %</td>
<td>-</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>III</td>
<td>IV</td>
<td>III</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>Ks %</td>
<td>III</td>
<td>III</td>
<td>II</td>
<td>III</td>
<td>III</td>
<td>III</td>
<td>II</td>
<td>II</td>
<td>III</td>
<td>III</td>
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<td>III</td>
<td>III</td>
</tr>
<tr>
<td>Pt %</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>I</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Phh %</td>
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<td>-</td>
<td>II</td>
<td>IV</td>
<td>-</td>
<td>IV</td>
<td>-</td>
<td>II</td>
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<td>-</td>
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<tr>
<td>Tp %</td>
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<td>II</td>
<td>II</td>
<td>II</td>
<td>V</td>
<td>III</td>
<td>III</td>
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</tbody>
</table>

- = species absent; I = 0 – 5 %; II = 5 – 15 %; III = 15 – 35 %; IV = 35 – 70 %; V = > 70 %

*Aa = A. andersoni; Ef = E. finlandicus; Ks = K. sp.; Pt = P. talbii; Phh = Ph. gr. horridus; Tp = T. pyri*

Table 4. Captures of Phytoseiid mites with the funnel traps.

<table>
<thead>
<tr>
<th>N° of mites</th>
<th>%</th>
<th>Captures – mites / m² day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total May June July August Sept. October</td>
</tr>
<tr>
<td>Row I - 5 m</td>
<td>113</td>
<td>57.6</td>
</tr>
<tr>
<td>Row II - 15 m</td>
<td>58</td>
<td>29.6</td>
</tr>
<tr>
<td>Row III - 35 m</td>
<td>16</td>
<td>8.2</td>
</tr>
<tr>
<td>Row IV - 75 m</td>
<td>9</td>
<td>4.6</td>
</tr>
<tr>
<td>Total</td>
<td>196</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The data confirms that passive dispersal is the main way through which the Phytoseiid mites reach the cultivation in areas where there is natural vegetation (Tixier et al., 1998, 2000). The wooded areas act as reservoir of predators as well as windbreaks. The captures were much lower than recorded in other areas in Northern Italy (Lozzia and Rigamonti, 2003). This is probably due to the adverse summer conditions, characterised by temperatures lower than usual and heavy rain (little more than 700 mm between June and September). This obstructed both the increase of the populations on natural vegetation and the dispersal of Phytoseiid mites. Aerial dispersal was effective only at a short distance. Already at 35 metres
from the trees the captures were quite low. The propensity to disperse seems to be uniform for
the various species. The scarce presence of Ph. gr. horridus is, with all probability, due to the
fact that it has practically colonised only the lower plants. The limited height of these
essences has, as a result, a reduced radius of diffusion. The great number of captures of T. pyri
is due instead to the fact that a part of it is made up of individuals from the populations of the
vine in active dispersal. The dominance of T. pyri on the vine, a rare species on natural
vegetation, together with the total absence of E. finlandicus, which is the most abundant
Phytoseiid mite, A. andersoni and Ph. gr. horridus show how there is a strong and quick
selection among the specimens that reach the cultivation. Clearly agricultural practices, the
characteristics of the varieties and interspecific competition make it possible for most mites to
succumb and only a few specimens of the most suitable species find a favourable environment
and manage to settle (Tixier et al., 2002). The essences present in wooded areas have,
therefore, an important role in the first phases of the colonisation of a vineyard, ensuring that
a sufficient number of individuals of the suitable species reach the culture. Just as important
are the factors that influence the settlement. Hence, further studies are necessary in order to
establish: 1) the influence of the impact of migrating mites and of the selection in the vineyard
for natural colonisation 2) which habitat management measures or cultural practices are the
most suitable in order to improve the natural colonisation.

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Kampimodromus aberrans (Oudemans) (Acari: Phytoseiidae): dispersal from surrounding
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The good plant protection practice for grape vine is more concerned, in relation to IPM, with the risk of resistance than the safety and other side-effects of pesticides

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Abstract: The EPPO Working Group on Plant Protection Products developed since 1987 the EPPO Standard PP 2/1 (1) Principles of Good Plant Protection Practice (GPP), published in 1994 and followed by GPP for all major crops of the EPPO region to cover methods for controlling animal pests, microbial pathogens and weeds. Since 1994, EPPO standards on GPP have been published for 25 individual crops. The standards for grapevine were just published in Bull. OEPP, Vol. 32 (2), August 2002. In January 2001 was published in Portugal “A protecção integrada da vinha na Região Norte”, distributed at the last meeting at Ponte de Lima of the IOBC/WPRS Working Group on Integrated Control in Viticulture. The comparative analyse of the recommendations about side-effects of pesticides concerning five diseases (downy mildew, powdery mildew, grey mould, esca and excorioid), three pests (berry moth, green leafhopper and mites) and the weeds considered in the guideline on GPP and on the IPM portuguese publication show clear differences. The IPM publication give detailed information about toxicity to man, domestic animals, natural enemies, fishes and aquatic organisms, phytotoxicity, resistance and, when available, about toxicity to bees and birds. The GPP guidelines give detailed development to resistance (73% of the side-effects information), some information (9%) about natural enemies and just 7% to toxicity to man (specially about sodium arsenate) and phytotoxicity. GPP guidelines ignore that three insecticides (dichlorvos, mevinphos and monocrotophos) are very toxic to man and nine insecticides (fenpropathrin, methidathion, methomyl, phosphamidon, quinalphos, rotenone, tiodicarb, tralomethrin and triazophos) and one acaricide (pyridaben) are toxic to man. The GPP guidelines ignore completely the side-effects of pesticides for aquatic organisms and bees. Must be stressed that among the 125 pesticides recommended in that GPP guidelines 18% of the insecticides are extremely dangerous and 31% of all pesticides are very dangerous to aquatic organisms and that 20% of the insecticides are very dangerous to bees. Such clear differences, confirming similar situation about the GPP guidelines for pear, show how it is impossible to accept the EPPO conclusion that GPP is the best and the optimum alternative in plant protection.

Key words: side-effects, pesticides, EPPO, IOBC/WSPR, toxicity to man, bees, aquatic organism, resistance

Introduction

At the Working Group Meeting in Florence (Italy), March 1999, in a paper on “The good plant protection practice (GPP) or integrated pest management (IPM)” (Amaro, 2000a) was stressed that OEP in the last 20 years didn’t take any initiative to develop IPM.

The great development of IPM, specially in the 90ties, had a very important contribution of IOBC/WPRS, namely through the activity of many working groups and commissions and the production: of the basic guidelines on Integrated Production in 1992 and the 2nd edition in 1999; and of guidelines for six crops (pome fruits, stone fruits, grapes, arable crops, soft fruits and olives). EPPO ignored IOBC/WPRS in the Guidelines on Good Plant Protection Practice, published in 1994 (OEPP, 1994) and stressed that “there are different concepts and definitions
of IPM that in general call for the compulsory integration of product application with other methods of protection, may involve complex and labour intensive decision-making system, and set an ideal of replacing the use of chemical products by other means”. And clarify that “the main purpose of EPPO recommendations on GPP is: to provide guidelines on whether and how to use products and ensure that they are used safely and effectively (OEPP, 1994).

The analysis made in 1999 (Amaro, 2000a) of the Principles of Good Plant Protection Practice has shown too much confusion about five questions related with de main purposes of EPPO recommendations on GPP, above cited:

Why GPP and not GPP of pesticides?
How it is possible that GPP recommend optimal practices?
Why EPPO ignore the IPM concept of IOBC/WPRS?
How it is possible that “IPM is evidently GPP”?
How it is possible that “Integrated Production is certainly GPP”?

As EPPO has announced the future publication of the GPP Guidelines on Grapevine and considering the confusions concerning GPP, the last words of the cited paper were: “Il est certainement convenable que le Groupe de Travail de Protection Intégrée en Viticulture de l’OILB/SROP n’ignore pas les résultats de cette initiative de l’OEPP” (Amaro, 2000a).

This paper was supported by the Project AGRO 13.

**The GPP Guidelines on Grapevine**

These guidelines were just published on the EPPO Bulletin in August 2002 (OEPP, 2002). Recommendations concerning 22 diseases, pests and weeds are presented.

It is considered quite valuable the information about biology and ecology and the basic strategy concerning the population assessments and the threshold values if available. Methods of cultural control for diseases and weeds and of biotechnical and chemical control are proposed and lists of pesticides are more abundant for berry moths (30 insecticides), powdery mildew (25 fungicides) and weeds (21 herbicides).

<table>
<thead>
<tr>
<th>Side-effects of pesticides</th>
<th>Guideline on Principles of GPP</th>
<th>GPP Grapevine Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxicity to man</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Phytotoxicity</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Toxicity to natural enemies</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Toxicity to bees and to wildlife</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Resistance to pesticides</td>
<td>53</td>
<td>73</td>
</tr>
<tr>
<td>Positive or negative effects on other enemies</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

The information about pesticide side-effects is rather poor in general with a exaggerated priority to the development of resistance and ignoring the side-effects on aquatic organisms, bees, birds and domestic animal, and with very limited information concerning natural
enemies and the toxicity to man. Considering the global information about side-effects of pesticides, 73% is about resistance, 9% related with natural enemies, 7% for both toxicity to man and phytotoxicity and 4% related with positive or negative effects on other enemies. Those values compared with the information of the Guidelines on Principles of GPP show a increase (53 to 73%) concerning resistance and a decrease about natural enemies (23 to 9%) (Table 1).

Details about fungicides and herbicides concerning resistance management of downy mildew (phenylamides and cyroxanil), grey mould (benzimidazoles, dicarboximides and anilinopyrimidines), powdery mildew (DMI, strobilurin group) and weeds (triazine, paraquat, diuron, glufosinate-ammonium) are considered. It is quite modest that kind of information concerning natural enemies (“some fungicides have a degree of action on mite populations”; “organophosphorus compounds and synthetic pyrethroids often have undesirable effects on beneficial”), specially if it take in attention that the majority (55%) of the 49 insecticides are very toxic to natural enemies and 10% of the insecticides and 16% of the 55 fungicides are toxic to natural enemies.

Information about toxicity to man is restricted to sodium arsanite (“not registered in most countries and have high human toxicity”). But the silence is total about other three very toxic insecticides (dichlorvos, mevinphos, monocrotophos) and about nine toxic insecticides (fenpropatrin, methidathion, methomyl, phosphamidon, quinalphos, rotenone, thiodicarb, tralomethrin and triazophos) and one toxic acaricide (pyridaben). The question of residues on the harvested crop is only limited to grey mould and berry moths treatments.

Table 2. Differences between integrated pest management (IPM) and good plant protection practices (GPP), with obvious consequences in the man and environment defence (Amaro, 2000b).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Integrated pest management</th>
<th>Good plant protection practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aim – reduction of pesticides use</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Use of pesticides as the last option</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Ban of pesticides (ex. mevinphos, paraquate) very toxic to man</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Ban of pesticides (ex. dimetuate, pyrethroids) very toxic and toxic to natural enemis.</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>ban of pesticides (ex. simazine) with great danger of water contamination</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>To increase farmer or other pesticide applicator security</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>

The problem of phytotoxicity is only considered about herbicides.

It is total the silence of information about toxicity to bees, but 20% of the insecticides are very dangerous. The same silence concerning toxicity to aquatic organisms but 18% of the insecticides are extremely dangerous and 31% of all pesticides are very dangerous.

The general information of the Principles of good plant protection practice (OEPP, 1994) explains that orientation adopted in the GPP guidelines to grapevine.

For “one of the most critical side-effects of product use”, the resistance of pest population, it is indispensable to give detailed information, but:
• **“side-effects on bees or on wildlife** are basically covered by the conditions of registered use, so GPP will automatically take account of them”;

• **safety**: “GPP requires that relevant official regulations and codes of practice for the safety of the operator, consumer and environment should have been respected” (OEPP, 1994).

And that is all!

In a Portuguese publication about *A protecção integrada da vinha na região Norte* the importance given to side-effects of 103 pesticides authorized in IPM was: phytotoxicity - 22.4%; toxicity to natural enemies - 18.1%; toxicity to aquatic organisms - 16.1%; resistance to pesticides - 15.8%; toxicity to man - 13.4% and toxicity to bees - 1.2% (Amaro, 2000b).

The great differences between GPP and IPM are synthesised in Table 2.

The IOBC/WPRS Guidelines for integrated production of grapes (Malavolta & Boller, 1999) are very clear about:

• **Direct plant protection measures**: *Priority* must be given to natural, cultural, biological and highly specific methods of pest, disease and weed control, and the use of pesticides must be minimised. Pesticides may only be used when justified. The most selective, least toxic, least persistent product or control procedure, which is as safe possible to humans and the environment, must be selected.

• **The criteria to adopt in the selection of pesticides** is cited including the pollution of ground and surface water and the toxicity to man, natural enemies and other natural organisms.

• **Examples of pesticides not permitted and permitted with restrictions** are presented.

It is very important to have always quite clear the real differences between the IOBC/WPRS Guidelines and the GPP Guidelines, that EPPO considers the optimum alternative in plant productions.

This explains, certainly, why the Commission of the European Communities consider, in the 4th objective of the Thematic Strategy on the Sustainable use of pesticides, “the promotion of good practices by further developing codes of Good Farming Practice integrating IPM concepts” (CCE, 2002).

**References**


CCE (Comissão das Comunidades Europeias) 2002: Towards a thematic strategy on the sustainable use of pesticides: 40 pp.


The pesticides very toxic to man, to natural enemies, to honey bees and to aquatic life must be prohibited or rigorously restricted for IPM in viticulture

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Abstract: According to the Guidelines of integrated production of grapes (2nd Ed. 1999), the selection of pesticides must respect several criteria, normally concerning toxicity to man, to key and other natural enemies and related to pollution of ground and surface waters. In those criteria there is no reference to toxicity to honey bees and to aquatic life. From country to country there is some variability concerning the pesticides toxic to man and to natural enemies prohibited or restricted for IPM in viticulture. To obtain a general acceptance of that IOBC/WPRS IPM guidelines from the EU, OEDC, FAO and other organizations is convenient to obtain a more detailed agreement of the application of the guidelines in different countries. Concerning the pesticides toxic to man it is proposed the prohibition of all pesticides toxic or very toxic if safer alternatives exist. The same criteria concerning toxicity to natural enemies and honey bees: prohibition of all pesticides toxic or very toxic if safer alternatives exist. The pesticides extremely toxic to aquatic organisms must be prohibited and the pesticides very toxic must be prohibited or the use conditioned if safer alternative exist. After the discussion in the Volos meeting of the Working Group, this question must be presented for analyse to the Commission “IP – Guidelines and Endorsement”.

Key words: unselective pesticide, honey bees, aquatic organism, birds, wildlife.

Introduction

The IPM concept has changed in the last 40 years and IOBC/WSPR in the 2nd Edition 1999 of the Principles and Technical Guidelines, in the Direct Control Measures consider that:

• “unselective pesticides with long persistence, high volatility, leachable or with other major detrimental characteristic are prohibited”;
• “for pesticides with high risk of resistance development of an adequate anti-resistance management scheme has to be established”;
• “the safety regulations for pesticides are to be stresses” (Boller et al., 1999).

In the Guideline on Good Plant Protection Practice (GPP), the EPPO stressed that “integrated pest control or integrated pest management (IPM) as it is also called, sets a different standard from GPP” (OEPP, 1994).

The so important selection of pesticides to prevent or attenuate the side-effects of pesticides is quite clear in the IOBC/WPRS Guidelines for Integrated Production of Grapes 2nd Ed. 1999: “the most selective, least toxic, least persistent product or control procedure, which is as safe as possible to humans and the environment, must be selected” (Malavolta & Boller, 1999).

It is surprising that in that Guidelines for integrated production of grapes there is no clear reference to prevent side-effects related with aquatic organisms and honey bees, certainly included in “other natural organisms” and to resistance (in general) and phytotoxicity. In contrast in the GPP Guidelines for Grapevine of EPPO (OEPP, 2002) great development is
dedicated to resistance (73%) and small reference to side-effects related with toxicity to man and natural enemies and phytotoxicity and positive or negative effect on other enemies (Amaro, 2003).

It is indispensable to give the most detailed information about side-effects of pesticides to technicians and to farmers to put IPM in practice. The next revision of the IOBC/WPRS Guidelines must give attention to that question, that was analysed at the last meeting of the Working Group, at Ponte de Lima (Mars 2001) (Amaro, 2001b). Now there is a good opportunity for a more detailed discussion.

This paper was supported by the Project AGRO 12.

Side-effects of pesticides in the Guidelines For Integrated Production of Grapes (2nd edition)

The general principle concerning the selection of pesticides states that must be chosen “the least hazardous to humans, livestock and environment whilst providing effective control of the pest, disease or weed problem” (Malavolta & Boller, 1999).

“In the classification of pesticides into “permitted”, “permitted with restrictions” and “not permitted categories”, the following criteria should be taken into account: selectivity, persistence and side-effects: toxicity to man, to key natural enemies and other natural organisms, the pollution of ground and surface waters and the ability to stimulate pests (Malavolta & Boller, 1999).

Concerning not permitted pesticides are cited:

• pyrethroid insecticides or acaricides;
• organochlorine insecticides and acaricides if safer alternatives exist;
• all acaricides toxic to phytoseiid mites;
• toxic, water polluting or very persistent herbicides (e.g. diquat, paraquat) (Malavolta & Boller, 1999).

Pesticides permitted with restrictions should be accepted when “either no ecologically safer alternatives are available or that the active ingredient is necessary for a planned resistance management”. Some examples are considered about number of applications and other questions related with:

• broad spectrum organo-phosphate and carbamate insecticides;
• acaricides harmful to phytoseiid mites;
• dithiocarbamate fungicides;
• sulphur and copper;
• fungicides with high potential to develop resistance;
• residual herbicides with dt90<1 vegetation period (Malavolta & Boller, 1999).

“Statutory maximum residue levels and adequate safe-to-harvest intervals must be observed” (Malavolta & Boller, 1999).

Restrictions to prevent side-effects related with toxicity to aquatic organisms, honey bees, birds and wildlife

The real importance and the frequency of the side-effects related with aquatic organism and honey bees justify that quite clear information must be included in the Grape Guidelines.

This question shall be exemplified with the information included in A Protecção Integrada da Vinha na Região Norte (Amaro, 2001a).
Table 1. Classification of toxicity to man, to aquatic organisms, to honey bees and to birds of pesticides permitted in IPM of grapes in Portugal.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Man toxic</th>
<th>Aquatic organisms</th>
<th>Honey bee</th>
<th>Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>extremely dangerous</td>
<td>very dangerous</td>
<td>very dangerous</td>
</tr>
<tr>
<td><strong>Insecticides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cihexatin</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dicofol</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dicofol+tetradifon</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>endosulfan</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>fenbutatin oxide</td>
<td>x</td>
<td></td>
<td></td>
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<tr>
<td>fenoxycarb</td>
<td>x</td>
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<td>fenpyroximate</td>
<td>x</td>
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<td></td>
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<td></td>
<td>x</td>
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<tr>
<td>lufenuron</td>
<td>x</td>
<td></td>
<td></td>
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<td>x</td>
<td>x</td>
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<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>Fungicides</strong></td>
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<td></td>
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<tr>
<td>azoxystrobin</td>
<td>x</td>
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<td>azoxystrobin+cymoxanil</td>
<td>x</td>
<td></td>
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<tr>
<td>cymoxanil+metiram</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>cymoxanil+zinebe+copper oxychloride</td>
<td></td>
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<td></td>
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<tr>
<td>cyprodinil+fludioxonil</td>
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<tr>
<td>dimetomorph + copper oxychloride</td>
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<td></td>
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<tr>
<td>dinocap</td>
<td>x</td>
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<td>fenarimol</td>
<td>x</td>
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<td>fenarimol+quinoxyfen</td>
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<td>fenbuconazole</td>
<td>x</td>
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<tr>
<td>fenbuconazol+dinocap</td>
<td>x</td>
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<td>fluquinconazole</td>
<td>x</td>
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<tr>
<td>fosetyl-aluminium+zineb</td>
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<td></td>
<td></td>
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<td>kresoxime-methyl</td>
<td>x</td>
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<tr>
<td>metalaxil-M+mancozeb</td>
<td>x</td>
<td></td>
<td></td>
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<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myclobutanil+dinocap</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>myclobutanil+pyrazophos</td>
<td>x</td>
<td>x</td>
<td></td>
<td>x</td>
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<tr>
<td>ofurace+cymoxanil+metiram</td>
<td>x</td>
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<tr>
<td>ofurace+folpet</td>
<td>x</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>penconazole</td>
<td>x</td>
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</tr>
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<td>pyrimethanil</td>
<td>x</td>
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<tr>
<td>quinoxyfen</td>
<td>x</td>
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</tbody>
</table>
Table 1 (continued). Classification of toxicity to man, to aquatic organisms, to honey bees and to birds of pesticides permitted in IPM of grapes in Portugal.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Man toxic</th>
<th>Aquatic organisms</th>
<th>Honey bee</th>
<th>Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>extremely dangerous</td>
<td>very dangerous</td>
<td>very dangerous</td>
</tr>
<tr>
<td>tetraconazole</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>zineb</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>zineb+copper oxychloride</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>zineb+copper sulphate</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td><strong>Herbicides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>linuron</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>linuron+glyphosate+terbuthylazine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pendimethalin</td>
<td></td>
<td></td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>quizalofop-P-ethyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The number of active ingredients not permitted is 35 (25% of the total) in consequence of: toxicity to man (14%) (amitrole, mevinphos and paraquat); contamination of water (14%) (simazine) and toxicity to beneficials (80%) (24 insecticides and 4 fungicides) (Table 2). Other 20% of the active ingredients are permitted with restrictions. Concerning the 24 insecticides not permitted, 33% are extremely dangerous and 33% very dangerous to aquatic organism, 42% are very dangerous and 29% dangerous to honey bees and just 4% very dangerous to birds and 13% very dangerous to wildlife (Amaro, 2001a).

The side-effects of 103 pesticides (18 insecticides, 72 fungicides and 13 herbicides) permitted without or with restrictions in IPM on grapes in Portugal in 2001 are quite frequent and important concerning aquatic organism, with some importance in relation to honey bees and very rare to birds (Table 1). Two insecticides (endosulfan, lufenuron), 11% of the total of insecticides are extremely dangerous to aquatic organism and 38% of all pesticides and 44% of the 18 insecticides, 38% of the 72 fungicides and 31% of the 13 herbicides are very dangerous to aquatic organism (Table 1).

Concerning the toxicity to honey bees, one fungicide (myclobutanil+pyrazophos) is very dangerous and five insecticides (28%) are dangerous and one fungicide (penconazole) is dangerous (Table1).

Just one fungicide (tetraconazole) is dangerous to birds (Table1).

It is clear that must be not permitted in IPM the use of extremely dangerous pesticides to aquatic organism and it is difficult not to permit it in consequence of the high number of pesticides. But only with restrictions could be authorized the use of very dangerous pesticides to organism aquatic.

Concerning honey bees must be not permitted the pesticides very dangerous to honey bees and only with restrictions the pesticides dangerous to honey bees.

Three insecticides (endosulfan, metidathion and phosphamidon) are toxic to man and must be not permitted in IPM.

It is suggested that this proposal must be discussed during the meeting and this paper and the result of the discussion must be presented for analyse to the Commission IP-Guidelines and Endorsement.
Table 2. Pesticides not permitted in grapes IPM in Portugal

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Insecticides</th>
<th>Fungicides</th>
<th>Herbicides</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha-cypermethrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>azinphos-methyl</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beta-cyfluthrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>carbaryl</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>carbofuran</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>cyfluthrin</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>cypermethrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>chlorpyrifos</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>chlorpyrifos+cypermethrin</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>chlorpyrifos+deltamethrine</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>chlorpyrifos+dimethoate</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>deltamethrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>diazinon</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>dimethoate</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flucythrinate</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>lambda-cyhalothrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>lindane</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>malathion</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>methidathion</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>methomyl</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>mevinphos</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>permethrin</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>quinalphos</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>trichlorfon</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benomyl</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cabendazim</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>thiophanate-methyl</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amitrole+duron</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amitrole+duron+winter</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>oil+simazine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amitrole+simazine</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>diuron+winter oil+simazine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>glyphosate+simazine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>paraquat</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>simazine</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ED – extremely dangerous; VD – very dangerous; D – dangerous
To obtain the general acceptance, from other organizations of the IOBC/WPRS IPM Guidelines in Grapes should be very important to obtain regularly information about the classification of pesticides adopted in different countries in Europe.

References

Amaro, P. 2001: La pratique des directives pour la production intégrée en viticulture doit être améliorée et s’imposent de nouvelles restrictions concernant quelques pesticides. – IOBC/wprs Bulletin 24 (7): 151-156.
Seasonal abundance of insect pests and their parasitoids in stored currants

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Abstract: Adhesive traps were used in order to monitor the seasonal occurrence of insect species that occur in stored currants. The traps were suspended at a horizontal, enclosed warehouse located at Corinth (Peloponnesse, Southern Greece), which is one of the main areas in Greece where currant is produced and stored. The storeroom was filled with approx. 1,200 tons of the product. Twelve traps were used, 6 of them were baited with TDA, the sex attractant for several stored-product pyralid species, and the other 6 were unbaited. The traps were inspected for captured insects at 10-day intervals, for 12 consecutive months, from February 2000 until January 2001. Thirty-four insect taxa were collected during the experimental period. The pyralid moths Ephestia cautella (Walker) and Plodia interpunctella (Hübner) were the most abundant Lepidoptera species, while adults of E. figulilella Gregson, E. kuehniella Zeller and E. elutella (Hübner) were also found in small numbers. The majority of the captured moths were counter during summer months, but no individuals of these species were found during winter. The most numerous beetle species were Cryptolestes ferrugineus (Stephens) (Cucujidae), Oryzaephilus surinamensis (L.) (Silvanidae), Carpophilus dimitiatus (F.) and C. hemipterus (L.) (Nitidulidae). Contrarily to moths, adult beetles were collected from the traps during the entire trapping period. Moreover, several Hymenopterous parasitoid species were found. The parasitoid Holepyris sylvanidis (Bréthes) (Bethylidae) was by far the most abundant parasitoid species in the warehouse, followed by, in decreasing order, Habrobracon hebetor Say (Braconidae), Venturia canescens (Gravenhorst) (Ichneumonidae), Cephalonomia tarsalis (Ashmead) (Bethylidae) and Theocolax (Choetospila) elegans (Westwood) (Pteromalidae).

Key words: Stored currant, Greece, monitoring, TDA, traps, stored-product insects.

Introduction

Greece is one of the main currant productive countries of the world. More than 40,000 tons are produced, processed and stored each year, in Greek storage facilities. Although several studies have been carried out so far concerning the phenology, biology and control of the main viticulture pests in Greece, little is known about the species that infest stored currant and sultanas. However, these species can be easily built up high population densities and cause irrecoverable damage to these products. More than twenty years ago, Buchelos (1980) conducted the first quantitative survey concerning the seasonal occurrence of beetle species that occur in Greek currant stores. Later, Buchelos (1985) lists 12 species, belonging to the orders Lepidoptera and Coleoptera, which are associated with these products. Nevertheless, until recently, nothing was known about hymenopterous species, despite the fact that these species are potential biocontrol agents. Eliopoulos et al. (2002a) listed a number of hymenopterous parasitoids which were found in dried fruit in Greece, including currant and sultanas. From that study, it became evident that these species are found in considerable numbers and very often in Greek storage facilities. Despite this, so far there is still inadequate information about the seasonal abundance of these species, and their associations with their hosts’
densities. The aim of our study was to examine the population trends of insect pests and their parasitoids, in stored currant in Greece. This knowledge is of practical importance, because it provides the inferences necessary for incorporating parasitoids when developing a pest management programme for these products, on the basis of IPM principles.

Table 1. Insect taxa and their numbers (% of the total number of individuals found) caught in traps during the entire experimental period.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>% of the total individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coleoptera</strong></td>
<td></td>
</tr>
<tr>
<td>Anobiidae</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Lasioderma serricorne (F.)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Anthicidae</td>
<td>11.28</td>
</tr>
<tr>
<td>Anthicus sp.</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Carabidae</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cleridae</td>
<td></td>
</tr>
<tr>
<td>Necrobia rufipes (Degeer)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cucujidae</td>
<td></td>
</tr>
<tr>
<td>Cryptolestes ferrugineus</td>
<td>2.55</td>
</tr>
<tr>
<td>Cryptolestes spp.</td>
<td></td>
</tr>
<tr>
<td>Curculionidae</td>
<td></td>
</tr>
<tr>
<td>Sitophilus oryzae (L.)</td>
<td>0.19</td>
</tr>
<tr>
<td>Cryptophagidae</td>
<td></td>
</tr>
<tr>
<td>Atomaria munda Erichson</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cryptophagius cellulis</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cryptophagius saginatus Sturm</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Derestidae</td>
<td></td>
</tr>
<tr>
<td>Anthrenus verbasci (L.)</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Trogoderma sp.</td>
<td>0.17</td>
</tr>
<tr>
<td>Lathrididae</td>
<td></td>
</tr>
<tr>
<td>Corticaria sp.</td>
<td>0.53</td>
</tr>
<tr>
<td>Mycetophagidae</td>
<td></td>
</tr>
<tr>
<td>Litargus balteatus Leconte</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Typhaea stercorea (L.)</td>
<td>1.84</td>
</tr>
<tr>
<td>Nitidulidae</td>
<td></td>
</tr>
<tr>
<td>Carpophillus dimittatus (F.)</td>
<td>1.97</td>
</tr>
<tr>
<td>Carpophillus hemipterus (L.)</td>
<td>1.29</td>
</tr>
<tr>
<td>Carpophillus ligneus Murray</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Taxa</th>
<th>% of the total individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lepidoptera</strong></td>
<td></td>
</tr>
<tr>
<td>Ephesia cautella (Walker)</td>
<td>2.11</td>
</tr>
<tr>
<td>Ephesia elutella (Hübner)</td>
<td>2.11</td>
</tr>
<tr>
<td>Ephesia figulilella Gregson</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Ephesia kuehniella Zeller</td>
<td>1.48</td>
</tr>
<tr>
<td>Plodia interpunctella (Hübner)</td>
<td>5.73</td>
</tr>
<tr>
<td><strong>Hymenoptera</strong></td>
<td></td>
</tr>
<tr>
<td>Bethylidae</td>
<td></td>
</tr>
<tr>
<td>Cephalonomia tarsalis (Ashm.)</td>
<td>2.11</td>
</tr>
<tr>
<td>Holecyrus silvanidis (Brethes)</td>
<td>14.37</td>
</tr>
<tr>
<td>Braconidae</td>
<td></td>
</tr>
<tr>
<td>Harbobracon hebetor Say</td>
<td>7.57</td>
</tr>
<tr>
<td>Ichneumonidae</td>
<td></td>
</tr>
<tr>
<td>Venturia canescens (Grav.)</td>
<td>2.91</td>
</tr>
<tr>
<td>Pteromalidae</td>
<td></td>
</tr>
<tr>
<td>Theocolax elegans (Westwood)</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>Diptera</strong></td>
<td>2.03</td>
</tr>
<tr>
<td><strong>Hemiptera</strong></td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Materials and methods

The study was carried out at a horizontal (flat) type rectangular warehouse located at Kalamata (Peloponese, Southern Greece). This storeroom was used every year for currant storage and it was made of bricks with a solid roof. On the first days of January 2000, the room was cleaned from old product residues. On late January, the warehouse was filled with approx. 1,200 tons of currants (from the 1999 harvest), which was placed in bulks, approx. 1.5 m high.
On early February, 12 traps were hanged, using wires across the store room’s roof. The distance between the lower trap parts and the bulk’s surface was 1.5 m. The traps were yellow rectangular cardboard stripes (size 27 x 8 cm), which were covered with Tanglefoot (The Tanglefoot Co, Crand Rapids, MI, USA). Six of them were baited with a replaceable polyethylene capsule (placed at the central point of the two diagonals of the stripes), containing 100 µg of TDA [(Z, E)-9,12-tetradecadien-1-yl acetate], the sex attractant for the male of several stored-product Pyralidae species (Buchelos and Levinson 1985, Buchelos 1998). The other six traps were left unbaited.

Fig. 1. Fluctuation of temperature and relative humidity into the currant warehouse, during the experimental period.

The traps were inspected at 10-day intervals, until January 2001, by removing and renewing the stripes. Then the stripes were taken in the laboratory for counting and identification of the captured individuals. The replacement of the pheromone capsules was
made every two trap-check dates. After each inspection, the traps were rotated clockwise, in order to minimize the influence of the individual trapping location. Air temperature and relative humidity indoors was recorded by using a digital thermohygrograph, placed at the center of the storeroom (Hobo H8, Onset Computer Co., USA). No insecticidal treatments took place during the experimental period.

Results

The seasonal fluctuation of the temperature and relative humidity indoors is shown in Fig. 1. Thirty-four insect taxa were found during the entire monitoring period, belonging to the orders Lepidoptera, Coleoptera, Hymenoptera, Hemiptera and Diptera (Table 1). Most of them were beetles, known stored-currant pest species (Nitidulidae, Cucujidae, Silvanidae), while others are mainly fungus feeders (Cryptophagidae, Lathrididae, Mycetophagidae). All the Lepidoptera species found were also known stored-product pyralid moths. In the case of hymenopterous wasps, all species were beetle or moth parasitoids. Finally, in the case of Hemiptera and Diptera, the identification was not possible up to the species level.

Fig. 2. Mean number of E. cautella adults/trap, on pheromone-baited and unbaited traps, during the experimental period.

More than 17% of the total number of individuals captured in traps were E. cautella adults. Most of them (>90%) were found in the pheromone-baited traps; however, E. cautella moths were also found in unbaited traps (Fig. 2). The majority of the adults of this species was found rather late in the storage season (September-October), and this species was found in traps until late November, while no adults were detected during winter months. Similar population trends were also recorded for P. interpunctella (Fig. 3). Nevertheless, P. interpunctella adults were found in traps one month later than E. cautella. In addition, no P. interpunctella adults were caught in traps from early November and on. Apart from Lepidoptera, all the other species were equally found in baited and unbaited traps and thus, the data are presented for the combined (baited and unbaited) trap counts.

The most abundant insect species were Cryptolestes spp., Oryzaephilus spp. and Carpophilus spp., given that the individuals belonged to these species correspond to more than 40% of the total number of individuals caught. From the species of the genus...
Cryptolestes, more than 80 % of the individuals were *C. ferrugineus* (the other species were not identified up to the species level). Similarly, approx. 80 % of *Oryzaephilus* adults were *O. surinamensis*.

Fig. 3. Mean number of *P. interpunctella* adults/trap, on pheromone-baited and unbaited traps, during the experimental period.

Fig. 4. Mean number of *Cryptolestes* spp. and *Oryzaephilus* spp. adults/trap, during the experimental period.

As far as the seasonal abundance of these species is concerned, *Cryptolestes* and *Oryzaephilus* indicated relatively dissimilar population trends (Fig. 4). The latter species were found mainly during summer months, in fact almost 85 % of the total was recorded early in the summer, during June and July. In contrast, very few adults were caught later in the season, while no adults were detected during November and winter months. In the case of *Cryptolestes* spp., the population outbursts were noted during late summer and September,
with more than 50 adults per trap, while adults of these species were continued to be present in traps, even during winter months. In the case of the nitidulids *C. dimitiatus* and *C. hemipterus*, the seasonal population trends were rather similar (Fig. 5). For both species, most of the individuals were found during late summer (late July-August). For *C. dimitiatus* a second peak was observed at late September. It must be noted that, these species were found in traps during the entire experimental period.

![Graph](image1.png)

Fig. 5. Mean number of *C. dimitiatus* and *C. hemipterus* adults/trap, during the experimental period.

![Graph](image2.png)

Fig. 6. Mean number of *H. sylvanidis* and *H. hebetor* adults/trap, during the experimental period.

From the wasp species found, more than half were *H. sylvanidis* adults. Despite the fact that this species was captured in traps during the entire trapping period, including winter, early in the season (until mid June) it was recorded at low numbers (<1 individuals/trap) (Fig. 6). However, from late July and, mainly, during August, September and until mid October,
this species was found in traps in extremely high numbers. Almost 85% of the total number of adults found was counted during this interval. From late October and until the end of the trapping period, the numbers of *H. silvanidis* dropped dramatically. The population fluctuation of the braconid *H. hebetor* was similar, as far as the single-outburst trend is concerned. Nevertheless, the peak of this species was observed much later (almost one month) than the respective one for *H. silvanidis*. More *H. hebetor* adults were found during September and October (more than 80% of the total). This species was also found during winter months.

**Discussion**

This study is the first quantitative work concerning the long-term monitoring of insect species in stored currant, during a full year season, by using traps. Colored sticky traps have been widely used for monitoring of stored product insect species, with or without the addition of an attractant (usually a pheromonic source). In our case, yellow was selected because it is generally the most commonly commercially available trap color, of the specific trap design. Buchelos and Levinson (1993) and Buchelos (1998) successfully used white sticky cardboard traps, for monitoring of several stored-product species. Johnson et al. (2000) used yellow sticky traps, for monitoring of several stored-product insect species in stored figs. However, the visual stimuli which is provided by the color of the sticky surface, consists an important component which affects captures, and has not been fully investigated in detail.

Pheromone-baited traps are a valuable tool when developing trapping protocols for stored-product insects. Despite the fact that moths are likely to be caught in unbaited traps, the results are often poor and of no practical importance, for the population trends of the species caught (Buchelos 1998, Pereira 1998). Our results stand in accordance with the above estimation. In light of our findings, several moth species coexist in stored currant. Buchelos (1980) also noted the coexistence of several puralid moths in flour mills. *E. cautella* seems to be the prevalent pyralid species in stored currant and sultanas in Greece. The highest numbers of these species’ adults in traps are observed at late summer-early autumn. On the other hand, *P. interpunctella* adults are generally appeared in traps much later than those of *Ephestia*, and they are not active at late autumn. Buchelos (1980 and 1998), using traps for monitoring of pyralid moths in flour mills and tobacco stores, reports similar observations concerning the seasonal abundance of *P. interpunctella* and *Ephestia* spp.

No relevant data is available about the flying activity of stored-product beetle species in currant stores. According to our results, some beetle species are more prone to fly than others, and monitoring with sticky traps above the product mass may be useful for control strategies. Eliopoulos et al. (2002b), using adhesive traps in raw grain reported that species like *Cryptolestes* spp. and *Oryzaephilus* spp. were found in these traps in high numbers. However, given that other traps types (perforated traps) which are inserted into the grain mass are more efficient, sticky traps are usually not suitable for these species (Hagstrum 2000). In the case of other bulked products, such as stored currant, sultanas or figs, where no alternative trap designs are available, aerial sticky surfaces are likely to give reliable results for these species. *Cryptolestes* and *Oryzaephilus* showed different seasonal trap performance. *Oryzaephilus* adults were found early in the season, when the presence of *Cryptolestes* in traps is rather limited. On the other hand, *Cryptolestes* builds up high densities later in the storage period. Surprisingly, these species are present in traps even during winter months, which is indicative of their full-year activity. The same holds for *C. dimittatus* and *C. hemipterus*. These two species are main stored-currant beetle species, and their appearance in high numbers is noted.
earlier than those of the other abundant beetle species. It is well established that, in the case of figs and related products, these species are able to infest the fruit outdoors before harvest, and can continue the infestation during storage (Aitken 1975). Nevertheless, it must be stated that, the decline that is observed in trap catches from mid autumn and on, could be mainly attributed to the decrease of the temperatures prevailing indoors, which influences negatively their flying activity. Moreover, increased insect activity in the traps do not necessarily correspond to high infestation levels into the product mass (Subramanyam and Hagstrum 1995). Concomittantly, for beetle monitoring, captures in aerial traps may not be representative at low temperatures.

One of the most interesting findings of this study is the seasonal occurrence of wasp parasitoid species, because no relative data is available. Among the species found, *H. hebetor* and *V. canescens* parasitize Lepidoptera, while the others Coleoptera. Comparing to the total number of insects caught, from Table 1 it becomes evident that hymenopterous individuals correspond to approx. one third of the total. In a recent survey in Greece, Eliopoulos et al. (2002a) found that, among several categories of stored products, dried fruit had the highest parasitoid presence. This is due to the fact that these products are usually heavily infested due to poor hygienic conditions, long-term storage etc. According to our results, adult wasps were active during the entire experimental period. *H. sylvanidis* is a species which usually parasitizes *Oryzaephilus* and *Cryptolestes* species. The decline of these species’ presence (mainly *Oryzaephilus*) could be partially attributed to the activity of this species. Similarly, the highest activity of *H. hebetor*, occurs during the high moth densities. These species may be very promising control agents, and the fact that they appear at high host densities, must be taken into consideration.

Reliable monitoring is essential for control strategies, under the IPM principles. The sticky traps (baited or not) can be utilized as decision tools, and the knowledge of insect activity in stored-currant facilities eliminates ‘blind’ insecticidal treatments. Apart from damaging pests, adhesive traps are capable of detecting parasitoid species as well. Due to their importance, these species can be incorporated in an integrated system of insect management in dried fruit.

**References**


Technical and economical validation of the integrated production of grape in the aquitaine vineyard
Report after three years of study (2000 - 2002)

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Context

After publishing the Integrated Production referential, we need to give to the vinegrowing path references which will allow to engage it through the vine farm in the IP way.

Definition of the technical way
Evaluation of the cost
Definition of the tools to drive this system of production (modus operandi, self checking of the technical ways...)
Definition of relevant criteria to evaluate

The tools created allow the traceability which is the only solution for the vinegrower to check what he is doing or what he has done, and to answer to these two questions:

a) feasibility of the IP in vinegrowing: is it possible on a technical level as well on an economical level
b) how vinegrowers adjust themselves, do they upgrade their methods. Then to optimize the transition of a lots of producers, we need to observe at first a population of leader.

Then we noticed that we must create all the necessary tools for our study, because we have not found any adapted one so far.

These convenient tools will be in a second time given to the vinegrowers in order to help them to built their own IP process for their farm.

I – Creation of the Tools for the Study

Creation of a network of “reference farms”

In 2002 this network was composed of 17 volunteer farms engaged in the IP concept. By their varied schemes of production (size – designation of origin...), these farms present a wide range of situations which fits the reality of the regional wine farms.

In this way, this network is used as an observatory. For each farm, a precise analysis of how the necessary adaptations are done, and how the practices are altered in which period of time, and type of organisation including the human logistic (working time – reorganisation of working schedule – training of the workers). The costs and the eventual savings are also analysed.

The drafting of the referential “Integrated production of grape in Aquitaine” by a working group from the National Referential of IP. It has been validated by the professional (2000 – 2001).

Creation of a technical diagnosis booklet which allow to situate the farm compared to the technical methods and requirements of the IP in vinegrowing. It has been published at the end of 2002 after its validation on tree campaign.
This Technical diagnosis booklet is a data entry form in which behavioural or equipment’s indicators are presented to the vinegrower. Divided into seven chapters, the indicators are a direct result of the reading of the referential for the IP.

The evaluation done allows to express in percentage the objectives filled compared to the laid down objectives of each chapter.

A picture of the situation of the technical level is given by a diagram which allows to show the situation at a given date but also the evolution obtained on many years compared to the lowest objectives of the IP.

Creation of a data entry form of the traceability
A data entry form of traceability has been established allowing to list all the information needed to be registered in order to fill out the technical diagnosis booklet but also to evaluate what is already registered and the quality of it.

Follow-up book
To registered all the interventions done on the farm, a follow-up book which is made of input charts, is necessary. These follow-up book is divided in two part one for the structural data on the farm (like age of each parcels, density ...), the other part for the data of the vintage (like spraying dates, pesticides used....). The creation of these tool has been started with the vinegrowers of the network.

Fig. 1: Functioning of an farm in integrated production of grape.

To ensure the viability and the cost. effectiveness
To ensure the viability and the cost. effectiveness

Financial

To control the cost.

Cost due to the IP referential

To preserve human health

Social

To select the workers

Marketing strategy

To know the good working methods

environment

To treat the garbage
To favor biodiversity
To take care of the landscape
To preserve the soil and the water

Economical diagnosis of the IP farm
To define a relevant system of information, you must first know the working of a farm in IP. A model is the simplified view of the functioning of a system.

In our case the system will be a farm in IP and the process is the evolution from a conventional vinegrowing to a integrated production of grape. The model give us the rules to run of the information system.

At first, the ITV wanted to evaluate the cost due to the application of the referential. The economists of the ENITA suggested us to also take in consideration the particular social and financial aspect of each farm. The model also includes an environment field which is evaluated through the IP technical diagnosis booklet.

The first illustration shows the links between the different fields. Each field is describe by indicators taken in the information system.
The implementation of the study

The implementation of the different tools described above, has allowed to evaluate the technical level of the farms and to show the evolving of each one. The first economical approach started in 2002 remains experimental.

II - References Acquired after Three Years of Study

Technical evaluation

Results after the first year

The results after the two first years show a good technical level on the farms of the network compared to the IP objectives. The percentage of filled up objectives is already high on the different technical chapters.

On certain aspects, methods must evolve like:

Fig. 2. Technical and economical validation of IP: Average of the engagements filled by chapter. Farms of references network - Aquitaine 2002

The fertilization is often carried out without doing the analysis of soil or foliar.

The choice of the weedkillers, of insecticides may be not in conformity with the positive list of plant health products.

The green cover, in a general way, often remains insufficient (turn spaces,...).

The methods of prophylaxis are not correctly implemented

But it is especially on the chapters "material of pulverization" and "management of the waste water and garbage" where the principal difficulties are.

The material of pulverisation would require to be better equipped and controlled. Broadly, the material is old and the design does not allow a sufficient control of the drifts during the applications of the pesticides.

The tanks flat-bottomed generate too important volumes of spray at the end of a treatment.

None of the exploitation is organized to control volumes of pulverization effluents, nor to recover or treat them. The existence of clear water tank assembled on the material allowing the rinsing of the pulverizer in the vineyard remains an exception.

Storage facilities of the pesticides are not in conformity with the legislation in force. The rules of individual protection are badly complied with, and felt like difficult to implement.
After the first assessment, a strong will of adaptation of the vinegrowers

Following the first diagnosis, after awareness of the problems and dialogue with the technical advisers of the farm, the vinegrowers have set improvement objectives. Our observations make it possible to better identify the progress achieved on one or two campaigns; all the farms are progressing.

Of course, the adaptations are done at the rhythm of each winegrower. Financial means of the exploitation influences the choices, in particular within the time period of certain investment (storage facilities of pesticides, ...).

Certain farms equipped themselves quickly, others adapt, organize themselves differently while waiting to carry out the investment (dilution and spraying of the left over at the end of a treatment,...). All the farms are approaching the advises presented in the IP Bordeaux referential.

Figure 3 illustrates the improvement noted between 2000 and 2002 for a farm of the network.

Fig. 3: Technical and economical validation of IP: Compared results of 2000, 2001, 2002 on the exploitation B

III - Transfer of the Tools towards the Development Agents

We make the report of a fast transfer of the tools "reference frame" and "technical diagnosis booklet" to the level of the development agents, and this, upon their request.

Prospects

Only the observation of the network over several years can make it possible to conclude as well on the effective feasibility of the IP in vine growing, as on the technical routes allowing an optimized transition from the "conventional" vinegrowing to an "integrated" vinegrowing.

In parallel, the economic stage of the study will be led on all the exploitations of the network. The finalization of the traceability data entry form and the follow-up book make it possible to have the complete panel of the tools, as much for our study than for the future development phase .

With this technical panel, the tools in working progress will be added, this will allow to establish a economical diagnosis of the IP farms. In addition a data base will be built allowing a better management and development of the information obtained.
Proposal of review of the third IOBC Technical Guideline for the Integrated Production of Grapes

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More than reconsidering, it concerns completing the technical guidelines published in 1999 (2\textsuperscript{nd} version), and more particularly:

\(\Rightarrow\) Chapter 10 – “Efficiency and security of the spraying methods”
- a better precision of the conditions to respect in the use of the pesticides treatments.
- to strongly encourage (at least when the equipment is renewed) the vine growers choice towards better adapted equipments to control the diffused or sporadic pollution and human security

\(\Rightarrow\) An other chapter (which would be the chapter 11) entitled: “health, security, and environment protection during the handling of the pesticides”

This chapter would complete the guideline in terms of:
- physical protection of the user exposed to the chemicals
- specific training on risks and way of controlling them
- material condition of stockage and handling of products, and the concoction of the chemicals in order to protect men and environment
- empty packaging management
- the management of the bottom of the atomizer tank and of the rinsing waste water

\(\Rightarrow\) The actual chapter 11: “Organisation inspection procedure” would become the chapter 12.

10 – Efficiency and security of the spaying methods

The atomizer must allow to treat each side of each row of vine. It must be equipped with stop drop system on the nozzles. If proper atomizer do not exist on the market, the direct treatment of each side of each vine is not obligatory. The regional guidelines will have to precise in a very restrictive way these kind of derogation.

The aimed spray on the grape bearing (grape caterpillar, grey mould…) will required to be realised by the mean of atomizers which will allow to treat each side of the row of the vine. The number of nozzles would be adapted to the treated leaves area.

The type of atomizer and using conditions must favour the security of the users, and minimize risks of spray drift. It is recommended not to spray by windy weather.

The atomizers must be fixed and tested at each beginning of the campaign and the good working must be verified before each treatment.

A diagnosis on the state of the atomizer will have to be realised every 3 years according to the CIETAP protocol by a registered agent. Other years, a diagnosis will be done by the vinegrower himself.
The impact on the environment can be minimised by calculating the dose/ha required for a given phenologic stage. The surface of the leave in complete vegetation and the vine training should be taken into account.

At the starting of the vegetation, only the necessary nozzles will be used.

In case the equipment is renewed, the choice will be oriented towards atomizers allowing treatments on each side of the row.

The atomizer must be equipped with a tank full of water, and the bottom of the tank must allow to finish the treatment with a tank practically empty.

The spray must be calculated precisely in order to minimise the left over at the end of the treatment.

The use of the helicopter and oscillating materials are forbidden excepted if a derogation is given:

  Derogation concerning the use of the helicopter :
  - If the access to the parcel is impossible because of exceptional weather conditions or
    if the topography of the parcel does not allow any other way of spraying.
  - In a collective fight against *Scaphoideus titanus*

Derogation concerning the use of the oscillating materials
  - If the slope of the parcel is over 20%

11 – Health, security, and environment protection during handling of the pesticides

To every workers using pesticide a training is given concerning the risks and the means to avoid them.

An individual and adapted protective equipment must be used during pesticide handling and spraying.

The storage of the pesticides must be done in a room specially arranged (waterproof, well ventilated...) and locked (see “TAM” document).

During the handling of the pesticides, in order to avoid a direct pollution of water, a sufficient distance must be respected from rivers or sewages.

During the fill up of the tank, be sure that there aren’t any risks of overflowing or contamination of the water system by sucking up.

The pesticides wrapping mustn’t be burnt or buried. They must be correctly washed out and thrown out with garbage according to the local laws or recycled when it is possible.

The pesticides which are not used and not taken back by the distributor must be treated by a specialized and authorized company.

The management of the left over and the first rinsing of the atomizer must be done by using a specific full of water tank and using nozzles installed inside the spraying tank. The diluted preparation must be pulverized on vine already treated (or on turn spaces with green cover)

The washing out of the external part of the atomizer mustn’t be done near human habitation, rivers or sewage, excepted if it exists a specific place to clean the atomizer.

**Conclusion**

The risk concerning the use of chemical products for men as well as the environment seems to justify a real rigor of each concerned users on the farm.
In fact, the improvement concerning the forecast of the risks methods and the alternative against pesticides already allow to reduce the volume of the spraying on the vineyard. However, the use of pesticide remains necessary in many “risky” situations for the vineyard. The number of treatments will remain important for a medium or even long term.

Thus, it would be appropriate to emphasize on the preciseness needed to accomplish the operations.
Pest Management and grape production quality

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Abstract: In this work we tried to evaluated differences between two systems: integrated production system and traditional way.

It was observed the evaluation of grapes’ diseases and pests and parameters from wine was measured.

The field observations were carried out from March to September and laboratory measurements were developed in August and in October 2002.

Key words: pest management, grape production.

Introduction

In Portugal, Guidelines for Integrated Production of Grapes, which allow farmers to produce under this production system and codify the associated cultural practices, were only officially published in March 2001, six years after the official adoption of IPM practice on grapes.

The aim of this study was to evaluate the effects of indirect measures and direct control measures on the grapes’ quality achieved through this production system.

Material and methods

This study was made in a vineyard located in Cartaxo, Ribatejo, during 2002.

During the vegetative growth phase the main diseases and pests infestation levels were observed in two fields, with 1,4 ha each: one of them followed the integrated production system and the other the traditional way.

Concerning diseases assessment, the risk estimate was determined including the risk periods, the nocivity factors and the intensity of diseases adopting the following scale: 0 – absent; 1 – incipient presence; 2 – median infestation; 3 – heavy infestation. After this first step it was analysed the need for a treatment, and if positive the pesticides most harmless to Human and environment measures were chosen.

Concerning pests, 100 grapes or leaves were observed, depending on the pest, to determinate the intensity of infestation and by comparison with the established thresholds levels it was made the decision about the necessity of a treatment.

In Table 1 are presented the treatments that were made in the integrated production system and in the traditional way. The decision to make the treatments in the integrated production system was taken after the observations in the field, for diseases and pests, and considering the climatic data, mainly temperature and precipitation. The treatments in the traditional way were made according with typical decision from the regions’ farmers and with the active substances that they use.
Table 1. Treatments in both fields during 2002.

<table>
<thead>
<tr>
<th>Enemy</th>
<th>Date</th>
<th>Active substance</th>
<th>Enemy</th>
<th>Date</th>
<th>Active substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>downy mildew</td>
<td>12-4-02</td>
<td>fosetyl+folpet</td>
<td>Downy mildew</td>
<td>26-3-02</td>
<td>cimoxanil+folpet+mancozeb</td>
</tr>
<tr>
<td></td>
<td>15-5-02</td>
<td>cimoxanil+folpet</td>
<td>09-4-02</td>
<td></td>
<td>fosetyl+folpet</td>
</tr>
<tr>
<td></td>
<td>12-6-02</td>
<td>copper hydroxide</td>
<td>23-4-02</td>
<td></td>
<td>cimoxanil+folpet+mancozeb</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>07-5-02</td>
<td></td>
<td>cimoxanil+mancozeb</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21-5-02</td>
<td></td>
<td>cimoxanil+copper oxichlorate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12-6-02</td>
<td></td>
<td>copper hydroxide</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28-6-02</td>
<td></td>
<td>copper oxichlorate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17-7-02</td>
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</tr>
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<td></td>
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<td>31-7-02</td>
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<td>dust sulphur</td>
<td>26-3-02</td>
<td></td>
<td>wp. sulphur</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
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<td>07-5-02</td>
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<td>myclobutanil</td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td>dust sulphur</td>
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<td>6</td>
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<td></td>
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<td>phosalone</td>
<td>grape berry moth</td>
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<td>dimethoate</td>
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<td></td>
<td></td>
<td></td>
<td>31-7-02</td>
<td>dimethoate</td>
</tr>
<tr>
<td>total pest</td>
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<tr>
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<td></td>
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<td>25</td>
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</table>
Table 2. Observation of diseases during the vegetative growth in the two fields, during 2002 (es – escorose, d - dry).

<table>
<thead>
<tr>
<th>Date</th>
<th>Integrated protection</th>
<th>Traditional protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>downy mildew</td>
<td>powdery mildew</td>
</tr>
<tr>
<td>25-Mar</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>01-Abr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>08-Abr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-Abr</td>
<td>0</td>
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</tr>
<tr>
<td>23-Abr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>29-Abr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>06-Mai</td>
<td>0</td>
<td>0</td>
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<td>14-Mai</td>
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<td>27-Mai</td>
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<td>0</td>
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<tr>
<td>26-Ago</td>
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</table>

Table 3. Efficacy evaluation on 1st July and 28th August in 2002.

<table>
<thead>
<tr>
<th></th>
<th>Incidence (%)</th>
<th>Severity (%)</th>
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<tbody>
<tr>
<td></td>
<td>1-7-03</td>
<td>28-8-03</td>
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<tr>
<td><strong>Integrated production</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>downy mildew leaf</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>downy mildew grape</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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In the tradition way dimethoate was used for grape berry moth, that is forbidden in the integrated production because it is very toxic to phytoseiid mites and for more two groups of beneficials. Also two active substances (cimoxanil+ folpet+mancozeb, cimoxanil+mancozeb) were used that have restriction, they can only be used twice because risk of resistance.

During grape maturation, weight of grapes, pH, total acidity and predictable alcohol content were measured from 100 grapes representative of the field.

After vinification, alcohol content, total acidity, volatile acidity and pH were also evaluated.

Results

The results of observations of diseases are presented on Table 2. The efficacy evaluation was made on 1st July and 28th August (Table 3). The results of observations of pests are presented on Table 4. The flight curves of grape berry moth and of leafhopper adults are presented in Fig. 1. Fig. 2 presents the grapes’ weight evaluations for both systems.

![Fig. 1. Flight curve of grape berry moth and of leafhopper adults, during 2002](image1)

![Fig. 2. Weight of 100 grapes in the two different production systems, during 2002 (IP – integrated production; TP – traditional production.)](image2)
Table 4. Observation of pests during the vegetative growth in the two fields, during 2002 (ca – infested grape; n – nest; e – egg; pf - perforation.; l – larvae; p –pupae; em – empty; d – dead; ny – nymph; if – infested leaf; fi – phytophagous; fs – phytoseid).

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**Integrated protection**

**Traditional protection**

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The evaluation of pH, total acidity and predictable alcohol during the maturation period for grapes produced in tradition way are presented in Fig. 3 and the same measures for the grapes produced in the integrated production can be observed in Fig. 4.

**Fig. 3.** Evolution of pH, total acidity and predictable alcohol during the maturation period from 100 grapes produced in the tradition way, in 2002 (TP – traditional production.)

**Fig. 4.** Evolution of pH, total acidity and predictable alcohol during the maturation period from 100 grapes produced in the integrated production, in 2002 (IP – integrated production.)
In Table 5 parameters are presented, measured immediately after the vinification and a few days later, in both systems.

Table 5. Parameters measured just after the vinification and a few days later (IP – integrated production; TP – traditional production).

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Conclusion

The efficacy evaluation on 1st July showed no losses related with downy mildew and grey mould in the integrated production field and the same for downy mildew in the traditional production. Losses in the integrated production were 0,20% of leafs and 0,68% of grapes caused by powdery mildew and in the traditional production were 0,31% on leafs and 0,30% of grapes caused by powdery mildew, too, and 0,062% of grapes caused by grey mould. On the 28th August, integrated production modality showed, on grapes, 0,68% of losses caused by grey mould and no other losses. In the traditional production modality the losses were higher: 0,31% of grapes with powdery mildew and 1,75% of leafs and 6% of grapes with grey mould. Concerning pests more than 59 perforations caused by grape berry moth were observed in the traditional production system.

The determination of wine parameters indicated no differences between the two alternatives.

Acknowledgments

We would like to thank the Laboratório Ferreira Lapa, specially Prof. Jorge Ricardo-da-Silva, Mr. António Marçal and Mrs. Graziela Rodrigues.

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References

Integrated Weed Management in *Oxalis* infested vineyards of Crete

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\textsuperscript{2} Higher Technological Educational Institute of Crete, School of Agricultural Technology, 711 10 Heraklion, Greece

**Description and biology of *Oxalis pes-caprae***

*Oxalis* (*Oxalis pes-caprae* L.) is a common weed of vineyards in many areas of Greece and especially of Crete. This weed is an indigenous species of South Africa and it was introduced to the Mediterranean area at the beginning of 19th Century probably as an ornamental plant. Except of vineyards, *Oxalis* is a common weed of olive and citrus orchards in Central and Southern Greece as well as in other Mediterranean countries. In many cases, animal poisoning has been reported especially in sheep fed on this weed. This is due to the high oxalate content of the plants interfering with the calcium metabolism of the animals.

Flower of *Oxalis pes-caprae* are tristylic and described as short, mid and long styled, depending on the position of the stigmas relative to the two whorls of anthers.

The species *Oxalis pes-caprae* rarely sets seeds and plants grow from underground bulbs. In some *Oxalis* plants, a number (9-10) of tumbleweeds (small bulbils) are grown on the axils of the rodax of the leaves. These small bulbils, together with the dried *Oxalis* leaves, form a mechanism of aerial dispersion, explaining the long distance dispersal of this not seed producing weed (Damanakis and Markaki, 1990).

The bulbs are believed to be truly annual, sprouting in one season and producing new bulbs of variable size to carry the population over to the next season (Marshall, 1987). The bulbs are formed at depths of up to 30 cm and these are important organs of growth, perennation and dispersal. A contractile root forms soon after sprouting by the enlargement of one of the roots arising at the base of the sprouted bulb. It has the dual purpose of water and nutrient storage and assisting in bulb dispersal (Peirce, 1997).

**Oxalis as a weed problem in vineyards of Crete**

*Oxalis* is a winter weed and in general it is not considered a serious weed problem in vineyards in Greece. However, *Oxalis* can disturb the utilization of the fertilizer applied in the vineyards at the end of winter as well as the effectiveness of residual herbicides if they are applied without previous destruction of *Oxalis* vegetation. In Crete and other regions of Southern Greece (Peloponissos, Cyclades etc.) there are serious reservations about the eradication of *Oxalis*, especially in cases of vineyards located on slopes of poor soil. The coverage of such places during the winter, by a thick carpet of *Oxalis* vegetation, protects the soil from erosion. Later, after the end of the biological cycle of the weed, in late spring, the foliar and root debris of *Oxalis* plants which remain on the soil, provide it with organic matter. The numerous underground tunnels, opened by *Oxalis* contractile roots, contribute to the aeration of the soil and help it to retain the water from the few rare spring rains.
The emergence of *Oxalis* in Crete begins at the middle of September - middle of October, depending on the occurrence of autumn rains. *Oxalis* bulbs are unable to grow earlier because of their dormancy. Flowering begins normally at the middle of December and it continues until April, depending on climatic conditions and location. By the end of April, the aerial part of the plant dries out and the formation of new bulbs in the underground part is completed. In many locations in Crete, a biological control of *Oxalis* vegetation is obtained due to parasitism of the weed by broomrape (*Orobanche* sp.). The parasite emerges and starts to parasitize *Oxalis* when the later is at the flowering stage, resulting to the earlier decline of *Oxalis* vegetation and the end of its biological cycle (Paspatis, 1987).

Allelochemical potential of *Oxalis* as a tool for establishing Integrated Weed Management System in vineyards

One of the most important advantages gained from the presence of *Oxalis* weed cover in vineyards, is the inhibition of emergence, growth and development of many difficult to control weeds such as *Parietaria* sp., *Amaranthus* sp., *Chenopodium* sp. etc. These inhibition phenomena occur possibly due to the strong allelopathic potential of *Oxalis*. Results of recent experiments showed that extracts of fresh *Oxalis* plants as well as root excretions of the same plants, caused a significant decrease of seed germinability and the seedling growth of oat and tomato. Water extracts of dried *Oxalis* have also proven very phytotoxic to aqueous plant *Lemna* sp. used as an index plant in the homonymous bioassay (Paspatis, 2002). Due to above mentioned characteristics of *Oxalis*, most vine growers of Crete and other areas of Greece consider it as a useful cover plant than a serious weed problem.

In *Oxalis* infested vineyards in Crete, where the weed vegetation forms a thick weed carpet covering the soil during winter, various weed control methods are applied. The combination of the cultural, physical, biological or chemical weed control methods applied in *Oxalis* infested vineyards, constitutes the basis of a practical, efficient and environmentally friendly Integrated Weed Management System. Main target of this system is to establish a dense carpet of *Oxalis* during the winter months (when the water needs of vines are limited) and the control of the weed at the beginning of the spring, after the formation of its propagation organs but before it begins to antagonize the vines for water and nutritional elements. Despite of its vigorous vegetative growth, *Oxalis* is a low fertilizer consumer in well fertilized vineyards (Damanakis and Markaki, 1990).

Integrated Weed Management in viticultural practice in Crete

The Integrated Weed Management System mentioned above, modified in each case according to weed problems and the local economic and climatic conditions, includes the following treatments:

A mechanical tillage of the middles, between vine rows (using harrows or rotary cultivators) is carried out at the end of February, aiming to control this natural cover plant and to incorporate the fertilizers added (Fisarakis, 1999). Control of *Oxalis* plants, grown on the vine trunks row, is carried out if necessary, using a contact herbicide (such as paraquat or glufosinate ammonium) or a rotary cultivator. The use for this purpose, of a row plow, is limited only to some big cooperative vineyards. In some cases, the mechanical tillage of the middles may be completely avoided, due to the natural aeration obtained by the *Oxalis* rooting system.
In some vineyards in Crete, conventional mechanical tillage of the middles is being replaced by the application, at the end of winter, of a contact herbicide (paraquat or gluphosinate ammonium) or by mowing *Oxalis* vegetation, in combination with the use of a cane shredder, after the end of pruning. In this way, a soil mulch from mowed *Oxalis* and cane shreds is formed. Non-tillage or mowing of *Oxalis* vegetation is very adaptable under drip irrigation status. The advantages of these techniques include lower management cost, reduced soil residues of herbicides and reduced erosion on steep terrain.

During summer months, annual weeds are controlled, if necessary, by mowing or a post-emergence directed chemical application, using a contact herbicide (paraquat or gluphosinate ammonium). In the case of presence of perennial weeds such as *Cynodon dactylon*, *Convolvulus arvensis*, *Sorghum halepense* etc., a post emergence spot application of a systemic, selective (fluazifop butyl) or non selective (glyphosate), herbicide is also carried out.

References


Integrated Pest Management in viticulture:
The current status in Greece

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Abstract: Vineyards represent about 4.5% of the cultivated surface of the country. Vine products represent 3.02% of the total agricultural product of our country. The key pest in Greece is Lobesia botrana (Lepidoptera: Tortricidae). Another pest that tends to be serious lately is Frankliniella occidentalis (Thysanoptera: Thripidae). Only small scale trials for application of Integrated Pest Management and also for Biological Control are conducted in vineyards in Greece. Conventional control, using plant protection products at the correct time, is the rule for vineyard protection. Mating disruption has been used and was effective when applied in isolated vineyards with low infestation but not in all cases. Priority is given to natural, cultural, biological, genetic and biotechnical methods at the establishing of new vineyards. To apply Integrated Pest Management we should have detailed and scientific information on insect biology, host preference and alternative hosts and ecology. On this field there was detailed data, many years of experience and very useful results. L. botrana completes 3-4 generations per year in Greece. L. botrana is capable of developing on several potential wild or cultivated host plants commonly found adjacent to the vineyards. They could serve as reservoirs for populations of L. botrana especially early in spring. Such a source of infestation should be taken into consideration when planning control methods. Scientifically established assessment methods appropriate to region or locality are necessary to be known and some trials are conducted. Development thresholds for Macedonia (northern Greece) showed that the lower development threshold was 7.3°C for egg to adult development. Degree days above 7.3°C required by L. botrana males and females to complete the egg, larval, pupal and egg to adult stages were 86.2, 461.2, 155.1 and 702.5 respectively. Non linear models predicted with sufficient accuracy the accumulated male moth catch and they accounted for the 60-78% and 86-91% of year-to-year variation in male flight activity in Thessaloniki and Naoussa, respectively. We also try to find out the harm and damage caused by the larvae to the berries of eleven varieties in the field and the damage in relation to Botrytis cinerea and other microorganisms that also developed on the grapes. One year experience showed that the economic thresholds for the 2nd generation fluctuated between 9-25% for application of wetable powder and 18-26% for dust. In the 3rd generation the respective percentages are 7-20 and 11-20. Non compact clusters showed higher thresholds than compact ones, when wetable powder was used. White varieties showed lower thresholds than the black ones in both generations tested. Having all the above and more research results, in order to organize an integrated pest control management, we have to examine the severity of the problem, to make a decision on the most appropriate method of control and to apply a treatment, having in mind the economic thresholds, timing etc. Where the use of plant protection products is necessary, the selected products must be the least hazardous to human beings, livestock and the environment, whilst providing the most effective control to the pest as well as being as safe as possible to key natural enemies.

Key words: Integrated Pest Management, Lobesia botrana, development thresholds, economic thresholds
**Introduction**

Vineyards cover about 132,000 ha in Greece. Wine grapes cover 53.5% of this surface, raisin grapes cover 35.0%, while table grapes cover the 11.5%. Vineyards represent about 4.5% of the cultivated surface of the country, 3.7% of vineyards of the European Union and 1.7% of the world vineyard.

Individual property is always small and covers 0.4-1.4 ha. Vine products represent 3.02% of the total agricultural product of our country. The wine varieties cultivated are white such as: Savatiano, Asirtiko, Debina, Athiri, Vilana, Robola or colored such as: Aigiorgitiko, Xinomavro, Linnio, Kotsifali, Mandilaria, Romeiko, Liatiko, Roditis, Moschofilero, Mavro Mesenikola. The table varieties cultivated are: Soultanina, Razaki, Victoria, Muscat d’Hambourg, Cardinal, Red Fraoula, Italia, Calmeria, Perlette, Ribier

**Pest control**

The key pest in Greece is *Lobesia botrana* (Lepidoptera: Tortricidae). Another pest that tends to be serious lately is *Frankliniella occidentalis* (Thysanoptera: Thripidae).

Only small scale trials for application of Integrated Pest Management and also for Biological Control are conducted in vineyards in Greece. Conventional control, using plant protection products at the correct time, is the rule for vineyard protection. For this, an Agricultural Warning Stations net, send instructions to the producers as to when and how to protect their vineyards.

Mating disruption has been used and was effective when applied in isolated vineyards with low infestation but not in all cases (Tsitsipis et al. 1995, Zartaloudis et al. 1997, Moschos et al. 1998). Therefore, more research needs to be done before we are sure that in the extremely dry and hot conditions during summer it could be an effective method for *L. botrana* control, which could be used in large surfaces from many producers.

Priority is given to natural, cultural, biological, genetic and biotechnical methods at the establishing of new vineyards.

**Research results available for organizing Integrated Pest Management**

To apply Integrated Pest Management we should have detailed and scientific information on insect biology, host preference and alternative hosts and ecology, and on this field we have detailed data, many years of experience and very useful results that I will try to present briefly:

Regarding the insect’s biology, information is available for Crete in the southern Greece (Stockel et al. 1990), Attiki in central Greece (Moschos et al.1998), and Macedonia (Thessaloniki, Naoussa) in northern Greece. Based on these observations, the insect completes 3-4 generations per year. Population fluctuation is regularly monitored and recorded for years also. Relationship between catches in traps and damage was found in the second generation (Savopoulou-Soultani et al. 1989).

Regarding the host plant preference much work has already been done and in some locations like Crete it is continued. Since *L. botrana* flight starts early in spring before the appearance of the appropriate stage for oviposition on vine, we try to understand the insect’s survival, searching for potential hosts that are in an appropriate stage before vine. Relation of insect’s development as a consequence of its ability to develop on other host plants was studied (Savopoulou-Soultani and Tzanakakis 1988, Roditakis 1989, Savopoulou-Soultani et al. 1990, Stavridis and Savopoulou-Soultani 1998) and it was found that the rate of larval
development was significantly faster on olive than on vine inflorescences. In the field, the number of eggs per female and the coefficient of multiplication of the insect’s population from generation to generation, was greater on olive than on vine inflorescences. In two-choice tests in the laboratory, vine inflorescences were preferred for oviposition to olive inflorescences, and to vine and olive leaves. In no-choice tests, vine leaves, vine inflorescences, olive leaves and olive inflorescences in the least advanced stage, were all equally accepted for oviposition. Among other plants tested, it was found that larvae of *L. botrana* had higher survival (30-35% in the laboratory and 10-15% in the field experiments) and shorter development time when reared on *Prunus persica nectarina*, *Prunus domestica*, *Taraxacum officinale*. In no choice tests in the laboratory the insect oviposits on flowers of these plants as well as on the fruits. More eggs were laid on fruits than on flowers. However, larval development was generally shorter on flowers than on fruits. The mentioned plants blossom earlier than vine and could be used as alternative hosts by the females. The above experiments demonstrate that *L. botrana* is capable of development on several potential wild or cultivated host plants commonly found adjacent to the vineyards. They could serve as reservoirs for populations of *L. botrana* especially early in spring. Such a source of infestation should be taken into consideration when planning control methods. Other potential hosts may also play a significant role in the maintenance of the insect and multiplication of its population, in areas where precocious grape varieties are cultivated or temperature conditions are suitable for insect’s development up to October or November. In these areas, males are caught in pheromone traps up to then, after the harvest of the grapes in late August-early September. This suggests that the females of the third and fourth generations may oviposit and the larvae may develop on alternative host plants.

Regarding the damage to grapes in relation to the variety and ripeness, research was done to find out the relation between duration of larval development on grapes of different stages of maturity of five of the cultivated varieties in Greece namely Sultanina, Muscat d’ Hambourg, Razaki, Italia and Xinomavro (Savopoulou-Soultani, Nikolaou and Milonas 1999). It was found that larval development was faster on midmature or mature berries depending on the variety. The fastest development was observed on Razaki. The observed differences were associated with differences in the sugar content and acidity during the process of berry maturity, although other unmeasured factors are likely to be important.

We also try to find out the harm and damage caused by the larvae to the berries of eleven varieties in the field (Ifoulis and Savopoulou-Soultani 2003).

The damage in relation to *Botrytis cinerea* and other microorganisms developed on the grapes was also investigated (Savopoulou-Soultani and Tzanakakis 1988, Savopoulou-Soultani et al. 1997). It was found that *L. botrana* larvae developed faster on grapes infected with the fungus *B. cinerea* and the yield in adults was higher than in healthy berries. This resulted in a 2.2 to 2.5 fold population increase on grapes infected as compared with fruits not infected with the fungus. Therefore, if the observed faster larval development due to the fungus, occurs also in the field, the infection of berries by it should be taken into consideration when developing predictive models.

Scientifically established assessment methods appropriate to region or locality are necessary to be known. To do so, we need data on flight monitoring with pheromone traps, relationship between catches of males in the traps and damage to grapes, if it exists, and observations. Furthermore, a Net of Forecasting Stations recording the climatic conditions, especially temperature, is also needed.

Development thresholds for Macedonia (northern Greece) showed that the lower developmental threshold was 7.3°C for egg to adult development and ranged from 6.3°C for larval stage to 7.4°C for egg stage. Degree days above 7.3°C required by *L. botrana* males and
females to complete the egg, larval, pupal and egg to adult stages were 86.2, 461.2, 155.1 and 702.5 respectively (Savopoulou-Soultani et al. 1996). Fecundity, preoviposition and oviposition period, and adult longevity were reduced with increasing temperature. Age-specific life-fertility tables and survivorship curves of adults were constructed for 15, 20 and 25ºC. Based on such data we try to construct prediction models in Northern Greece (Milonas et al. 2001). The generation time of L. botrana was estimated as the number of day-degrees required between the start of the flight and the start of the following flight in two regions. The day-degrees required for the first generation were 339.3 and 275.6 for Naoussa and Thessaloniki respectively and they were significantly shorter than for the 2nd and 3rd generations. Non linear models were developed using trap catches of males for predicting its flight activity. A lower threshold of 6.45ºC was used in calculating daily day-degrees from the 1st of March. The models predicted with sufficient accuracy the accumulated male moth catch and they accounted for the 60-78% and 86-91% of year-to-year variation in male flight activity in Thessaloniki and Naoussa, respectively. This level of accuracy is probably sufficient when using long-residual materials but is probably insufficient for short–residual materials such as microbials. This might indicate that a warm period could lead to prolonged flight activity, as expressed in terms of day-degrees, and the flight curves became flatter. It is also possible that the lack of an upper threshold decreases the predictive ability of the model. The better accuracy of the model in Naoussa could be due to a lower mean air temperature and a shorter flight period than in Thessaloniki. Furthermore, vineyards in Naoussa consist of one variety while in Thessaloniki there is a complex of varieties resulting in differences in duration of larval development. Considering the increasing interest for biorational insecticides where precise timing of treatments is extremely important, day-degree models could be a useful tool improving their efficacy. This first model is the base for such improvements in order to cover more locations and be more predictable. Some more trials are conducted in Crete and Attiki by other scientific groups on this subject.

We also tried to estimate the approximate level of infestation or the damage by establishing thresholds for 2nd and 3rd generation of L. botrana on eleven vine varieties and 2 formulations of Bacillus thuringiensis, to have a base for the decision as to whether or not treatment is required. We also tried to find out the relationship of the economic thresholds with the morphological characteristics of the cultivated varieties (Ifoulis and Savopoulou-Soultani, unpublished data).

One year experience showed that the economic thresholds for the 2nd generation fluctuated between 9-25% for application of wettable powder and 18-26% for dust. In the 3rd generation the respective percentages are 7-20 and 11-20. Non compact clusters showed higher thresholds than compact ones, when wettable powder was used. White varieties showed lower thresholds than the black ones in both generations tested. (Ifoulis and Savopoulou-Soultani, unpublished data).

For Frankliniella occidentalis as far as I know the chemical control is always used. We can see the kind of damage on grape berries. In compact clusters the point where berries touch one another is shown. In a preliminary experiment we found that the presence of weeds in the vineyard is related with the population of the insect.

Other potential pests are the following: Viteus vitifoliae, Sparganothis pilleriana, Theresimina ampelophaga, Noctuidae (Agrotis sp.), Anomala vitis, Bytiscus betulae, Otiorrhynchus sp., Planococcus sp., Cicadellidae.

Some beneficial insects and mites found in vineyards are the following: Dibrachis affinis, Ichneumon deceptor, Eulophus polychrosis, Phytomyptera nigrina, Crysoperla carnea, Phytoseius finitimus (Roditakis 1983, Papaioannou-Soulioti 1996). The first two species are
nymph parasitoids, the following two larval parasitoids. There were no data on their biology and effectiveness.

Conclusions

Having all the above and more research results, in order to organize an integrated pest control management, we have to examine the severity of the problem, to make a decision on the most appropriate method of control and to apply a treatment, having in mind the economic thresholds, timing etc. Where the use of plant protection products is necessary, the selected products must be the least hazardous to human beings, livestock and the environment, whilst providing the most effective control to the pest as well as being as safe as possible to key natural enemies.

It is the duty of scientists like us here, to collaborate in order to provide, through our research work, the new knowledge that will ultimately lead to a better protection of the vineyard from insects, mites, diseases weeds and other enemies. Such protection should be environmentally acceptable and in addition effective and economically bearable.

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Integrated production of grapes in Slovenia - experiences and some questions

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Abstract: The total surface of vineyards in Slovenia is approximately 25,000 ha and about 6,000 ha of them are included in the system of integrated production of grapes (IPG) until now. The system of integrated production, which is harmonised with Directive 2078/92 EEC, has been introduced in Slovenia in 1999 and for last two years the Slovenian Ministry of agriculture, forestry and food has financially supported it. Before this system was introduced, the prevailing system of grape production in Slovenia already contained the majority elements of IPG, but it was not officially controlled. The technical guidelines for integrated production of grapes are divided into two main parts. Its first part treats the use of fertilizers and soil management. The practical experiences in the last two years have shown that some technical questions are still are not clear, particularly the restrictions of nitrogen fertilization and changes of land techniques such as entire grass-covering or grass-covering in alternate rows. Much more complex is the situation concerning the integrated pest management, which is treated in the second part of the guidelines. Due to the reduction of insecticides against grape moths (Eupoecillia ambiguella and Lobesia botrana) or use of high selective insecticides from the group of insect growth modulators or Bacillus thurgiensis, the incidence and importance of some minor pests, particularly Neopulvinaria innumerabilis and Parthenolecanium corni has increased. The consequence of reduced use of sulphur against grapevine powdery mildew (Uncinula necator) the cases of significant damages of rust and gall mites (Calepitrimerus vitis, Colomerus vitis) are much more frequent. The result of grass covering as important issues of IPG, seems to be increased the incidence of stolbur phytoplasma (Grapevine bois noir phytoplasma) in some regions. The preliminary investigations of leaf- and planthoppers have shown the increased population of vector Hyalestes obsoletus in vineyards with entire grass-covering system. In the submediterranean region of Slovenia the grapevine powdery mildew (Uncinula necator) is the most important disease. Due to resistance problems on some IBE fungicides and strobilurines the use of dinocap was introduced again in the IPG strategy with maximum two treatments per year. Respecting the environmental and production factors the use of dinocap in our vineyards has no unacceptable negative consequences on beneficial fauna of arthropods. Several trials in Slovenia have shown that dinocap under the higher temperature conditions can reduce the populations of Phytoseiid mites in vineyards, but at the same time it also reduces the proliferation of phytophagous spider mites (Panonychus ulmi, Eotetranychus carpini) so in practise there is not observed significant change of biological equilibrium. The most common predaceous mites of the family Phytoseidae in Slovenian vineyards are Typhlodromus pyri and Amblyseius andersoni in the continental part of Slovenia, but Kampimodromus aberrans and Amblyseius andersoni in southwest (submediterranean) region. These mostly control the phytophagous spider mites enough, so that the use of acaricides in vineyards has decreased drastically in last few years. The acaricides are used periodically only, mostly against grapevine rust mite (Calepitrimerus vitis).

Key words: integrated grape production, integrated pest management, diseases, insects

Introduction

Slovenia is defined as a wine growing country - not because of its total surface of 25,000 ha, but of its structure. In all, 41 % of farms in Slovenia have vineyards and the share of wine-
growing in total agricultural output is approximately 9%. The share of agriculture and fishing in total domestic product is around 5%. The wine growing area in Slovenia is divided into three wine-growing regions as a consequence of the great variety of the landscape, different climatic and soil conditions or so-cold natural conditions and particularly there is a great variety of wine cultivars and technologies.

The Primorska wine-growing region is located in the west part of Slovenia where it bordered with Italy and the Adriatic Sea. The influence of the sea is mostly felt in the Koper district by the sea, but also in the three others districts further away from the sea. Most vineyards are situated on terraced slopes and planted with red and white varieties in equal shares. The Posavje wine growing region with three districts is located in south of Slovenia by the Sava river, bordered by Croatia. The Podravje wine growing region covers the north-eastern part of Slovenia. A great part of vineyards are located on steep hillsides planted with white varieties. It is divided into seven districts with their specific characteristics.

Up to three-quarters of Slovenia involves areas with limiting factors where the conditions of agricultural production are relatively challenging. Difficult natural conditions of Slovenian small parcels of agricultural land characterize agriculture and specially viticulture, as on average each farm only cultivates 0.7 ha of vineyard and only 1.2% of all wine-growers cultivate more than 5 ha of vineyards.

The second important characteristic of Slovenian viticulture is a great part of steep hillside viticulture – rightly defined as heroic viticulture. There are more than 31% of vineyards with incline over 30%. These two parameters - small parcels and inclination, which are linked to the quite low-level production intensity of viticulture in Slovenia lead to integrated grape production. In the last twenty years the system of production, which involves the majority of elements of integrated pest management, has been characteristic for Slovene grape production.

**Integrated grape production**

Since 2001 integrated grape production has been one of the twenty-two measures associated in the Slovene Agro-Environmental Programme. This programme was adopted by the Government of the Republic of Slovenia as the implementation component of the Programme of Agricultural Policy Reform. It is also an important part of the EU Common Agriculture Policy. SAEP is oriented towards the sustainable use of natural resources, preservation of biodiversity and characteristics of Slovene landscape and also towards decreasing negative impacts of agriculture on environment. The system of integrated grape production is harmonised with the Directive 2078/92 EEC. It has been introduced in Slovenia in 1999 and for the last two years the Slovenian Ministry of agriculture, forestry and food has financially supported it. The concept of integrated production from some European countries with similar environmental conditionals was applied in the preparation of the guidelines for Slovenia. The guidelines are divided into two parts. The use of fertilisers and soil management is defined in the first part. The instructions for integrated pest management are precisely described in the second part. The practical experiences in the last two years have shown that there are still some problems left. The main problem in the area of technology is the limitation of fertilisations and grass covering of vineyards.

In general, when the soil contains normal amounts of mineral elements, the general rule is to return the share of nutrients absorbed by the plants every year. It is necessary to take into account also the nutrients from the pruned wood, decomposed and mineralised leaves and shoots and the mineralised grass. Fertilisation with nitrogen fertilisers depends on plant growth and productivity of vines. It is limited to 50 kg per ha for one application and must be
applied in the period from 15th April to the end of June. The total amount of nitrogen should not exceed 80 kg per ha. This limitation was increased last year because there were many suggestions and complaints from producers about the limitation of 50 kg per ha. The use of leaf fertilisers is restricted. They can be used only for supporting plants that are suffering from drought and in cases when deficiency symptoms of some nutrients on vines appear. Soil cultivation is limited in the integrated grape production. It is necessary to keep the entire land surface in vineyards covered with grass or with others organic materials such as straw and bark during wintertime. This request represents a great change for traditional viticulture. Till now winter tillage has been a common practice in vineyards. From April to November at least one half of vineyard surface must be covered and not cultivated. The most recommended technique is permanent grass covering with mulching but unfortunately it is not suitable for all vineyards under various natural conditions. Wine growing district Kras is an exception. It is situated on Karst plateau where calcareous rock is covered with a thin layer of soil named terra rossa. Wine growers in this district are allowed to cultivate their vineyards during the summer to prevent the drought.

**Integrated pest management (IPM)**

The situation concerning integrated pest management (IPM) is much more complex. It is treated in the second part of the guidelines. The authors compared the related guidelines from the neighbouring countries (Italy, Austria and Switzerland). Due to the climatic and production characteristics and diversity of wine growing regions in Slovenia the rules have been adapted to our circumstances. The experiences acquired in the practice in the last three years have shown that the whole system of IPM works quite well. However, some problems have already arisen, which seem quite serious, at least at the local level. Some of them will be explained here.

The grapevine powdery mildew (*Uncinula necator*) has become more and more serious disease, especially in the southwest submediterranean region of Slovenia. A large number of factors are responsible for that, but two of them seem to be the most important:

- The increased introduction and spreading of sensitive cultivars like 'chardonnay' in the last 20 years;
- The regular and mass appearance of cleistothecia in autumn, which increases the genetic diversity of the fungus.

In the conditions of high infection pressure of the grapevine powdery mildew the sulphur fungicides in recommended doses of 2-3 kg per hectare are mostly not effective enough. Furthermore, the cases of resistance to IBE fungicides (e.g. triadimephon, myclobutanil, penconazol) and strobilurines are well known and have been repeatedly reported also in Slovenia. In accordance to the FRAC recommendation their use is now limited to maximum of three treatments per year. Due to these resistance problems, the use of dinocap was introduced in the IPM strategy again. Only two treatments per year are allowed. Respecting the environmental and production factors the use of dinocap in our vineyards has not expressed unacceptable negative consequences on beneficial fauna of arthropods, even when it was used 4 or 5 times per season. Several trials in Slovenia and in Italy have shown that under the higher temperature conditions the dinocap can reduce, but not compromise the populations of Phytoseiid mites in vineyards (Miklavč, not published data; Girolami, 1998, Pasenato & al., 1995). At the same time it also reduces the proliferation of phytophagous spider mites (*Panonychus ulmi, Eotetranychus carpini*) and gall mites (*Calepitrimerus vitis, Colomerus vitis*). Therefore, significant changes of biological equilibrium have not been observed in practice up to now, especially where *Kampimodromus aberrans* and *Amblyseius...*
andersoni are predominant predaceous mites. Resistant populations of these species on dinocap, dithiocarbamates and organophosphorus insecticides are already known (Pasenato & al., 1995). Further investigations are needed to find out an appropriate answer regarding the number of treatments and the time of use of dinocap as a very effective fungicide against the grapevine powdery mildew.

There are important differences between wine growing regions in occurrence of predaceous mites of the family Phytoseidae. In the continental (eastern) part of Slovenia the most common predaceous mites are Typhlodromus pyri and Amblyseius andersoni, but Kampimodromus aberrans and Amblyseius andersoni are the predominate species in southwestern (submediterranean) part of Slovenia. They mostly control the phytophagous spider mites sufficiently. Consequently the use of acaricides in vineyards has decreased drastically in the last few years. They are used only periodically, mostly against the grapevine rust mite (Calepitrimerus vitis). In general the apperance of grapevine rust mite (Calepitrimerus vitis) in our vineyards has increased in the last 15 years and it remains a problem also in IPM. Economic damages are much more frequent then in the past, especially in the earlier growth stages in spring when its predators are not sufficiently active yet. The increased use of organic fungicides like IBE and strobilurines instead of sulphur against the grapevine powdery mildew seems to be one of the most important causes for increased occurrence of these mites, but not the only one. Treatments with specific acaricides are sometimes necessary. High doses of wettable sulphur (6-12 kg/ha) in the wool growth stage can also give good results, if the whether is warm after the treatment.

Occurrence of the grapevine yellows seriously complicates the integrated pest management in Slovenian viticulture. They have been present in Slovenian vineyards since 1983, but only for the last two years they have taken an epiphytotic extension in some parts of the country. In some vineyards of the cv. 'Chardonnay' the infection rate goes up to 85 % and the yield loss was consequently very high. Systematic laboratory testing in 2001 and 2002 has shown that Grapevine bois noir phytoplasma (BN) is the only one certainly present in Slovenia. The preliminary investigations of leaf- and planthoppers in our vineyards have also shown that the vector of BN Hyalesies obsoletus is widely spread in all three winegrowing regions (SELJAK and al., 2003). Generally its population rate and consequently the incidence of stolbur phytoplasma (Grapevine bois noir phytoplasma) seem to be much higher in vineyards where grass-covering system has been introduced. The researches in course should make this relation more clear. They should also show if different agricultural techniques and phytosanitary measures can limit the populations of Hyalesies obsoletus and their main hosts, which can be an important source of stolbur phytoplasma in vineyards of sensitive cultivars. The Flavescence doree phytoplasma is not present in Slovenia yet. Its natural vector Scaphoideus titanus is only distributed in southeast part of Slovenia by the Italian border (Seljak, 2002).

Insecticides have never been used in very large amounts in Slovenian vineyards even in conventional production. Nevertheless important limitations have been provided in IPM. Only high selective insecticides of the group of growth modulators of insects and Bacillus thurgiensis are allowed against the grape moths (Eupoecillia ambiguella and Lobesia botrana). Some more selective organophosphorus insecticides (chlorpyriphos-methyl, phosalone, diazinon) can also be used under special conditions. The incidence and importance of some minor pests, particularly Neopulvinaria innumerabilis, Theresimima ampolphaga, Peribatodes rhomboidarius, Drepanothrips reuteri, Empoasca vitis, Lygus spinolai and some Noctuides has increased as a consequence of drastic reduction of organophosphorus insecticides and carbamates. Neopulvinaria innumerabilis is a relatively new pest of vine in Slovenia and its distribution is limited to the southwestern part of our country (Seljak, 1995).
At the moment the control of the pests mentioned above is regulated with special permissions, which are given individually by the control organisation.

Conclusions

In spite of some unsolved or potential new issues and difficulties, which go hand in hand with integrated grape production, the whole system should not be applied merely as a spice or as cheap advertising. On the contrary, they should reflect a real shift of mind and technology towards the viticulture more friendly to both, the environment and the wine grower.

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The change of copper concentration in leaves, grapes, must and wine of biological viticulture

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Abstract: This paper presents the change of copper concentration in leaves and vinegar products of two biological viticultures of Magnesia region. The determination of copper concentration has been achieved by using the method of Atomic Absorption Spectroscopy with flame (Perkin-Elmer apparatus). The samplings of leaves and unripe grapes started in August and finished in December with the determination of copper concentration in wine that was produced in the wine factories of the two biological viticulture. According to that procedure, it was made possible to monitor the change of copper presence and concentration in all the phases of viticulture and wine production. In comparison to previous measurements, which took place in our laboratory, in stumps from the same field that had not been sprayed, we can make conclusions as far as it concerns the copper behavior in the viticulture – wine production. The above analysis would be very useful in sight of the international demand for reduction in copper application concerning the biological and integrated production.

Key words: copper, biological viticulture, grape, must, wine.

Introduction

In Greece, vineyard consists the 10% of biologically cultivated area. The wine making grapes cover 2,500 hectares. 3% of the wine making grapes is cultivated in the area of Thessaly. Copper application is legal. There are different kinds of fungicides based on copper. Samples were taken from fields, where Bouillie Bordeaux, copper oxycloride etc, were added. Copper acts as a fungicide by changing cellular membrane permeability. That causes abnormalities in basic functions, such as formation of proteins. Diseases like: Downy mildew, gray mold and sour bunch rots can be controlled by copper application. Excessive concentrations can cause reduction of respiration, scalds, growth inhibition and other abnormalities. The above mentioned phenomenon could be aggravated by the environmental conditions such as humidity and low temperatures.

During the period of our experiment (July, August, September of 2002) the height of rainfall was greater in comparison to previous years. As a result the grapeharvest period was delayed.

The Regional Center of Magnesia suggested spraying with copper. The infections of Downy mildew, and Powdery mildew caused injuries in grapes which resulted to the growth of Botrytis and Sour bunch rots.

The samples come from five different fields as shown in table 1 and concern six different varieties.

As far as it concerns the final product (grape and wine) factors that influence it’s quality are:
a) Subjective, which include sensory factors, such as: odor, color, flavor and taste.
b) Physical, color for example.
c) Chemical, which include all safety factors, like: i) presence of residues, ii) presence of heavy metals (Cu, As, Cd, Pb), iii) presence of toxins, iv) presence of methanol, v) presence of ethylcarbamides, vi) nutritional factors, and
d) Microbiological.

Copper could have a toxic action for men when excessive quantities are consumed. Consumption of 3-5 gr causes gastroenteritis, consumption of 8-12 gr causes immediate death.

In view of regulation changes concerning the integrated and biological viticulture, as well as the general tendency and suggestions about the reduction in applied copper’s quantity, the laboratory of the Regional Center of Plant Protection and Quality Control of Magnesia was occupied with the determination of copper (Cu) in the chain: «grape leaves – grape – must – wine». That project was the succession of a previous one, which concerned the presence of copper in fresh grape leaves and in grape leaves in brine as well.

Samples come from 13 hectares which belong to five different farmers. (Table 1)

Table 1. Samples.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>GRAPE VARIETIES</th>
<th>REGION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rhoditis</td>
<td>M. Almyros</td>
</tr>
<tr>
<td>2</td>
<td>Syrah</td>
<td>»</td>
</tr>
<tr>
<td>3</td>
<td>Rhoditis</td>
<td>M. Argalasti</td>
</tr>
<tr>
<td>4</td>
<td>Xinomavro</td>
<td>»</td>
</tr>
<tr>
<td>5</td>
<td>Rhoditis</td>
<td>M. Anghialos</td>
</tr>
<tr>
<td>6</td>
<td>Cabernet Sauvignon</td>
<td>M. Kamena Vourla</td>
</tr>
<tr>
<td>7</td>
<td>Asyrtiko</td>
<td>»</td>
</tr>
<tr>
<td>8</td>
<td>Moschato</td>
<td>M. Nea Ionia</td>
</tr>
</tbody>
</table>

Materials and methods

Two kilos of grapes and half a kilo of leaves were taken from each field, taking care to have some grapes from every position of the field and from different heights of the plant. The sample was prepared by the method of wet digestion as following: half a kilo of the grape was pulped. The pulped grape was filtered. 20ml of the filtered juice was collected in a volumetric flask of 100ml. 10ml of HCL was added and the flask was fulfilled with distilled water. That was the procedure followed for the preparation of must and wine samples too. For the grape leaves a drying was preceded. 1gr of the dried leaves was digested with the addition of 10ml of nitric acid and an overnight heating at 45°C. The solution was filtered and collected in a volumetric flask of 50ml. The flask was fulfilled with distilled water. The measurement of copper concentration was done by Atomic Absorption Spectroscopy (Perkin-Elmer 3300, with air-acetylene flame, relative noise: 1.1, characteristic concentration: 0.17mg/lt, linear range: 5 mg/lt).
Results and discussion

Copper concentration in leaves found in our samples is shown in table 2 and figure 1. As it is shown there is a gradual reduction in copper concentration with a maximum on the first sampling (22 of August) and a minimum at the last sampling, right before grape-harvest (11 or 24 of September). It is also obvious that samples from Argalasti, which have not been sprayed, have much lower concentration than samples from Almyros and Aghialos.

Table 2. Copper concentration in grape leaves.

<table>
<thead>
<tr>
<th>Date</th>
<th>Almyros Rhoditis</th>
<th>Almyros Syrah</th>
<th>Argalasti Rhoditis</th>
<th>Argalasti Xinomavro</th>
<th>Aghialos Rhoditis</th>
<th>Kamena Vourla Asyrtiko</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 August</td>
<td>1215,1</td>
<td>680,6</td>
<td>8,3</td>
<td>9,9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29 August</td>
<td>680,4</td>
<td>593,5</td>
<td>5,2</td>
<td>6,7</td>
<td>510,7</td>
<td>310</td>
</tr>
<tr>
<td>11 September</td>
<td>340</td>
<td>411,4</td>
<td>4,7</td>
<td>3,2</td>
<td>344,1</td>
<td>252,1</td>
</tr>
<tr>
<td>24 September</td>
<td>332,2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Copper concentration in grape leaves.

In table 3 and figure 2 we can see that the copper concentration was reduced during the wine-making procedure. Moreover, measurements were made in lees. The result was
304.1 ppm, which shows that a great part of the copper is removed partially from the grape juice to the lees due to the precipitation. We should notice the gradual reduction of copper concentration which is very important for the safety of the final product and the consumer’s health.

Table 3. Evolution of copper concentration from grape juice to wine (samples 1 and 2).

<table>
<thead>
<tr>
<th>Date/sample</th>
<th>Almyros Rhoditis</th>
<th>Almyros Syrah</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 August/ Grape Juice</td>
<td>6.8</td>
<td>7.8</td>
</tr>
<tr>
<td>29 August/ Grape Juice</td>
<td>6.9</td>
<td>8.5</td>
</tr>
<tr>
<td>11 September/ Must</td>
<td>1.5</td>
<td>0.5</td>
</tr>
<tr>
<td>13 December/ Wine</td>
<td>0.6</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Evolution of copper concentration from grape juice to wine (samples 1 and 2).

In table 4 and figure 3 we have the copper concentration from grapes to wine from the area of Argalasti. We notice that those two samples were not sprayed and as a result the copper concentration is very low in the grape juice, as well as in the wine. The concentration of the above samples was much lower than in the samples from Almyros (figure 2), which were sprayed.

In Table 5 we have the evolution of copper concentration in grape and wine from three different areas. It is obvious that during the wine-making procedure there is a reduction in copper concentration. In wine the concentration was very low, not detectable.
Table 4. Evolution of copper concentration from grapes to wine (samples 3 and 4).

<table>
<thead>
<tr>
<th>Date/ Sample</th>
<th>Argalasti Rhoditis</th>
<th>Argalasti Xinomavro</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 August/ Grape Juice</td>
<td>0</td>
<td>0.015</td>
</tr>
<tr>
<td>6 September/ Grape Juice</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>10 September/ Grape Juice</td>
<td>0.35</td>
<td>0.5</td>
</tr>
<tr>
<td>19 September/ Grape Juice</td>
<td>0.23</td>
<td>0.7</td>
</tr>
<tr>
<td>13 December/ Wine</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 3. Copper concentration in grape juice and wine from the area of Argalasti.

Table 5. Evolution of copper concentration (ppm) (Samples 5-8).

<table>
<thead>
<tr>
<th>Area of sampling/ Sample</th>
<th>Grape</th>
<th>Wine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamena Vourla/ Asyrtiko</td>
<td>11.5</td>
<td>0</td>
</tr>
<tr>
<td>Kamena Vourla/ Cabernet Sauvignon</td>
<td>4.5</td>
<td>0</td>
</tr>
<tr>
<td>Anghialos/ Rhoditis</td>
<td>19.3</td>
<td>0</td>
</tr>
<tr>
<td>N.Ionia/ Moshato</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusions

As a result we can conclude that the copper concentration in the grape leaves was greater than in the grapes. In both cases the concentration of copper was reduced gradually as time was coming by, and the lowest concentration was measured right before grape-harvest. In the vineyards where there was no copper application the copper concentration was lower than in
the vineyards where copper was used as a fungicide. We can also see that there is a gradual reduction of the copper concentration from the grape juice to the must and finally to the wine. The copper concentration in all cases in wine sample was not detectable, so it was much lower than the upper limit (1 ppm). As a result, the use of copper as a fungicide, in relation to the climate conditions (sunlight, low humidity for the majority of grape cultivating areas in Greece), cannot be considered as a potential hazard for the consumer’s health and safety.

References