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Working Group “Integrated Protection and Production in Viticulture”

OILB / SROP

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Compte Rendu de la Réunion**

– Appendix –

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**Edited by
Carlo Lozzia**

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Editorial

The objective of this new edition of the OILB Bulletin number 26 of 2003 is to update the list of participants and to include some new papers that were omitted in the original Bulletin.

I apologise for the misunderstanding that there has been with the local organization. I hope that in the future the collaboration between the local committee will be stronger in order to avoid further problems.

Our studies have contributed to the development of integrated protection and production in European viticulture and I hope that these studies will continue with the same spirit and professionalism, which have characterized us in the past.

See you in the future in this fantastic group.

Carlo Lozzia
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Contents

Editorial.....	i
List of participants.....	v
Effectiveness of different insecticides incorporated into artificial diets on larvae of the grapevine moth <i>Lobesia botrana</i> and the grape berry moth <i>Eupoecilia ambiguella</i> <i>P. J. Charmillot, D. Pasquier, S. Verneau</i>	1
Early evaluation of grape berry susceptibility to <i>Botrytis cinerea</i> <i>B. Dubos and J. Roudet</i>	7
Precursory climatic indices of <i>Botrytis</i> rot development in mature grapes <i>M. Fermaud, P. Piéri, F. Mimiague</i>	11
Plant parasitic nematodes in vineyards under different agricultural management <i>B. Manichini and S. Landi</i>	15
Un nouveau dépérissement de la vigne en France: le Black Dead Arm causé par <i>Botryosphaeria</i> spp. <i>P. Larignon, R. Fulchic et B. Dubos</i>	21
“Young grapevine decline” associated with defected propagated material <i>I.C. Rumbos</i>	27
Ten years investigations for the promotion of integrated viticulture in Greece <i>I.C. Rumbos, A.G. Koutroubas, E.J. Navrozidis, Z. Zartaloudis, P. Papaioannou-Souliotis, D. Markoyiannaki-Printziou, A. Sachinoglou, G. Salpiggidis and I. Adamopoulos</i>	37
Cooperation between wine industry and research centers for the promotion of integrated control systems in Greece <i>I.C. Rumbos, A.G. Koutroubas, G. Salpiggidis, P. Papaioannou-Souliotis, and Y. Ranos</i>	45

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Effectiveness of different insecticides incorporated into artificial diets on larvae of the grapevine moth *Lobesia botrana* and the grape berry moth *Eupoecilia ambiguella*

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Abstract: Insecticides were incorporated into an artificial diet at different concentrations to determine their larvicidal effectiveness on larvae of the grape berry moth *Eupoecilia ambiguella* and the grapevine moth *Lobesia botrana*. A first evaluation was made after 14 days of rearing from newborn larvae. Surviving individuals were then kept until adult emergence. These data should provide a better understanding of the insecticides with regard to their respective effectiveness on both species, their larvicidal properties and rapidity of action and thus enable their utilisation to be optimised in relation to the phenology of the pest. Furthermore, these data could be used in the future as a rapid reference for identifying cases of resistance.

Dose-mortality curves were established for 9 insecticides, on laboratory-reared strains of both species originally collected in the region beside Lake Geneva. Efficiency varied greatly among the insecticides tested. Seven out of the 9 products had a good potential for the control of grape moths as their LC₅₀ values ranged from 0.02 to 1 ppm: namely, methoxyfenozide, flufenoxuron, indoxacarb, tebufenozide, spinosad and chlorpyrifos-methyl. Concerning chlorpyrifos-ethyl (Pyrinex), its high LC₅₀ of approximately 60 ppm was surprising because this product is found to be effective in vineyards. This poor performance can be attributed to its microencapsulated formulation that probably inhibits diffusion of the active ingredient into the diet. The effectiveness of teflubenzuron was good on *L. botrana* but low on *E. ambiguella*. Diflubenzuron did not present any interest for the control of either grape moth species.

Introduction

Trials to determine the effectiveness of several insecticides on larvae of two species of moths, the grapevine moth *Lobesia botrana* and the grape berry moth *Eupoecilia ambiguella*, were conducted. The insecticides (some already homologated and others currently being developed) were incorporated into an artificial breeding diet. The effectiveness-concentration curves thus obtained allow a comparison of products to be made in addition to providing a reference for ulterior detection of resistance.

Materials and methods

L. botrana and *E. ambiguella* larvae were reared on an artificial diet "Manduca - Heliothis Premix" (Stonefly Industries, USA) into which the insecticides to be tested were incorporated. Using a spatula, a little of the contaminated diet (1-2 g) was placed into each of 30 small plastic boxes. One newborn larva was then laid in each box. The larvae were reared under laboratory conditions at 25°C. After 14 days, a first check was made to count the number of surviving larvae. Rearing then continued to the adult stage.

The trial was carried out using 9 products (Table 1), at 3-10 different concentrations ranging between 0.005 and 300 ppm (mg/kg). The larvicidal effectiveness of the products was calculated in relation to the corresponding control experiment. The POLO-PC programme

(LeOra, 1987) was used to determine « dose-effect » parameters for both the larval period up to the first check and also for the whole duration of the trial up to moth emergence.

Table 1. List of products tested in the artificial diet

Product	Trade name	Formulation
tebufenozide	Mimic	SC 240 g/l
methoxyfenozide	Prodigy (RH-2485)	SC 240 g/l
diflubenzuron	Dimilin	SC 480 g/l
teflubenzuron	Nomolt	SC 150 g/l
flufenoxuron	Cascade	EC 100 g/l
chlorpyrifos-methyl	Reldan	EC 400 g/l
chlorpyrifos-ethyl	Pyrinex	ME 250 g/l
indoxacarb	Steward	WG 300 g/kg
spinosad	Audienz	SC 480 g/l

Results and discussion

Grapevine moth Lobesia botrana

Rearing during the first 14 days: In the control, average survival rates reached 87.8%. Methoxyfenozide was by far the most effective product tested with an LC_{50} at 0.05 ppm (Fig. 1). LC_{50} values varied between 0.1 and 0.3 ppm for flufenoxuron, indoxacarb and tebufenozide, and between 0.3 and 0.7 ppm for teflubenzuron, spinosad et chlorpyrifos-methyl. As for the results of chlorpyrifos-ethyl, its LC_{50} value of 63 ppm was surprising as this product is found to be effective when used in the vineyards. This apparent contradiction may be explained by its microencapsulated (ME) formulation which probably slows down liberation of the active ingredient in the artificial diet or in the digestive tube of larvae. After insecticidal treatment in the vineyard, on the other hand, the insecticide would be absorbed by the plant and more readily available to the insect. Lastly, diflubenzuron, which was tested at 3 different concentrations only, was practically totally ineffective even at concentrations of 10 ppm.

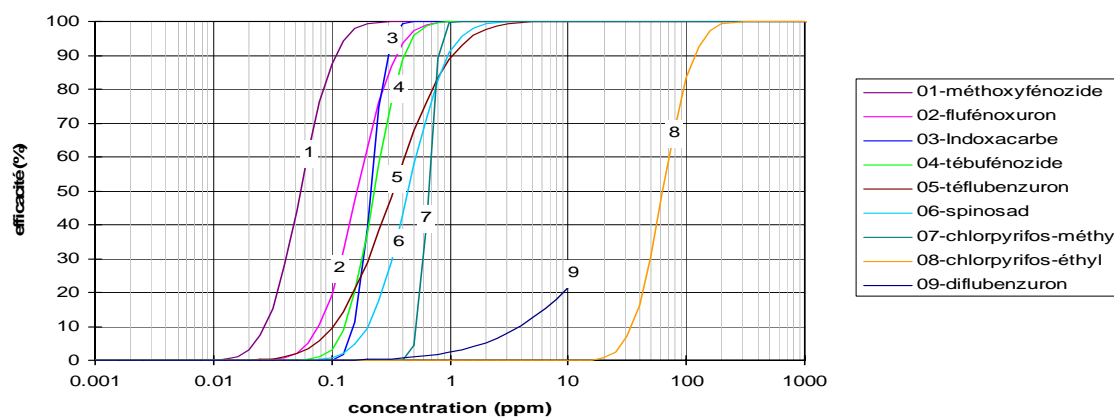


Figure 1. Effectiveness of products incorporated into an artificial diet on *L. botrana*, according to concentration. Effectiveness checked after 14 days rearing.

Rearing up to adult stage: Average survival rates of 83.1% were found in controls. Methoxyfenozide was the most effective product with an LC_{50} value of 0.04 ppm (Fig. 2). The LC_{50} values of 6 other products, between 0.07 and 0.66 ppm, ranked in order of effectiveness, were flufenoxuron, teflubenzuron, indoxacarb, tebufenozide, spinosad and chlorpyrifos-methyl.

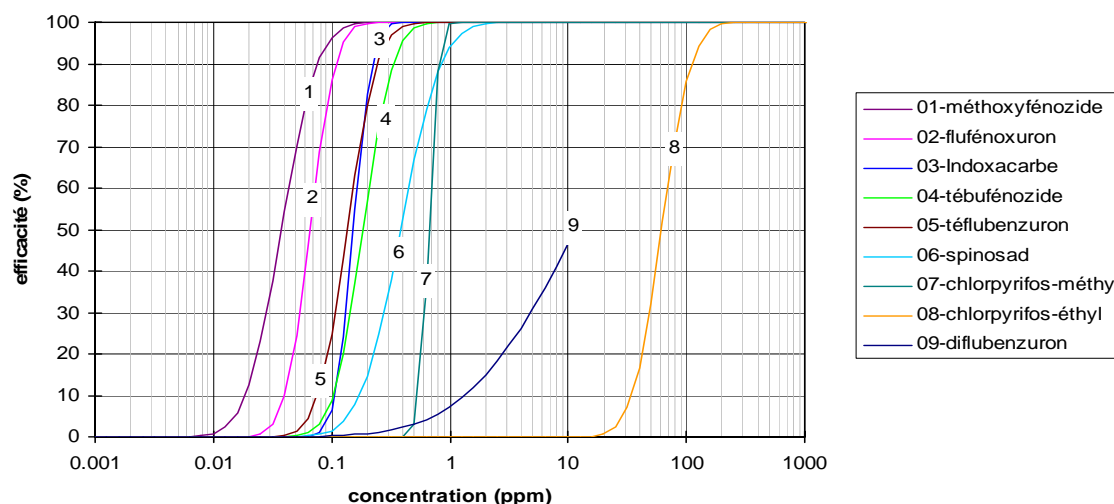


Figure 2. Effectiveness of products incorporated into an artificial diet on the grapevine moth *L. botrana*, according to concentration. Effectiveness checked from rearing to adult emergence.

Grape berry moth Eupoecilia ambiguella

Rearing during the first 14 days: In the control experiment, average survival rates reached 94.8%. Methoxyfenozide was by far the most effective product with an LC_{50} value of 0.03 ppm (Fig. 3). LC_{50} values for spinosad, tebufenozide and flufenoxuron ranged between 0.09 and 0.17 ppm and for chlorpyrifos-methyl, indoxacarb and teflubenzuron, they were between 0.9 and 1.5 ppm. Diflubenzuron, which was tested at 3 different concentrations only, was hardly effective at all, even at a concentration of 10 ppm. This result confirms expectations that this product is ineffective in the control of grape moths. Concerning chlorpyrifos-ethyl, the high concentration results of 59 ppm at the LC_{50} level of effectiveness can probably be explained by its ME formulation.

Rearing up to the moth stage: Average survival rates of 91.9% were found in the controls. Methoxyfenozide was by far the most effective product with an LC_{50} value of 0.02 ppm (Fig. 4). The LC_{50} values of 6 other products, between 0.08 and 1.05 ppm, ranked in order of effectiveness, were spinosad, flufenoxuron, tebufenozide, indoxacarb, teflubenzuron and chlorpyrifos-methyl.

Comparison of product effectiveness after 14 days rearing and up to butterfly emergence.

Insect growth inhibitors (IGIs), flufenoxuron, teflubenzuron and diflubenzuron, which prevent the synthesis of chitin, have an influence during moulting process only and acted rather slowly. A significant proportion of the larvae died after the first check at 14 days. Alternatively, this late mortality rate could also be explained by a higher sensitivity to these products in the final larval stages than in the early stages. The other products acted rapidly on both species.

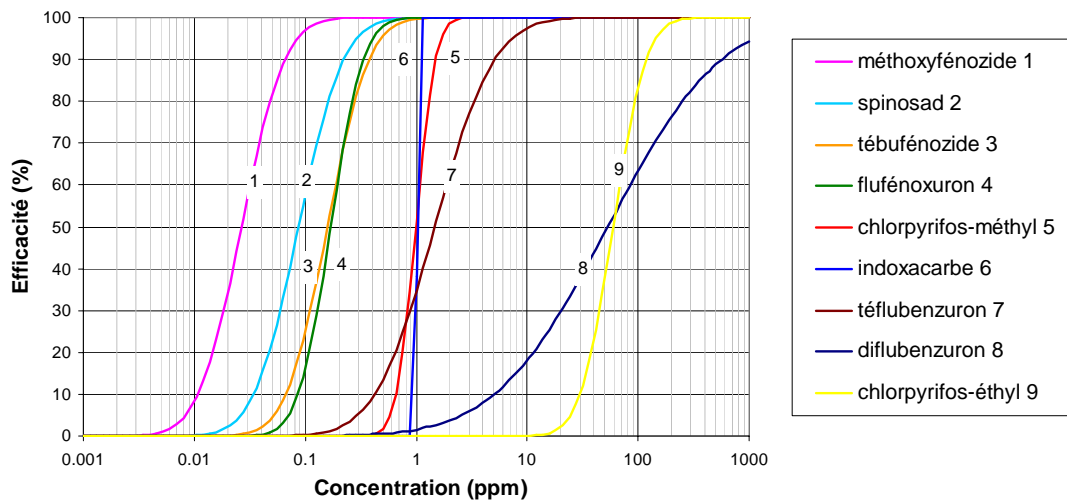


Figure 3. Effectiveness of products incorporated into an artificial diet on *E. ambiguella*, according to concentration. Effectiveness checked after 14 days rearing.

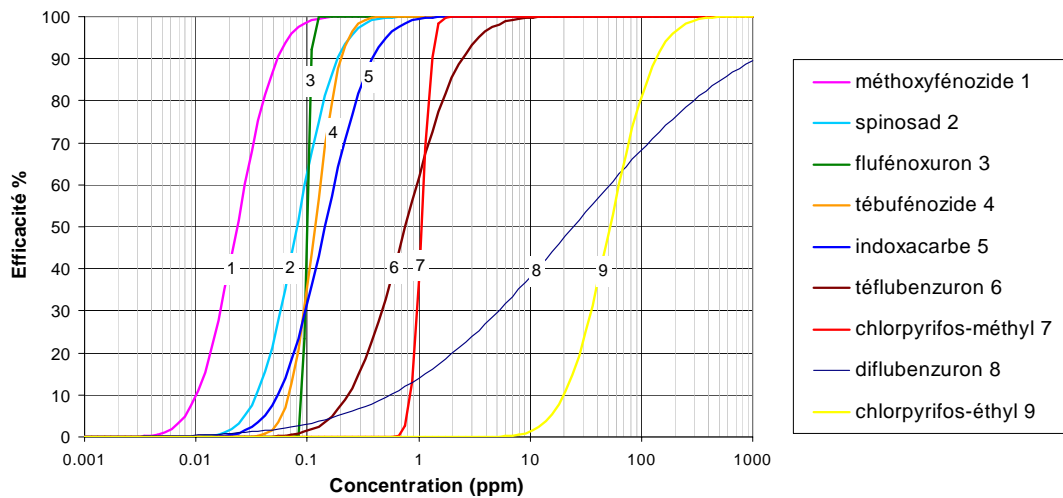


Figure 4. Effectiveness of products incorporated into an artificial diet on the grape berry moth *E. ambiguella*, according to concentration. Effectiveness checked from rearing to adult stage.

Comparison of product effectiveness on grapevine and grape berry moths

Tebufenozide and methoxyfenozide were potentially slightly more effective on the grape berry moth than on the grapevine moth. Teflubenzuron, however, was clearly more effective on the grapevine moth. Indoxacarb acted more effectively on young grapevine larvae than on grape berry larvae. On the other hand, effects of indoxacarb on both species reared up to the adult stage were practically identical. Spinosad was clearly more active on the grape berry moth than on the grapevine moth. All the other products tested gave comparable results for both species of moth.

Conclusion

Seven of the insecticides tested in this trial gave potentially good effectiveness results, although there was quite a variation in their LC_{50} values, between 0.02 and 1 ppm concentration of product. These differences cannot be explained by the mode of action of the insecticide. Indeed, the LC_{50} values of IGI's (flufenoxuron, teflubenzuron, diflubenzuron) were interposed between those of IGRs (methoxyfenozide, tebufenozide), that of the organophosphate chlorpyrifos-methyl and those of products which act on the nervous system, such as indoxacarb or spinosad. The high LC_{50} value of chlorpyrifos-ethyl (Pyrinex) was surprising as this product is found to act effectively in vineyards. Its poor performance in this particular study can probably be explained by its microencapsulated formulation which may hinder release of the active ingredient in the artificial breeding diet, which does not occur when they are applied directly onto plant surfaces.

Tebufenozide and methoxyfenozide showed a potentially greater effectiveness against the grape berry moth than the grapevine moth. However, the insecticide teflubenzuron was much more effective on the grapevine moth whereas spinosad was much more effective on the grape berry moth.

It should nevertheless be noted that the effectiveness levels determined in this study apply not only to newborn larvae. The larvae initially placed on the contaminated diet at the L_1 stage do in fact go through 4 ulterior stages before metamorphosis into pupae and adult. Consequently, the measured effectiveness reflects sensitivity of the most vulnerable stage. Finally, we should remember that LC_{50} values are not the only parameters to consider when comparing insecticide properties. In practical terms, compensation for a relatively low potential effectiveness can be made by increasing dosage, not forgetting persistence of product which is also an essential factor to be taken into consideration.

Acknowledgements

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Early evaluation of grape berry susceptibility to *Botrytis cinerea*

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Abstract: Two indicators of susceptibility of berries to *Botrytis cinerea* were identified. 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 cultivars of vines, sensitive Sauvignon, and Arriloba (crossing of Sauvignon and rafiato 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 obtain:

- 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 guaiacol 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.

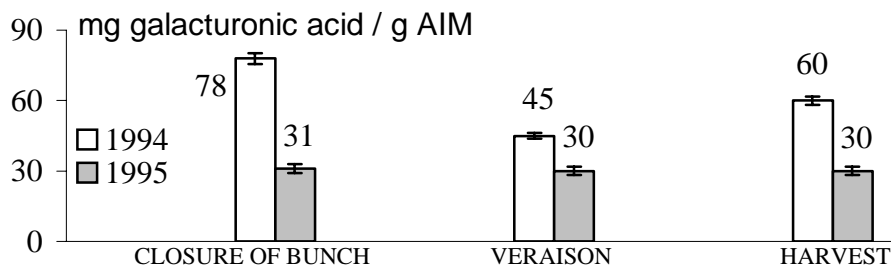


Figure 1. Evolution of SPW of the skin of healthy berries of Sauvignon during their development in 1994 and 1995.

Evolution of the phenolic compounds (PC)

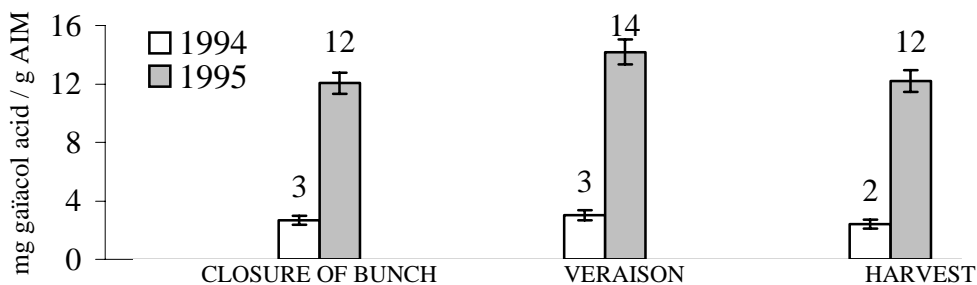


Figure 2. Evolution of the PC of the skin of healthy berries of Sauvignon during their development in 1994 and 1995.

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.

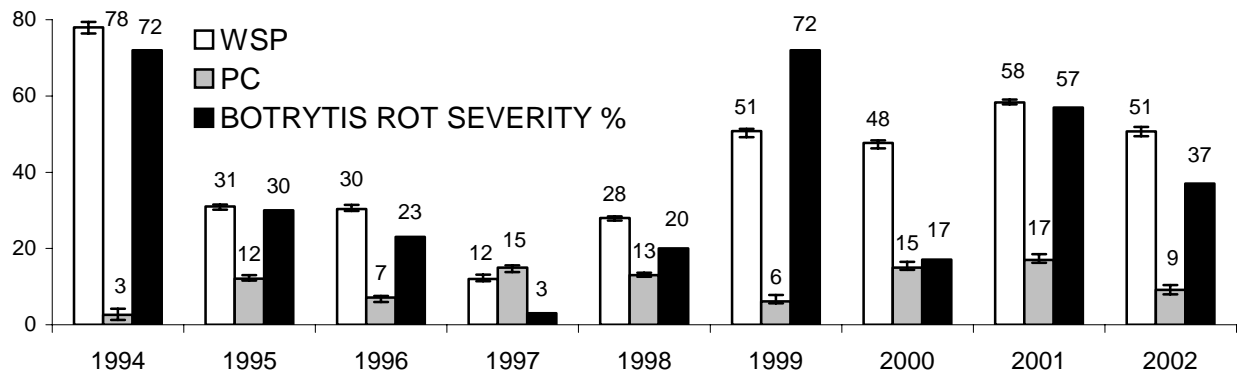


Figure 3: WSP and PC contents in relation to grey mold in the test plot at harvest from 1994 to 2002. Grey mold damage are visually assessed one week after technological maturity in order to better observe differences.

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)

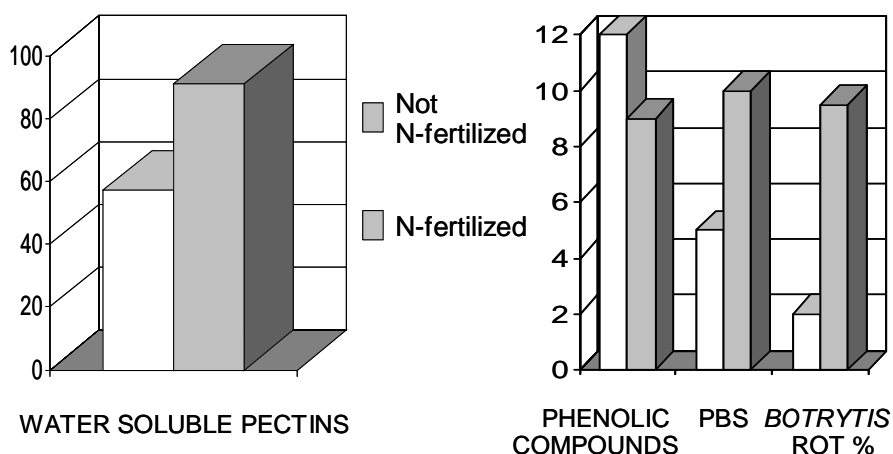


Figure 4: Influence of nitrogenous fertilization on potential of susceptibility of berries to *B. cinerea* (PSB)

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).

Acknowledgements

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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.

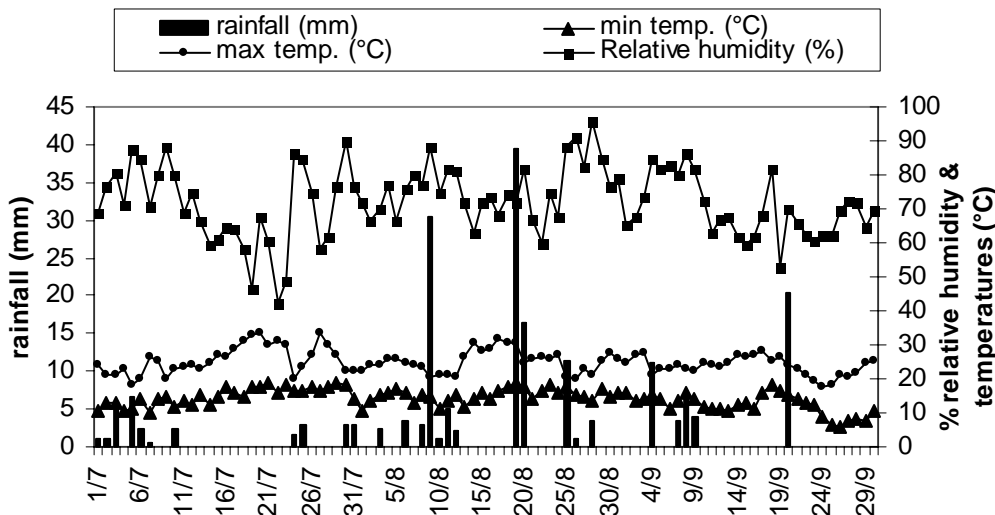


Figure 1: Climate features in 2002 (INRA, Bordeaux)

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). The 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.

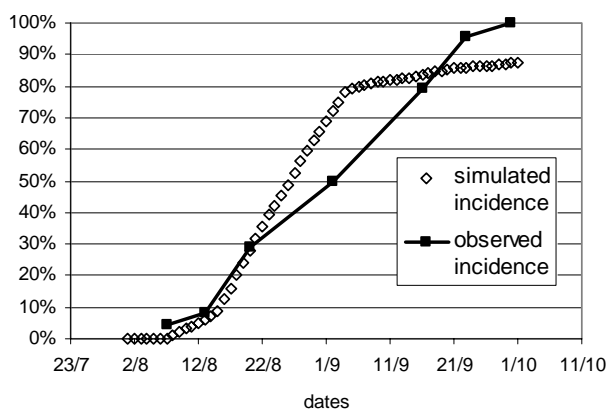


Figure 2: Percentage of rotted bunches in 2002 on Merlot

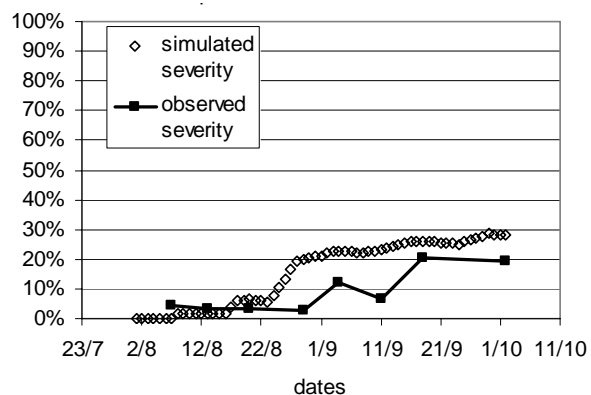


Figure 3: Grey mould severity, i.e. percentage of rotted berries per rotted bunch in 2002 on Merlot

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).

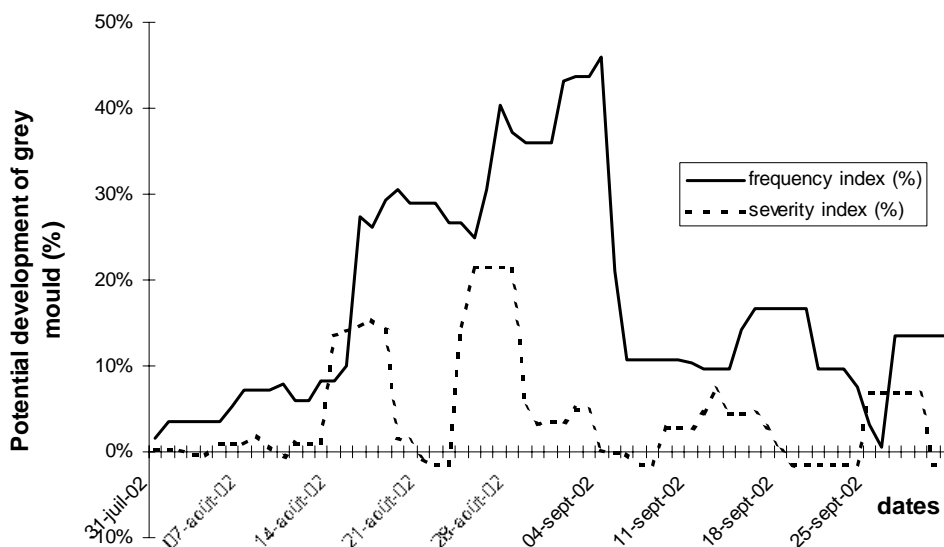


Figure 4: Variations of the climatic indices for Merlot in 2002

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 M., 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.

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Plant parasitic nematodes in vineyards under different agricultural management

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Abstract: A study was carried out in 1999 and 2000 to investigate the plant parasitic nematodes of vineyard. Four different agricultural management vineyards were considered: uncultivated, new implanted, with green cover and the last one with weed management treated with the herbicide Glyphosate.

Nematodes were extracted from soil samples by Baermann funnel method and Ludox centrifugation. Afterwards plant parasitic nematodes were identified to genus level. The seasonal distribution and the effects of the different agricultural management on phytophagous nematodes were investigated. The plant parasitic nematodes collected belong to 16 genera and 11 families. The most abundant genus was *Aphelenchus* followed by *Filenchus*, *Pratylenchus* and *Helicotylenchus*. *Pratylenchus* reached the injury level in the uncultivated vineyards. It was recorded the presence of other quite serious pests, but in low abundance, as *Paratylenchus* and *Tylenchorhynchus*.

Key words: Agricultural management, Phytophagous nematodes, root lesion nematodes.

Introduction

Nematodes are a serious threat to vineyards worldwide (Brown 1993; McClure, 1999; D'Errico, 2000). Plant-parasitic nematodes pierce plant cell walls with their stylet, which is formed by some of the mouth and oesophagus parts. Then they pump up the plant cell just into their digestive system. These nematodes only feed on a certain part of the plant. Most feed on the roots. Some of kinds of nematodes feed only on the outer tissue of the root, others penetrate more deeply, and some completely enter the host. There are different ways they harm the plants. Some induce their hosts to produce nutrients, which the nematode can survive on, or enlarged structures in which the nematodes live, or both. Some produce metabolites, which kill host tissue. These activities all result in energy being removed from plants to support nematodes. Some plant-parasitic nematodes can cause severe damage that can result in unmarketable, yield decrease, or even total crop failure. In addition to direct damage caused by their feeding, some nematodes transmit virus diseases of grapevines, which can be devastating. Much information is available for the vector nematodes as *Xiphinema* spp. and *Longidorus* spp. (Arias *et al.*, 1994; Tzortzakakis *et al.*, 2001), while little is known about the other phytophagous nematodes present in the vineyards.

A lack of information does not necessarily imply a lack of damage (McClure, 1999). The objective of this study was to survey different vineyards under different agricultural management determining the kinds and numbers of plant parasitic nematodes present. This study focuses on the nematodes, which may be potentially damaging the vineyards and establishes the effect of different management on their presence.

Material and methods

The objective of this study was to investigate the plant parasitic nematodes present in the vineyards under different agricultural management: uncultivated (U), new implanted (N), with

green cover (W+) and the last one with weed management treated with the herbicide Glyphosate (W-). The sampling was carried out in June and October 1999 and in June 2000, using a cylindrical probe of 4 cm diameter and 30 cm length, to a depth of 30-40 cm.

To collect general phytophagous nematodes longer probes were not applied; in fact, they are used only to collect the genus *Xiphinema* and *Longidorus*. These genera have the maximum population density under 400mm (Quader *et al.*, 2003).

Six hundred soil samples per hectare were collected following a random scheme, to account the typical clustered nematodes distribution (Moens, 1993). Nematodes were extracted from 150g of soil using Baermann funnel method and Ludox centrifugation. Then they were killed with warm (+72°C) formaldehyde and counted. Four replicates for each vineyard were done. Afterwards the nematodes were processed by De Grisse-Cobb method's (1969). Nematodes were randomly picked out and transferred on slide to the permanent mounts. The specimens were observed, using a WILD M 12 microscope at 100X and identified to the genus level.

Data were subjected to ANOVA and Duncan test (SPSS 8.0 for Windows).

Results and discussion

On the total nematofauna plant parasitic nematodes were the 8% in the new implanted vineyard (N), the 11% in the green cover field (W+) and the 13 % in the weed management (W-). The highest percentage of phytophagous was found in the uncultivated field (15%).

Table 1 reports plant parasitic nematodes collected in the four vineyards. Nematodes belong to sixteen different genera and eleven families.

Table 1. Total number of nematodes collected from soil of four different vineyards: new implanted (N), with green cover (W+) and the last one with weed management treated with the herbicide Glyphosate (W-), uncultivated (U).

Families	Genera	N	W +	W -	U
Anguinidae	<i>Ditylenchus</i>	+	-	-	+
Tylenchidae	<i>Filenchus</i>	+	+	+	+
Tylenchidae	<i>Coslenchus</i>	-	-	+	-
Tylenchidae	<i>Lelenchus</i>	-	-	+	-
Tylenchidae	<i>Basiria</i>	-	+	-	-
Neotylenchidae	<i>Neotylenchus</i>	+	+	-	-
Belonolaimidae	<i>Tylenchorhynchus</i>	+	+	-	-
Belonolaimidae	<i>Merlinius</i>	+	-	-	-
Hoplolaimidae	<i>Helicotylenchus</i>	+	+	+	+
Pratylenchidae	<i>Pratylencoides</i>	-	-	-	+
Pratylenchidae	<i>Pratylenchus</i>	+	+	+	+
Criconematidae	<i>Criconemoides</i>	-	-	+	-
Tylenchulidae	<i>Paratylenchus</i>	-	-	+	+
Tylenchidae	<i>Tylenchus</i>	-	+	+	-
Aphelenchidae	<i>Aphelenchus</i>	+	+	+	+
Aphelencoididae	<i>Aphelencoides</i>	+	+	+	+

Only five genera were present in the all vineyards studied: *Aphelenchus*, which was the most abundant, followed by *Filenchus*, *Helicotylenchus*, *Pratylenchus* and *Aphelenchoides*.

Aphelenchus had the highest percentage (19.3%) in the vineyard N, however there were not founded statistical differences ($F=0.29$; $df=3, 11$; $p=0.83$) among the different vineyards. No statistical differences were also resulted for *Aphelenchoides*, present with the highest percentage (1.6%) in the uncultivated field ($F=0.95$; $df=3, 11$; $p=0.46$).

Nevertheless, there were some genera present only in one particular vineyard. *Coslenchus*, *Lelenchus* and *Criconemoides* were collected only in the vineyard W-. *Basiria* was present in the vineyard W+. *Merlinius* in the new implanted field (N) and *Pratylenchoides* was collected only in the uncultivated one (U).

The research was carried out during two different years; so it was possible determine seasonal distribution of plant parasitic nematodes (Fig. 1). In three vineyards studied (U, W+, W-) an increment of population was observed during the months of autumn. This result confirms what found by other researchers (Pinochet & Cisneros, 1986; Quader *et al.*, 2003) and it is probably correlated with high precipitation of the period. In addition, it is reported that plant parasitic nematodes are more abundant when the grapevines are dormant (Quader *et al.*, 2003). Different was the state of new implanted vineyard. The lowest presence of phytophagous nematodes could be correlated to the stress that new installation of vineyard had made to the nematofauna.

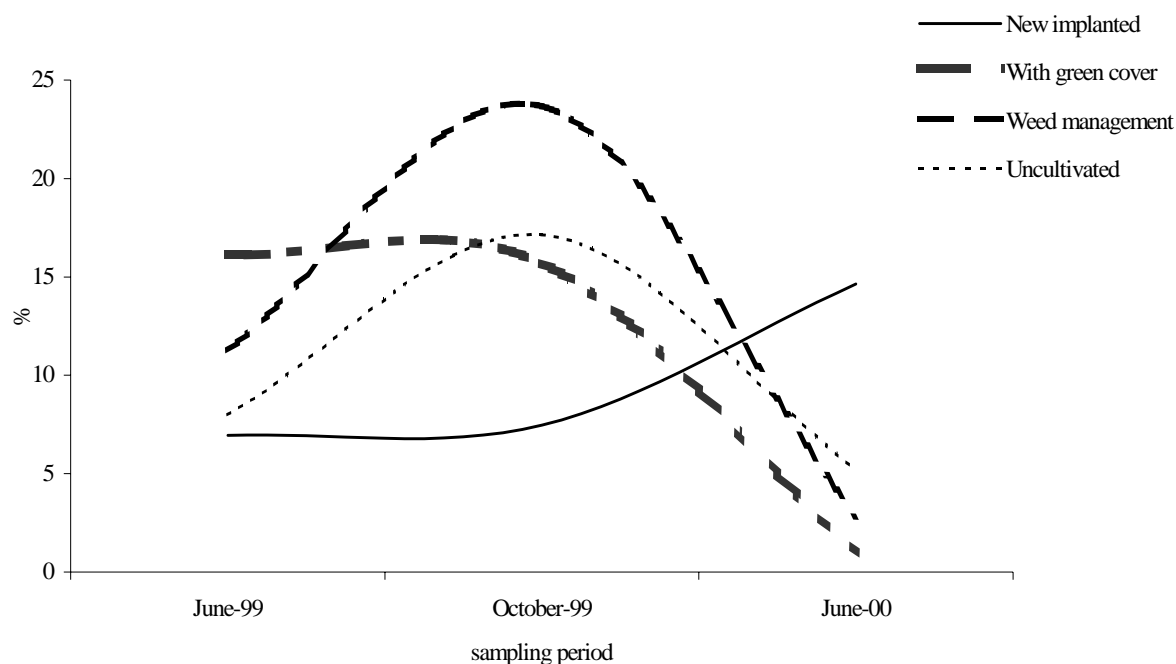


Figure 1: Seasonal distribution of plant parasite nematodes during the sampling periods.

It was also considered the seasonal fluctuation of the most abundant and potentially dangerous genera as *Pratylenchus*, *Helicotylenchus* and *Filenchus* (Fig.2, 3, 4). The first genus had the maximum percentage (10.8%) in the uncultivated vineyard (U) and the lowest abundance in the new implanted one (8%). However the result of ANOVA had established that there are no statistical differences ($F=1.12$; $df=3, 11$; $p=0.40$) in the abundance of this nematodes in the four different vineyards studied.

The population density of *Pratylenchus* had a peak around October probably at the time of the main root flush (Fig. 2).

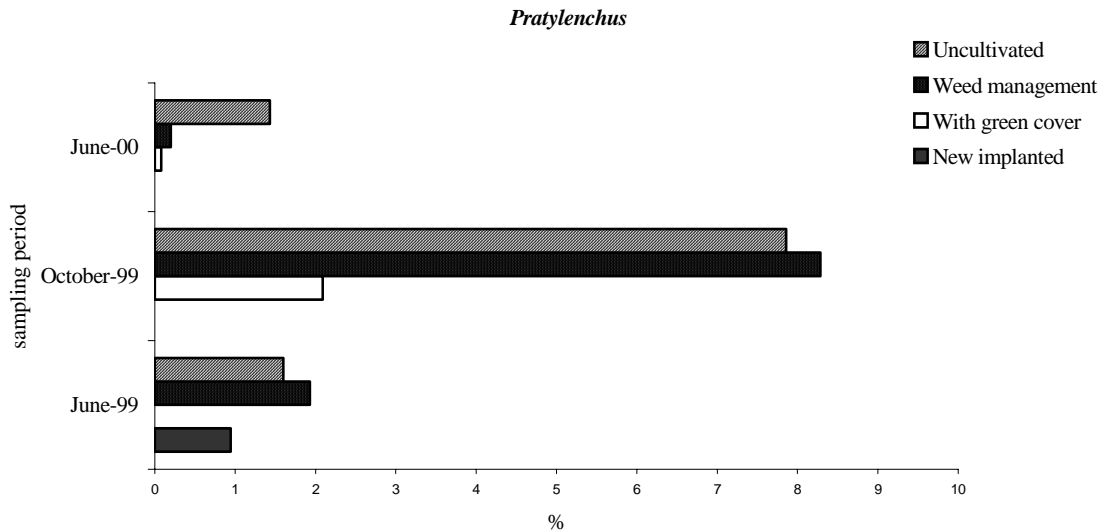


Figure 2. Seasonal distribution and percentage of *Pratylenchus* on the total abundance of nematodes.

On October *Pratylenchus* was mostly more numerous in weed management and uncultivated vineyard but only in the last one it was found above the injury level (50 *Pratylenchus*/ 300g of soil). Walker and Morey (2000) suggested that root lesion nematode (*Pratylenchus*) might multiply on susceptible cover crops, leading to continual reinvasion of grapevine roots.

However in this study was not possible to determine if the high abundance of this nematode was due to the abandon or the vineyard was uncultivated because of the presence of nematode above the injury level. It is important to notice that root-lesion nematodes rip a hole in the sides of root cells and crawl inside. They move through the root, piercing, sucking, and leaving behind a trail of both cell-killing metabolites and eggs. Cell death results in brown lesions on the roots. Lesions begin on one side, but may encircle a root and thereby girdle it. The overall effect is a weak, shallow root system with many dead areas.

In fact, *Pratylenchus* spp. has been associated with poor growth in grapevines (Tacconi, 1984; Brown *et al.*, 1993). McClure (1999) found that *Pratylenchus* and *Criconemella* occur, alone or in combination with *Xiphinema* and *Meloidoygine*, and that these nematodes are probably experiencing some degree of damage and yield loss. The author also claims that measures to limit spread of these pests in the vineyard are warranted. Moreover care should be taken when moving soil or rooted plants to newly planted areas or areas intended for future production.

Helicotylenchus (Fig. 3) feed semi-endoparasitically in root tissues, cells physically disrupted during penetration become necrotic. It is present naturally in the soil of vineyards like reported in numerous studies (Stephan *et al.*, 1985; Coiro, 1998; Wang *et al.*, 2001; Belair *et al.*, 2001), but only in high abundance can be dangerous. In this study *Helicotylenchus* was never found above the injury level, in fact the highest percentage (4.7%) was recorded in the vineyard with weed management. The statistical analysis had not found for significant differences in the abundance of *Helicotylenchus* ($F= 0.38$; $df= 3,11$; $p= 0.77$) among the four vineyards.

Filenchus (Fig.4) had highest percentage (4.9%) in the new implanted and the lowest in the uncultivated vineyard (0.4%). No statistical differences were found for its abundance among the different vineyards ($F= 0.63$; $df= 3,11$; $p= 0.62$). This phytophagous is not reported as a serious dangerous nematode for the grapes however, different species of this genus were often found in the vineyard soils (Al Banna *et al.*, 1996). The seasonal fluctuation

of *Helicotylenchus* and *Filenchus* had a peak in October, in the weed management and uncultivated vineyard respectively (Fig. 3, 4). Similar situation as found for the other plant parasite nematodes.

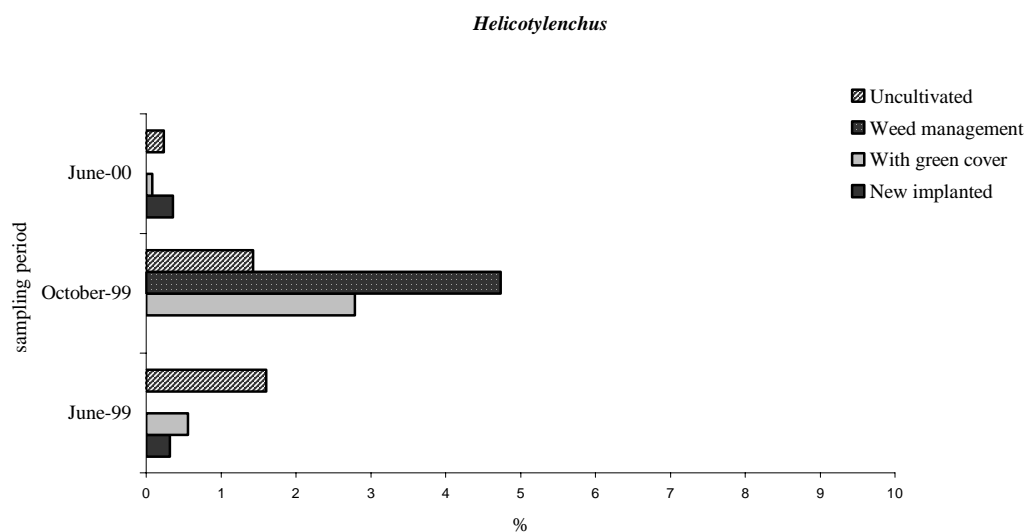


Figure 3. Seasonal distribution and percentage of *Helicotylenchus* on the total abundance of nematodes.

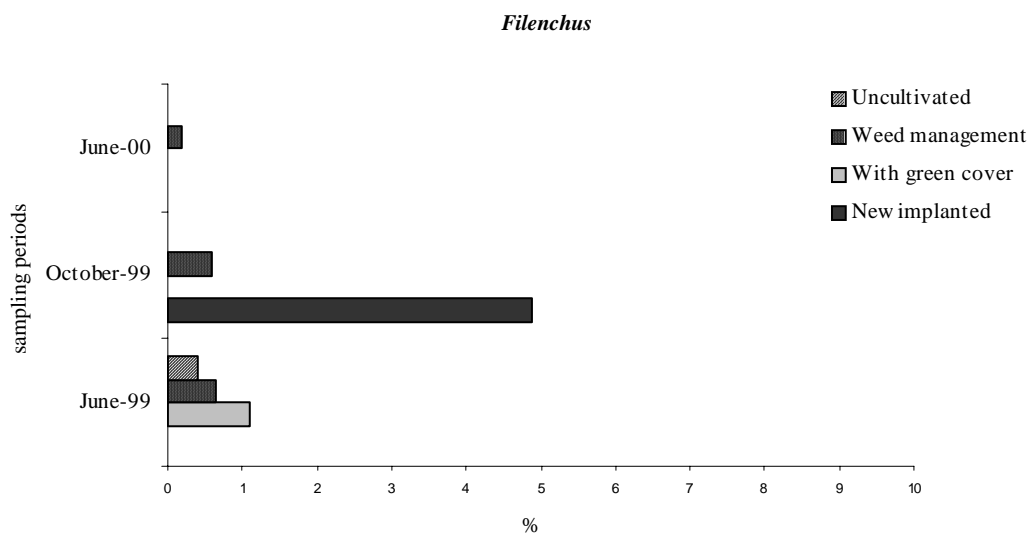


Figure 4. Seasonal distribution and percentage of *Filenchus* on the total abundance of nematodes.

Other nematodes, which are reported, be serious pest for vineyards were collected in all the fields studied always under the injury level: *Paratylenchus*, *Criconemoides* and *Tylenchorhynchus*.

Pine nematodes (*Paratylenchus*) pierce root cells from the soil outside of the plant. Low numbers may appear inconsequential, but in high enough numbers, pin nematodes can damage crops. Ring nematodes (*Criconematidae*) behave similarly. In spite of this little is known about ring nematode damage on many crop plants. *Tylenchorhynchus* is of minor economic importance however some stunting of grape can causes a reduction in host vigor. Stunt nematodes, favored in sand loam or loam soil, are widely distributed.

Conclusion

Plant parasitic nematodes, reported to damage grapevine, were found in all vineyards. Their population density was always lower than the injury level, except in the case of *Pratylenchus* in abandoned (old) vineyards. However as aged vineyards are replanted on infested sites it is probable that these nematodes could cause yield loss in new replanted vineyards. For this reason monitoring plant parasitic nematodes could be suggested before replanting vineyards in the same site of aged grapevines. Knowledge of nematode species present in damaging numbers could also provide a basis for assessing the need for chemical controls. The next goal will be providing information that could be utilized by wine grape producers for rational decision-making regarding nematode management, including the selection of rootstocks and the limitation of nematode spread through prudent cultural practices.

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Un nouveau dépérissement de la vigne en France : le Black Dead Arm causé par *Botryosphaeria* spp.

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Résumé: Le Black Dead Arm a été identifié dans le vignoble français en 1999. Il s'attaque à la charpente de la plante dont il provoque la mort à plus ou moins long terme. Les premières analyses microbiologiques ont montré que deux principaux champignons sont associés à cette maladie : *Botryosphaeria obtusa* et *Botryosphaeria dothidea*. La biologie de *B. obtusa* a été plus particulièrement étudiée. Ces études réalisées pendant la période de repos de la vigne dans le vignoble bordelais pendant l'hiver 99/00 ont montré qu'il peut être considéré comme un champignon à dissémination aérienne pendant une partie de son cycle biologique. Les blessures de taille seraient une des voies de pénétration du champignon dans la plante. La source d'inoculum (pycnides) est localisée à la surface de chancres au niveau des troncs et des bras et sur les sarments. Ce champignon peut également se propager par des sarments de greffons en pépinières.

Mots-clés: Black dead Arm, *Botryosphaeria obtusa*, cycle biologique, bois, vigne.

Abstract: A new grapevine decline in France: Black dead Arm caused by *Botryosphaeria* spp. Black Dead Arm has been identified in the french vineyards in 1999. It attacks the wood of the plant, causing decline and eventually death. First microbiological analyses have shown that two main fungi were associated with this disease: *Botryosphaeria obtusa* and *Botryosphaeria dothidea*. The biology of *B. obtusa* has been more particularly studied. These studies carried out during the dormancy period in the Bordeaux vineyard in winter 99/00 showed that it can be considered airborne fungus during a period of its life cycle. The pruning wounds should be one of the ways by which *B. obtusa* penetrates into the plant. The source of inoculum (pycnidia) occurred at the surface of cankers on arms and trunks, and diseased canes.

This fungus can be propagated by scions in nursery.

Key words: Black Dead Arm, *Botryosphaeria obtusa*, life cycle, wood, grapevine.

Introduction

Le Black Dead Arm est une maladie de dépérissement, identifiée pour la première fois dans le vignoble de Bordeaux en 1999 (Larignon *et al.*, 2000). C'est une maladie qui s'attaque à la charpente de la souche dont il provoque la mort à plus ou moins long terme. Ce dépérissement a été attribué à l'Esca à cause de la similitude des symptômes se manifestant sur la végétation. Selon les enquêtes jusqu'à maintenant réalisées, il semble toucher les vignes âgées au moins de huit ans et n'est observé sur des vignes qui n'ont pas été traitées à l'arsénite de sodium.

Les symptômes au niveau de la partie herbacée apparaissent à partir du début juin dans le vignoble bordelais. Ce sont les feuilles de la partie inférieure qui sont touchées les premières. Pour les cépages noirs, des taches de couleur rouge vineux apparaissent en bordure des feuilles ou à l'intérieur du limbe. Ces taches s'agrandissent, fusionnent pour donner de plus grandes plages. Elles prennent ensuite une teinte "feuille morte", ne laissant ainsi qu'un liseré rouge entre cette partie et celle de la feuille encore verte. Concernant les cépages blancs, des

décolorations pâles et orangées apparaissent en bordure des feuilles ou sur le limbe, puis s'étendent et fusionnent pour donner des plages sèches et friables entre les nervures toujours vertes. Dans les cas les plus graves, elles se dessèchent complètement, se recroquevillent, puis tombent. Les rameaux se dessèchent. Selon la gravité de la maladie ou la période où elle se manifeste, elle peut toucher les inflorescences ou alors les fruits conduisant à leur dessèchement.

Le décollement de l'écorce à la main montre une bande brune d'une largeur de quelques centimètres, qui part du rameau atteint pouvant aller jusqu'au niveau de la soudure.

Cette maladie fut pour la première fois décrite dans le vignoble du Tokay (Lehoczky, 1974) dans les années 70, puis par Cristinzio (1978) et Rovesti et Montermini (1987) en Italie. Elle fut attribuée à *Botryosphaeria stevensii* dans le vignoble hongrois et à *Botryosphaeria obtusa* dans le vignoble italien. Dans le vignoble français, deux champignons semblent être associés à cette maladie. Il s'agit de *Botryosphaeria obtusa* et de *Botryosphaeria dothidea*. La biologie de ces champignons est actuellement peu connue. Leur connaissance est pourtant nécessaire pour mieux diriger la lutte à l'égard du Black Dead Arm et trouver une solution de rechange aussi efficace et de moindre toxicité que l'arsénite de sodium. Les travaux rapportés dans cet article présentent les premiers résultats concernant la biologie de *B. obtusa*, champignon qui est plus particulièrement isolé dans le vignoble de Bordeaux. Ces études concernent plus particulièrement les points suivants : sa recherche au niveau des plaies de taille et à l'intérieur des sarments pendant la période de repos de la vigne, l'étude de la réceptivité des plaies vis-à-vis de ce champignon et le suivi de sa sporée aérienne.

Matériel et méthodes

Les études sont réalisées pendant la période hivernale 1999/2000 sur une parcelle caractérisée par un très fort taux de la maladie. Elle est située sur la commune de Naujan-et-Postiac, en Bordelais (Entre-Deux-Mers), et plantée avec le cépage Cabernet Sauvignon, greffé sur porte-greffe SO4. Elle est conduite en cordon latéral et âgée de 35 ans en 1998 (date de plantation : 1963). Cette parcelle n'a jamais été traitée à l'arsénite de sodium.

Identification des sources d'inoculum

Les fructifications des champignons sont recherchées sur le tronc, les bras et les bois de taille laissés sur le sol.

Sporée aérienne

Elle est réalisée à l'aide de pièges qui sont constituées de lames vaselinées, placées à 1-2 cm de la surface de zones excoriées, la face vaselinée dirigée vers le bois. L'expérimentation a commencé le 16 décembre 1999 et s'est terminée le 25 avril 2000. Chaque semaine, 30 lames sont prélevées et examinées au laboratoire selon la méthode décrite par Larignon et Dubos (2000). La suspension ainsi obtenue est analysée à l'aide d'une cellule de Malassez. Les spores sont dénombrées sur dix champs, soit 10 µl.

Recherche de Botryosphaeria obtusa au niveau des plaies de taille et dans les sarments

Pour chaque période de taille (16 décembre 1999, 1 février et 21 mars 2000), les sarments sont coupés à environ 20 cm de leur base. Chaque semaine et pour chacune des périodes de taille, 40 sections de 10 à 15 cm de long sont prélevées au hasard jusqu'au 25 avril 2000. La taille réalisée en mars correspond à la période des pleurs. Les sarments sont analysés selon la méthode de Paillassa (1992) qui consiste à les écorcer sur 1 cm à partir de la plaie et à les découper en 10 rondelles qui sont ensuite mises sur le milieu de culture (malt-agar).

La recherche de *B. obtusa* à l'intérieur des tissus ligneux est effectuée sur des sarments non taillés. La zone analysée a la même localisation que celle des sarments taillés. Quarante rameaux sont ainsi prélevés chaque semaine et cela jusqu'au 25 avril 2000.

Réceptivité des plaies de taille vis-à-vis de Botryosphaeria obtusa

Elle a été réalisée pendant l'hiver 1999/2000 aux mêmes dates de taille que précédemment. Chaque semaine, 30 plaies sont inoculées avec une suspension de conidies de *B. obtusa* à raison de 50 par plaie jusqu'au 25 avril 2000. Quinze jours après l'inoculation, elles sont prélevées et analysées selon la méthode de Paillassa (1992).

Résultats

Recherche des sources d'inoculum

Les pycnides sont situées sur le bois de vigne, plus particulièrement sur les parties qui sont excoriées. Elles sont également observées sur les bois de taille laissés au niveau du sol.

Sporée aérienne (Fig. 1)

La sporée aérienne a commencé lors de la semaine du 1 février 2000 et s'est poursuivie toutes les semaines jusqu'à la fin de l'expérimentation, à l'exception de la période allant du 7 au 21 mars.

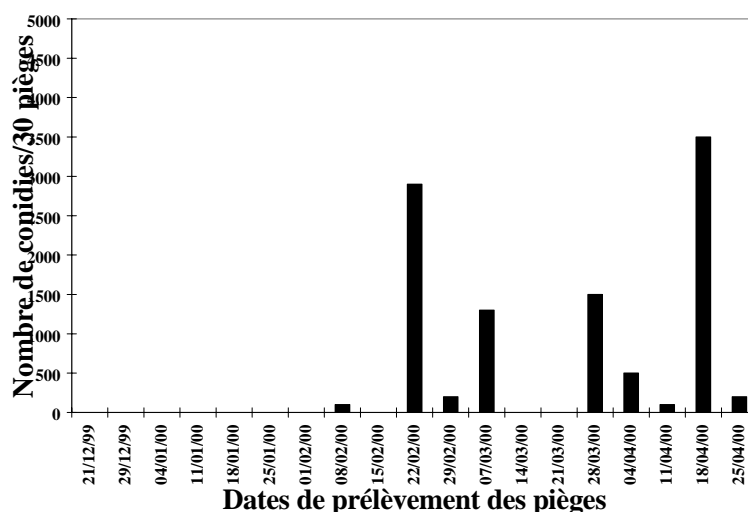


Fig. 1. Suivi de la sporée aérienne de *Botryosphaeria obtusa* pendant la période du 16/12/1999 au 25/04/2000

Recherche de B. obtusa au niveau des plaies de taille et dans les sarments (Fig. 2)

Il est isolé dans 3 % des plaies analysées pour la taille de décembre, 7,7 % pour celle de février et 3,5 % pour celle de mars. Il est aussi isolé dans les sarments non taillés (2,38 %).

Pour la taille de décembre, il est isolé toutes les semaines à partir de la 4^{ème} semaine de prélèvement des plaies de taille (à l'exception du 01/02, 14/03, 4 et 12/04). Pour celle de février, il est isolé toutes les semaines depuis le jour même de la taille. Pour la taille de mars, il est isolé dans 5 %, 2,5 % et 10 % des plaies prélevées respectivement le 28/03, le 18/04 et le 25/04.

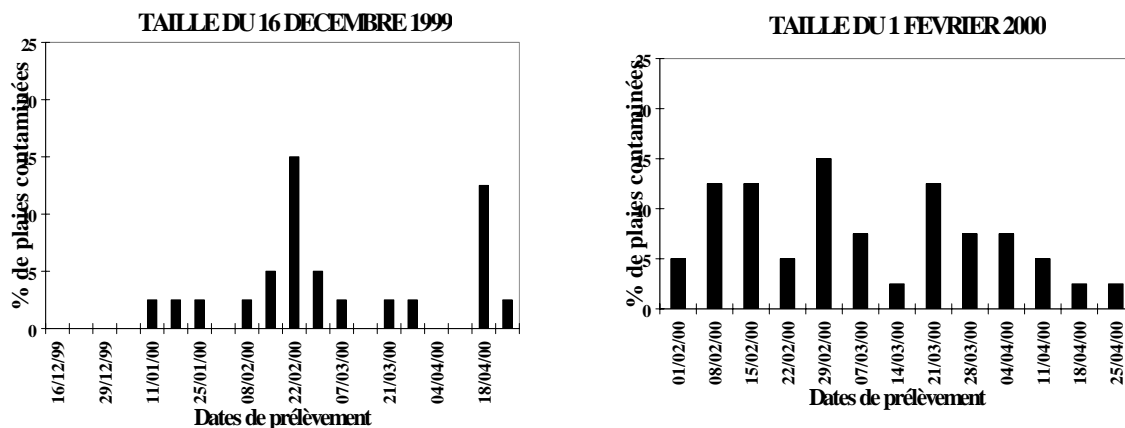


Fig. 2. Périodes de contamination des plaies de taille par *Botryosphaeria obtusa* pour l'hiver 99/00.

Etude de la réceptivité des plaies de taille vis-à-vis de Botryosphaeria obtusa (Fig. 3)

Le taux de réisolement du champignon est très élevé le jour même de la taille (90 %) pour devenir très faible à partir de la 2^{ème} semaine (10%) pour la taille de décembre, et à partir de la 1^{ère} semaine pour les tailles de février (17 %) et de mars (3%).

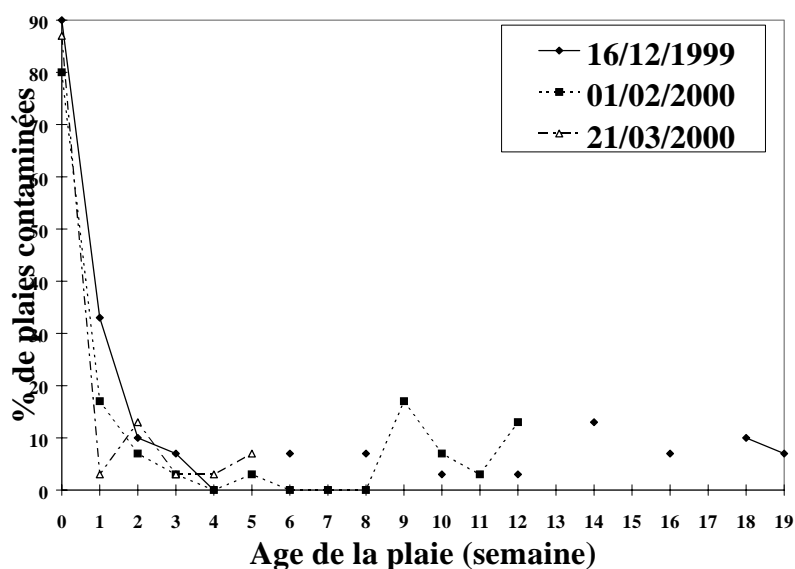


Fig. 3. Etude de la réceptivité des plaies de taille vis-à-vis de *B. obtusa* lors de l'hiver 1999/2000.

Discussion

La source d'inoculum de *B. obtusa* a été observée sous forme de pycnides sur des troncs et sur les bois de taille laissés au niveau du sol. Ce résultat est lourd de conséquences dans la pratique pour les viticulteurs. Dans les parcelles atteintes par le Black Dead Arm, il serait peu souhaitable de laisser les bois de taille sur le sol.

L'analyse de la microflore des plaies de taille pendant la période hivernale semble montrer que ce champignon peut contaminer les plaies, notamment suite à la taille de février. En effet, le pourcentage de son isolement dans les tissus sous-jacents à la plaie (7,71 %) est

plus élevé que celui des sarments non taillés (2,38 %). Ces contaminations ont eu lieu grâce aux émissions de spores observées début février, moins d'une semaine après la taille et donc à une période durant laquelle les plaies sont encore réceptives.

Lors de la taille de décembre, il est difficile de conclure que cette taille a été à l'origine de contaminations puisque le pourcentage de son isolement dans les sarments taillés (3,03 %) est similaire à celui des sarments non taillés (2,38 %). De plus, les études sur le suivi de la sporée aérienne montrent que les premières émissions avaient eu lieu seulement quatre semaines après la taille. Or, à cet âge, les plaies ne semblent plus être réceptives comme le montrent les résultats sur la réceptivité des plaies de taille (durée de réceptivité : 1 à 2 semaines pour les tailles précoces).

Pour la taille de mars, en dépit de l'émission de spores à cette période, le pourcentage de sarments contaminés (3,50 %) n'est pas significativement supérieur à celui des sarments non taillés. Cela s'expliquerait par le fait que les pleurs empêchent la pénétration des spores.

Cette étude a montré également que *B. obtusa* est isolé dans les sarments non taillés. Cela suggère qu'il peut se propager en pépinières par les bois infectés.

Cette étude a permis d'améliorer les connaissances au sujet de la biologie de *B. obtusa*, qui était jusqu'alors inconnue. Cependant, de nombreuses questions restent à élucider. Pour cela, les actions futures devront porter sur la détermination des facteurs favorables à la formation des pycnides, la dissémination des spores et la contamination des plaies (si celle-ci se vérifie), et sur la recherche d'autres possibles voies de pénétration du champignon dans la plante. De plus, sa propagation par les bois en pépinières devra être confirmée.

Remerciements

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Ten years investigations for the promotion of integrated viticulture in Greece

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Abstract: During the last decade the promotion of integrated systems for the control of pests and diseases of grapevine has been the first priority in Plant Protection Institute of Volos. The period 1992-2002 several activities have been undertaken in order to persuade the Greek growers to adopt components of IPM. The central point of the success of these efforts were the training on the principles of integrated viticulture of more than 100 high qualified agronom engineers coming from most grapevine growing areas of Greece and secondly the creation of a scientific advisory network between Research Centers and enterprises. The outcomes of these efforts were: a) The diffusion of research in the main viticultural areas of Greece. More than 40 pilot vineyards were established in order to demonstrate the advantages of the integrated viticulture. b) The creation of the map of the phytoseiids occurring on 37 grapevine cultivars in most grapevine growing areas of Greece. c) Data from experiments which were carried out under different environments in order to define the side effects of the various agrochemicals on phytoseiids. d) Data of experiments for lower use of agrochemicals and promotion of natural, cultural, biological and highly specific methods for the control of pests and diseases. The method of mating disruption for the control of berry moth *Lobesia botrana* was successfully applied for 3 subsequently years in large grapevine areas in the islands of Samos and Lemnos. The last achievement was the creation in the Plant Protection Institute of the "Genetic Collection of the Greek Cultivars *in vitro*" which will be the origin for the production of certified propagation material of the Greek cultivars. The above mentioned activities were supported by the EU projects ETIC-vigne, EPET II, INTERREG II and ADAPT II.

Key words: IPM, cultivars, predatory mites, *Lobesia botrana*, agrochemicals, powdery mildew

Introduction

During the period 1992-2002 the promotion of IPM systems for the control of diseases and pests of grapevine has been the first priority at the Plant Protection Institute of Volos (Rumbos and Koutroubas, 1995; Rumbos, 1996; Rumbos & Koutroubas, 1996; Rumbos, 2000; Koutroubas et al., 2000; Rumbos et al., 2001). The most important think was to persuade the Greek vine growers to adopt components of IPM. On the other had it was necessary to carry out experiments and to establish pilot vineyards in different vine growing areas of Greece in order to reduce the use of dangerous agrochemicals and to promote natural, cultural, biological and highly specific methods for the control of pests and diseases. It was also important to define the different phytoseiids occurring on the different grape cultivars at the different grape growing areas of Greece. Another important point was to define the side effects of the various agrochemicals on phytoseiids and to provide to the grape growers

healthy grape propagative material of the different local cultivars. All these targets were achieved by participating in several national or EU projects (ETIC vigne, ADAPT II, EPET II, INTERREG II, DIMITRA)

Materials and methods

During the period 1992-2002 about 40 pilot vineyards of 37 different cultivars were established in various grape growing areas of Greece in order to study the existing phytoseiids species, the side effect of the different agrochemicals on the phytoseiids and to determine the most ecological and effective spray programs for the control of pests and diseases of grapevine. All trials were carried out three years long under field conditions in vineyards of Central, North and South Greece, as well as in several Greek islands (Samos, Lemnos, Paros, Santorini, Cephalonia). For the most important local grape cultivars sanitary methods were applied for the production of healthy propagative material.

The pest control protocols included several fungicides, as copper compounds, sulphur (dust and wettable) triazoles, dithiocarbamates and 10 different insecticides preferably Bt. All treatments were applied in four repetitions. Twenty five leaves were collected per repetition in 2 week interval, resulting $4 \times 25 = 100$ leaves per treatment. Sampling started mid April and finished mid November. Leaves were sent to laboratory in paper bags. Phytoseiids were recorded under the stereomicroscope.

For the control of the berry moth *Lobesia botrana* integrated control programs were applied in various pilot vineyards. In a vineyard of 25ha in the island Lemnos and in a vineyard of 30 ha in the island Samos the method of mating disruption was applied for 3 subsequent years against the first and second generation, while products of *Bacillus thuringiensis* (Agree, Novartis) were used twice against the 3rd generation of the insect. Fifty dispensers per 1000 m² were used in equable pattern. The dispensers, type RAK-Z PLUS, contained E, Z-7, 9 DD A (E,Z), 7-9- dodecadienyl acetate of BASF company. A 10 acres vineyard which was 600 m away from the experimental plot was used as control. There were four pheromone traps in the experimental vineyard and one trap in the control vineyard for a closer watch of the method.

For the control of the powdery mildew fungus *Uncinula necator* (Schw.) 10 experimental vineyards in 10 different grape growing areas of Greece were used. Protection programmes were based mainly on powder sulphur, and secondarily on wettable sulphur and some triazoles. Depending on the grape growing area, the number of the treatments carried out every year was fluctuated between one (island Santorini) and seven (Volos). The doses of the fungicides used were the recommended ones. For the powder sulphur a dose of 20kg/acre was applied. Leaf samples were collected every 2 weeks and examined for mites through out the vegetation period. The trials were carried out for 3 subsequent years .

The sanitary program for the production of healthy grape propagative material was applied on 37 local cultivars and was included: a) the detection of six virus diseases, namely: Grapevine fanleaf virus (GFLV), Grapevine Leafroll associated Virus 1 (GLRaV-1), Grapevine Leafroll associated Virus 3 (GLRaV-3), Grapevine Virus A (GVA) and b) the isolation on PDA nutrient medium of the fungi *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., *Botryosphaeria dothidea* and *Cylindrocarpon destructans*.

Results

Phytoseiids

The survey conducted in the main viticultural areas of Greece showed that a total of 20 species of Phytoseiidae occur. The predominant species was *Phytoseius finitimus* which was

found in proportional frequencies between 90-100%, followed by *Amphyseius californicus*, *Typhlodromus hellenicus*, *Amphyseius finlandicus* and *Typhlodromus exhilaratus* (Table 1). The distribution and the occurrence of the various phytoseiids on the different Greek cultivars is presented in Figure 1.

Table 1: Phytoseiids occurring in different grapevine growing areas of Greece

Region	Phytoseiidae	% Occurrence
Nemea	<i>Typhlodromus hellenicus</i> Swirski & Ragusa	99,0
	<i>Phytoseius finitimus</i> (Ribaga)	
	<i>Seiulus amaliae</i> Swirski & Ragusa	1,0
Santorini	<i>Phytoseius finitimus</i> (Ribaga)	99,5
	<i>Amblyseius andersoni</i> (Chant)	
	<i>Typhlodromus Kerkirae</i> Swirski & Ragusa	0,5
	<i>Typhlodromus commenticiosus</i> Liv. & Kuzn.	
Samos	<i>Phytoseius finitimus</i> (Ribaga)	65,0
	<i>Amblyseius californicus</i> (Mc Gregor)	32,5
	<i>Amblyseius stipulatus</i> Athias-Henriot	2,0
	<i>Amblyseius finlandicus</i> (Oudemans)	
	<i>Seiulus amaliae</i> Swirski & Ragusa	0,5
Lemnos	<i>Typhlodromus perbibus</i> (Wainstein)	
	<i>Phytoseius finitimus</i> (Ribaga)	100,0
Kavala	<i>Amphyseius californicus</i> (Mc Gregor)	99,5
	<i>Typhlodromus hellenicus</i> Swirski & Ragusa	
	<i>Typhlodromus intercalaris</i> Liv. & Kuzn.	0,5
	<i>Typhlodromus cotoneastri</i> Wainstein	
Crete	<i>Typhlodromus exhilaratus</i> Ragusa	60,0
	<i>Phytoseius finitimus</i> (Ribaga)	40,0
Volos	<i>Phytoseius finitimus</i> (Ribaga)	99,0
	<i>Typhlodromus athiasae</i> Porath & Swirski	0,4
	<i>Seiulus amaliae</i> Swirski & Ragusa	0,3
	<i>Kampimodromus aberrans</i> (Oudemans)	
	<i>Typhlodromus perbibus</i> (Wainstein)	
	<i>Typhlodromus kerkirae</i> Swirski & Ragusa	0,3
	<i>Typhlodromus cotoneastri</i> Wainstein	
Karditsa	<i>Typhlodromus hellenicus</i> Swirski & Ragusa	
	<i>Phytoseius finitimus</i> (Ribaga)	99,0
	<i>Seiulus amaliae</i> Swirski & Ragusa	0,5
	<i>Amphyseius marginatus</i> (Wainstein)	0,2
	<i>Amblyseius andersoni</i> (Chant)	
	<i>Amblyseius barkeri</i> (Hughes)	
Mantinia	<i>Kampimodromus aberrans</i> (Oudemans)	0,3
	<i>Typhlodromus hellenicus</i> Swirski & Ragusa	
	<i>Phytoseius finitimus</i> (Ribaga)	99,5
	<i>Amblyseius californicus</i> (Mc Gregor)	
Naoussa	<i>Typhlodromus kerkirae</i> Swirski & Ragusa	0,5
	<i>Typhlodromus involutus</i> Liv. & Kuzn.	
	<i>Amblyseius finlandicus</i> (Oudemans)	98,5
Naoussa	<i>Amblyseius andersoni</i> (Chant)	1,0
	<i>Typhlodromus hellenicus</i> Swirski & Ragusa	
	<i>Typhlodromus kerkirae</i> Swirski & Ragusa	0,5
	<i>Phytoseius finitimus</i> (Ribaga)	

Side effect of agrochemicals on phytoseiids

In the Tables 2-5 are presented the results of the side effect of the application of different copper, dithiocarbamates, conventional insecticides and Bt products on the predatory mite *Phytoseius finitimus*

Table 2: Influence of copper products used against *Plasmopara viticola* to the predator mite *Phytoseius finitimus* (1997)

Treatment ¹	g ή ml/ 100 lt	Measurements of <i>P. finitimus</i> ²		
		Before treatment	5 days after the 1 st treatment	34 days after the 3d treatment
Kocide	150	20.50 a ³	16.25 a	19.25 a
B.B.S.	250	19.75 a	16.25 a	18.25 a
Tenn-cop	150	22.75 a	17.75 a	26.00 a
Viricuire	150	17.50 a	16.00 a	16.50 a
Quinolate	150	28.50 a	12.50 a	8.75 a
Control	--	26.50 a	20.00 a	31.50 a

1. Dates of treatments: 13/6/97, 24/6/97, 7/7/97, 21/7/97

2. Means of 4 repetitions of 25 leaves

Table 3: Influence of fungicides used against *Plasmopara viticola* to the predator mite *Phytoseius finitimus* (1997)

Treatment ¹	g ή ml/ 100 lt	Measurements of <i>P. finitimus</i> ²		
		Before treatment	5 days after the 1 st treatment	35 days after the 3d treatment
Alliete	300	16,25 a ³	8.25 bc	5.50 b
Antracol+Bayfidan	200	13,25 a	1.50 c	0.50 b
Polyram+Combi	200	18,25 a	11.25 b	4.00 b
Euparen	200	28,00 a	16.25 b	2.50 b
Dithane	200	23,50 a	8.75 bc	5.25 b
Control	--	24,00 a	26.50 a	21.25 a

1. Dates of treatments: 13/6/97, 24/6/97, 7/7/97

2. Means of 4 repetitions of 25 leaves

Table 4: Influence of insecticides used against *Polychrosis botrana* to the predator mite *Phytoseius finitimus* (1997)

Treatment ¹	g ή ml/ 100 lt	Measurements of <i>P. finitimus</i> ²		
		Before treatment	8 days after the 1 st treatment	36 days after the 3d treatment
Sulphur powder + <i>B. thuringiensis</i>		55.00 a ³	48.75 a	19.00 ab
Dipel	100	62.50 a	32.00 b	3.75 d
Insegar	40	55.00 a	6.25 c	15.50 abc
Sevin	150	58.25 a	1.50 c	3.00 d
Polimal	150	60.25 a	0.25 c	4.75 d
Zolone	200	52.75 a	0.50 c	1.75 d
Thiodan	150	53.50 a	0.00 c	7.75 cd
Mavrik	30	57.75 a	0.75 c	1.00 d
Pennicap	120.5	63.50 a	2.50 c	10.50 bcd
Decis	50	49.25 a	1.75 c	1.00 d
Control	--	45.00 a	46.25 a	24.25 a

1. Dates of treatments: 8/7/97, 17/7/97, 4/8/97, 12/8/97

Table 5: Results of the effect of six microbial compounds based on *Bacillus thuringiensis* on the population fluctuation of the predator *Ph. finitimus*
 Counts of moving forms of *Ph. finitimus* on grapevine leaves²

Treatment ¹	Before treatment	5 days after the 1 st treatment	10 days after the 2 nd treatment	21 days after the 3 ^d treatment
Xen-tari	201.00 a ³	211.50 a	168.50 a	164.00 a
Bactospeine	205.75 a	189.00 a	154.25 a	169.50 a
Agree	172.50 a	166.25 a	114.25 b	148.75 a
BMP	189.25 a	182.25 a	132.25 b	171.50 a
Dipel	218.00 a	213.00 a	86.00 b	188.00 a
Bactecin	221.50 a	201.75 a	96.50 b	182.25 a
Control	200.25 a	201.25 a	115.00 b	168.75 a

¹ Spraying days: 14/7/99, 23/7/99, ² Average of four repetitions of a sample of 32 leaves, ³ Levels of statistical significance are P=0.05

Control of berry moth

The dry and warm climate of the islands resulted in a significant and gradual reduction of the stored pheromones in the dispensers. After 15th July the pheromone reserves was almost exhausted.

The effective protection of the grapes from the berry moth was achieved when the mating disruption method was combined with *Bacillus thuringiensis* treatments. In both cases-mating disruption alone or combined with Bt treatments - the results were satisfactory when compared with the usual chemical control where several applications of wide spectrum insecticides were applied (Table 6).

Table 6: Comparative pest control-classical, integrated and biological - in vineyards of Samos (1998)

Biological		Integrated		Classical	
06.05.98	Sulphur	06.05.98	Sulphur	27.04.98	Sulphur
21.05.98	»	21.05.98	»	08.05.98	Topas
07.06.98	»	07.06.98	»	22.05.98	Systhane
22.06.98	»	22.06.98	»	10.06.98	Sulphur
				21.06.98	Systhane
				03.07.98	Topas
12.05.98	Copper	12.05.98	Copper	02.05.98	Copper
28.05.98	»	28.05.98	»	15.05.98	»
15.07.98	»	15.07.98	»	27.05.98	Ridomyl
				12.06.98	Alper
				23.06.98	Copper
				12.07.98	»
mating	disruption	14.06.98	B.t	01.06.98	Carbaryl
»	»	24.06.98	»	10.06.98	Reldan
»	»	27.07.98	»	18.06.98	Pennacap
»	»	29.07.98	»	25.06.98	Thiodan
				05.07.98	Reldan
				15.07.98	Carbaryl
				22.07.98	Pennacap
				30.07.98	Reldan

Control of powdery mildew

Results showed that the fungus could be efficiently controlled by using powder sulphur without any negative influence on the predatory mites. The use of powder sulphur seems not to be influenced by the high temperatures, even during the summer months. No phytotoxicity symptoms were observed in any area during the three years of the trials. In the Figure 2 is presented the fluctuation of the population of *Ph. finitimus* after four sulphur applications.

From each of the 37 Greek local cultivars tested for their sanitary status, 5-12 genotypes which were found healthy were established *in vitro* culture. Their multiplication *in vitro* enabled the production of healthy propagative material and the establishment in some regions of healthy vineyards which will be used for the production of healthy material.

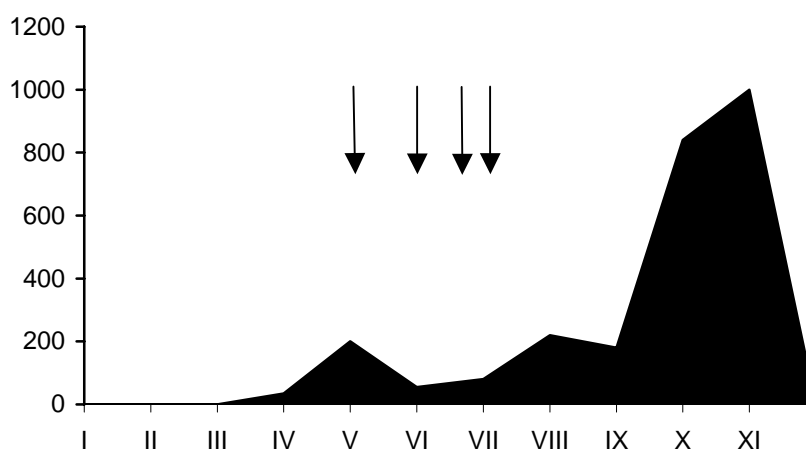


Fig. 2. Fluctuation of the population of *Ph. finitimus* after four sulphur-dust applications

Economical aspects

The efforts for the establishment of IPM systems on grapes were concentrated not only to the use of ecologically safer methods for the protection of the environment and the human health, but also to achieve a better economical benefits. It was achieved a 38,46% mean reduction of the number of the treatments (Figure 3) and a 37,6% reduction of the costs for buying the agrochemicals (Figure 4).

Discussion

During the period 1992-2002 for the promotion of the IPM on grapes in Greece several attempts were undertaken. In order to persuade the Greek grape growers to accept the principles of IPM at least 40 pilot vineyards in the different grape growing areas of Greece were used. This was done after teaching the basic principles of IPM to the local agronom engineers. Over 100 agronom engineers working for years in different viticultural areas came in Plant Protection Institute of Volos for 7 days and obtained valuable knowledges on IPM in grapes.

The development of regional research in the main viticultural areas of Greece enabled: a) the demonstration of the favorable economical aspects of IPM to the growers, b) to obtain valuable knowledge on the phytoseiids occurring in Greece, c) to study the side effect of the agrochemicals used in viticulture on the phytoseiids, d) to control the berry moth using new technology, e) to control powdery mildew – which is the most important fungul disease in

Greece – only with powder sulphur and f) to establish new vineyards with healthy propagative material.

The survey on the phytoseiids showed that at least 20 species occur in the viticultural areas of Greece. The predominant species is *Phytoseius finitimus* occurring in high percentages (90-100%).

The fungicides containing copper compounds (copper oxychloride, copper hydroxide), sulphur dust and wettable, the pyrimidines (fenarimol) and the triazoles (myclobutanil, triadimefon) have an indifferent to slightly negative effect on *Ph.finitimus*. The dithiocarbamates proved to have a toxic effect on the predatory mites. Similar toxic effect proved to have the classical insecticides when applied in three or four repetitions. (Rumbos *et al.*, 1997; Papaioannou-Souliotis *et al.*, 1998; 1999; Rumbos *et al.*, 2000). The combination of *Bacillus thuringiensis* plus sulphur dust (Bacticin) proved to have a neutral effect on the population of phytoseiids. The use of powder sulphur seems not to be influenced by the high temperatures, even during the summer months. No phytotoxicity symptoms were observed in any area. (Rumbos, 2000; Rumbos *et al.*, 2000).

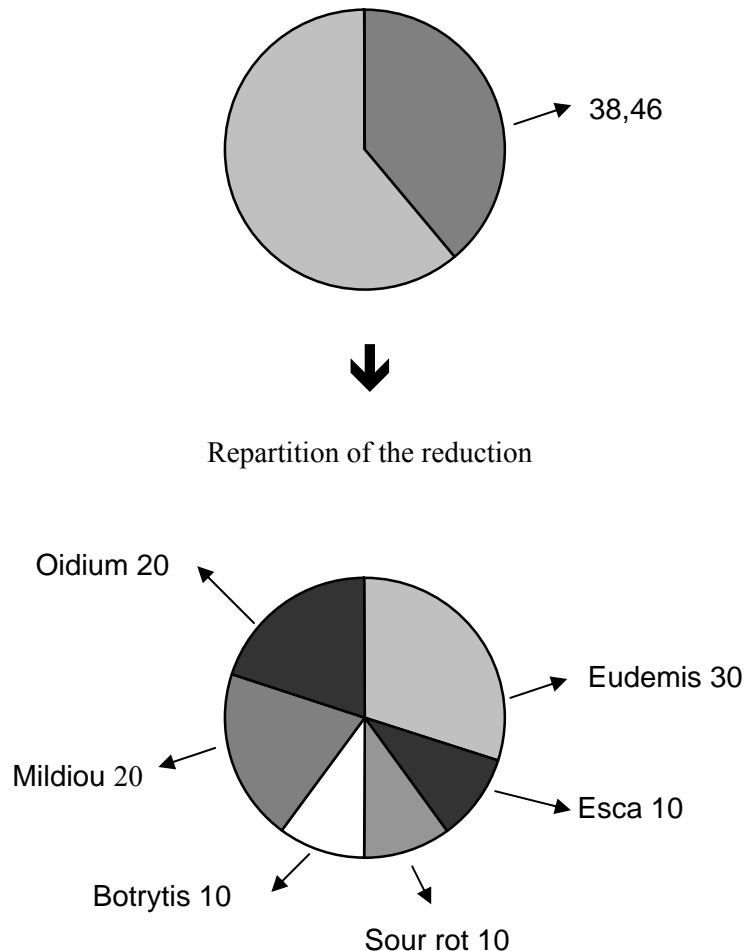


Fig. 3. Mean reduction % on the number of the treatments

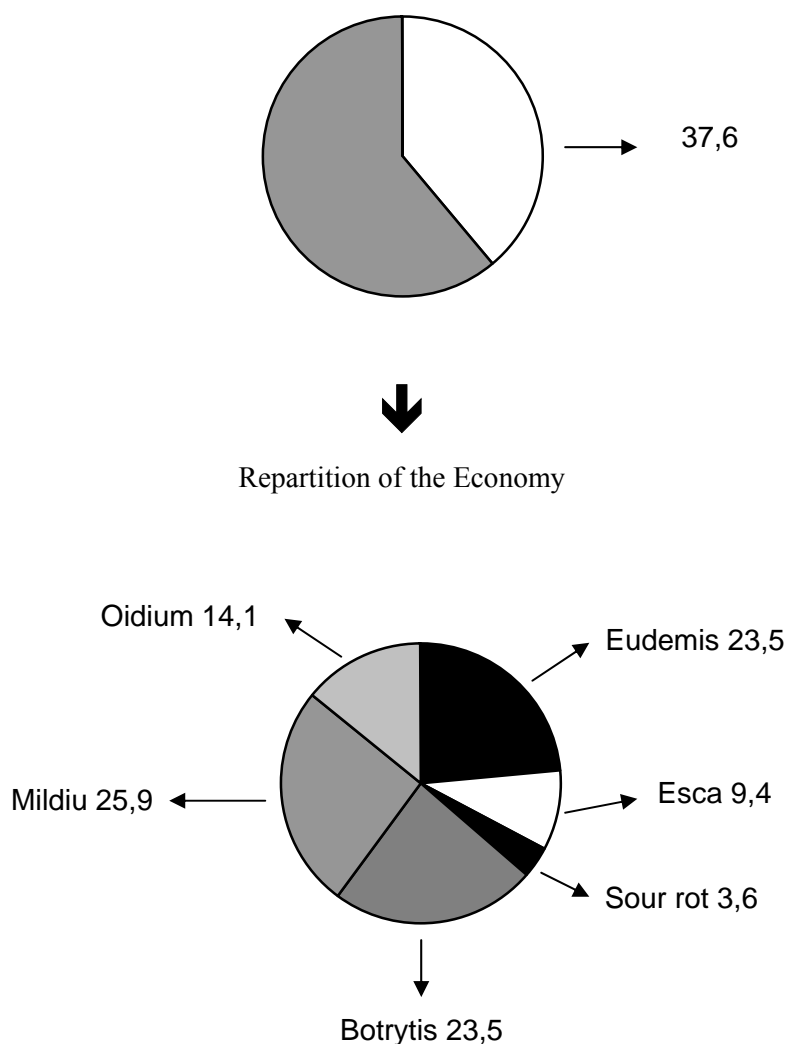


Fig. 4. Mean economy % on the cost of the chemicals

For the control of berry moth with the method of mating disruption it is important to take in consideration the dry and warm climate of Greece which is the main reason for the reduction of the pheromones content in the dispensers. The combination of mating disruption with Bt applications during the 3d generation gives the best results. (Koutroubas et al., 2000).

The problem concerning the use of defected grape propagative material which exists not only in the Greek, but also in the European and international grape market was confronted by applying sanitary programs and created a “Genetical Bank of Greek Grape Cultivars *in Vitro*” in Institute of Plant Protection in Volos. (Rumbos et al., 2000; Rumbos and Rumbou, 2001).

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Cooperation between wine industry and research centers for the promotion of integrated control systems in Greece

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Abstract: In the period 1998-2000 and in the frame of the project ADAPT II four pilot vineyards were established in different grape growing areas of Greece in order to promote integrated control systems against pests and diseases. The vineyards belonged to the winery E. TSANTALIS and were located in the regions of Saint Mount (cultivars: Grenache and Athiri), Maronia (cultivars: Chardonnay, Syrah, Limnio), Rapsani (cultivars: Xinomavro, Stavroto and Krasato) and Chalkidiki (cultivars: Asyrtico and Sauvignon). To reduce, pest disease and weed impact different natural, cultural and biological measures were applied, as for example appropriate choice of training systems, proper canopy management and avoidance of excessive nitrogen. The decision for the application of direct control measures was based on tolerance levels, risk assessment and the meteorological data which were provided by automatic weather station. Populations of pests and diseases were monitored and recorded. A warning, forecasting and early diagnosis system was established. Plant protection products were applied only when it was justified. Where the use of plant protection products was necessary, the most selective, least toxic, least persistent products, which were as safe as possible to humans, the beneficials and the environment were selected. The side effect on the beneficials of the different control programs applied in each area was also recorded. For this purpose from mid April till mid November leaf samples were collected every fortnight and the population of spider mites and predators were recorded. In each experimental plot 10 randomly picked vines were sampled, of which 10 leaves per stock were taken each resulting in samples made up of 10X10=100 leaves per plot. The measurements concerned the predatory mite *Phytoseius finitimus* which is prominent in these areas.

Key words: IPM, cultivars, agrochemicals, predatory mites

Introduction

The grape cultivated area in Greece is about 130.000 ha and the wine producing about 69.500 ha. The big wine producing enterprises (wineries) with their highly qualified technical personnel, their big in surface vineyards which they possess and their ability to have a different politic on the grape prices may play an important role in the distribution of new ideas and new technologies. This fact was used for promoting the principles of IPM in the frame of project ADAPT II. In total, 25 wine producing enterprises were participated.

The main target was to change the traditional viticulture into integrated by following means:

- * By persuading the grape growers for the advantages of IPM (economical, ecological)
- * By establishing pilot vineyards for demonstrating the principles of IPM.
- * By creating an information and communication network between Research Centers and wine enterprises in order to confront all existing or appearing problems.

The most important grape disease in Greece is powdery mildew (*Unicinula necator*). Downy mildew (*Plasmopara viticola*) and gray mold (*Botrytis cinerea*) can cause severe damages only in years with rich rainfalls (Rumbos, 2000; Rumbos et al., 2001).

The main pest is berry moth (*Lobesia botrana*). Flight activity monitoring is done by pheromone traps. The obtained flight curves help the advisory services to determine the spray schedule. In the 1st generation of the insect, sprays are not recommended. For the 2d and 3d generation two treatments are usually performed (Koutroubas et al., 2000).

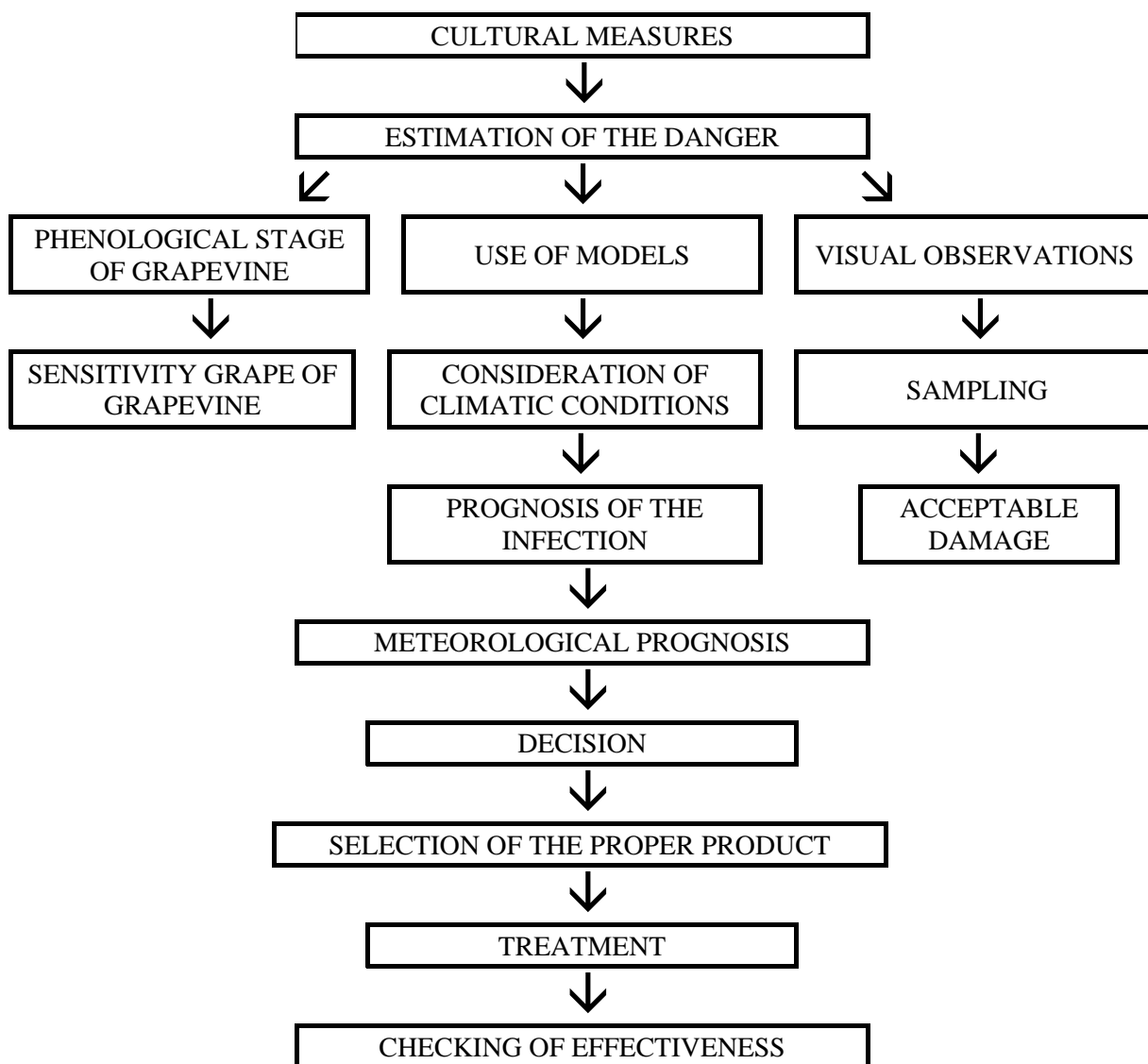


Fig. 1. Scheme showing the production adapted for taking the decision to apply or not against the diseases and pests of grapevine.

Material and methods

Pilot vineyards

In the period of 1998-2000 and in the frame of the project ADAPT II four pilot vineyards were established in different grape growing areas of Greece in order to promote principles of IPM. The vineyards belonged to the winery E. TSANTALIS and were located at the region of Saint Mount (cultivars: Grenach and Athiri), Maronia, North-East Greece (cultivars: Chardonnay, Syrah and Limnio), Rapsani-Olympus Mount area, Central Greece (cultivars: Xinomavro, Stavroto and Krasato) and Chalkidiki (cultivars: Asyrtico and Sauvignon).

Protection spray programs against pests and diseases

To reduce pest disease and weed impact different cultural measures were applied as for example appropriate choice of training systems, proper canopy management and avoidance of excessive nitrogen.

The decision for the application of direct control methods was based on tolerance levels and meteorological data provided by automatic weather stations (Fig. 1).

Populations of pests and diseases were monitored and recorded. A warning, forecasting and early diagnosis system (ATCON) was established and evaluated.

Plant protection products were applied against pest organisms only when it was justified. In order to minimize the use of agrochemicals priority was given to natural, biological and highly specific methods. Where the use of plant protection products were necessary the most selective, least toxic, least persistent products, were selected (Table 1 and 2).

The most suitable for each grape area control programs were determined.

Table 1. Copper content of phytosanitary products in the Greek market.

Product	Dosage in g / 100 l	Active ingredient	Cu %
Tenn-cop	225	5.14	11.5
Champ 24,4 sc	180-215	24.4	44
Funguran-OH	150-175	30	45
Kocide 15 sc	300-350	15	45
Quinolate	150	40	60
Cupravit 35 wp	220-350	35	77
Vorpo	500-700	13.6	88
Viricuvre	200-500	50	100
Nordox	200-300	50	100
BBS	400-500	25	100
Bouillie Bordelaise	500-600	20	100
Caldo Borde Les Valles 200 wp	550-650	20	110
Cupranorg 35 wp	500	35	175

Table 2. Biological products for the pest control of grapevine available in the Greek market.

Product	Name of microorganism	Pest	Company
Agree	<i>Bacillus thuringiensis</i>	Eudemis	Sygenta
Bactospeine	» »	»	Hellafarm
Xentari	» »	»	Bayer
Dipel	» »	»	Eythymiadis
BMP 123 WP	» »	»	Intrachem
Bactecin	B.t. + sulphur dust	»	Hellafarm
Trichodex	<i>Trichoderma harzianum</i>	Botrytis	Alpha

Side effect on beneficials

Each control program applied against pests and diseases was evaluated for its effect on the phytoseiids. For this purpose from mid April till mid November leaf samples were collected every fortnight and the population of spider mites predators were recorder. In each experimental plot 10 randomly picked vines were sampled, of which 10 leaves per stock were taken, each resulting in samples made up of 10x10=100 leaves per plot. The measurements on predators concerned the predatory mite *Phytoseius finitimus* which is predominant in these areas.

Results

Scientific advisory network

A scientific advisory network was created in order to supply to the participating enterprises all necessary technical and scientific knowledge for applying the principles of IPM. The advisory net consisted of 15 scientists of different science sectors e.g. plant pathology, entomology, acarology, vinology, nematology weed science soil science grape physiology, wine science etc. the center receiving all requests and inquiries was in Plant protection Institute of Volos (Figure 2).

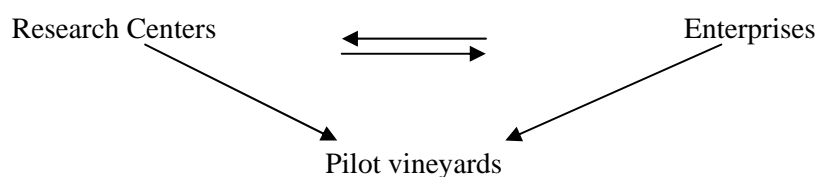


Fig. 2. Scientific advisory net.

Protection spray programs – Side effects on phytoseiids

During the period 1998-2000 several spray programs were applied against pests and diseases in the four main grape growing regions selected for our trials (Saint Mount, Maronia, Rapsani and Chalkidiki). In the figures 3,4,5 and 6 are presented the results concerning the fluctuation of the populations of the predator mite *Phytoseius finitimus* after the application of a specific protection spray program in each area.

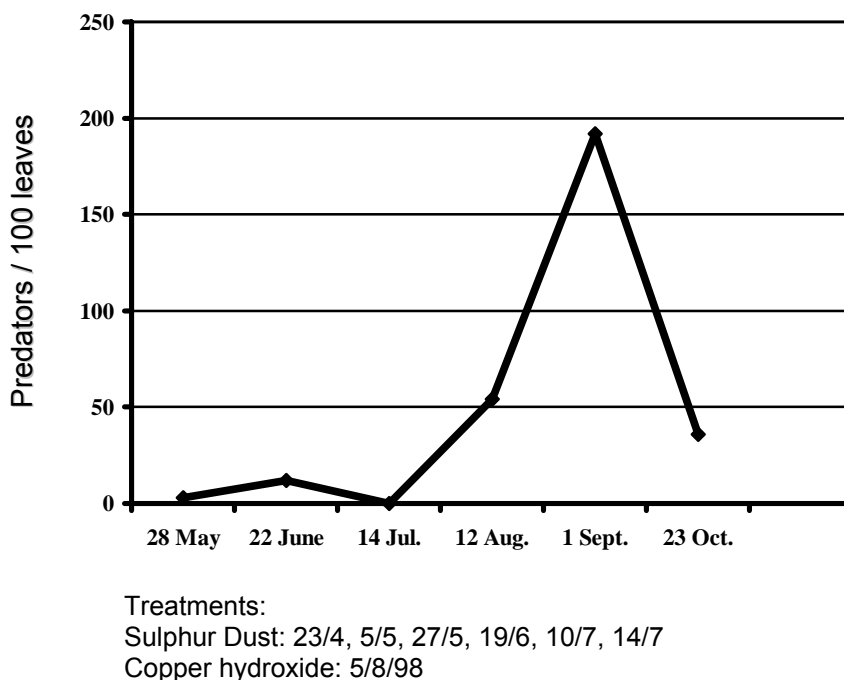


Fig. 3. Effect of fungicides on the population fluctuation of the predator *Phytoseius finitimus* in an experimental vineyard in the grape growing area of Saint Mount (cv. Athiri).

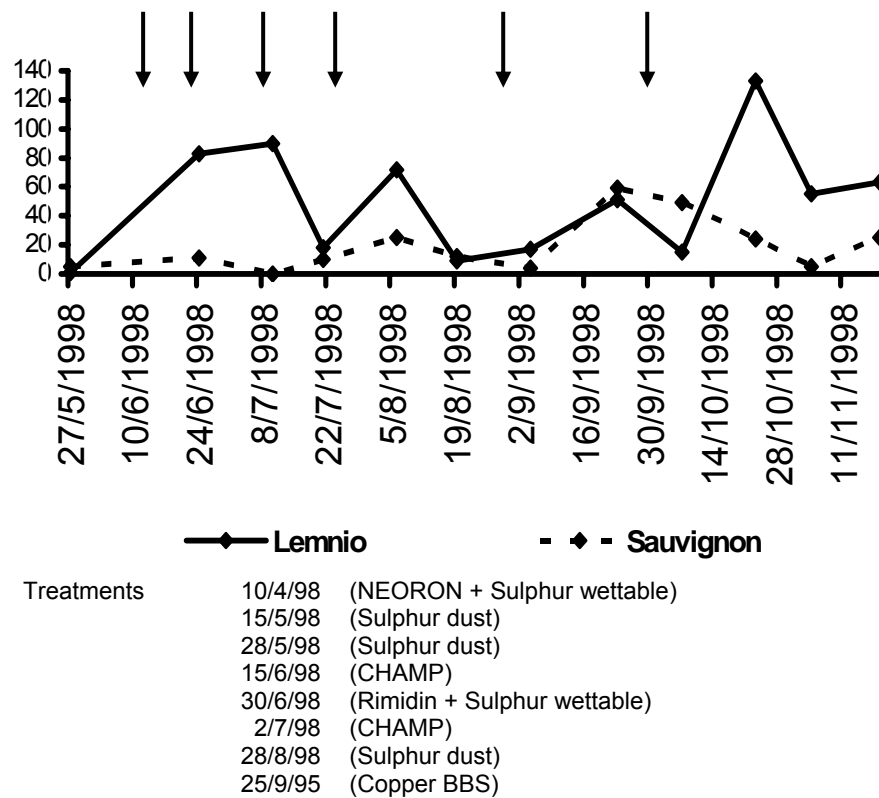


Fig. 4. Fluctuation of the population of *Phytoseius finitimus* in an experimental vineyard in the region of Maronia.

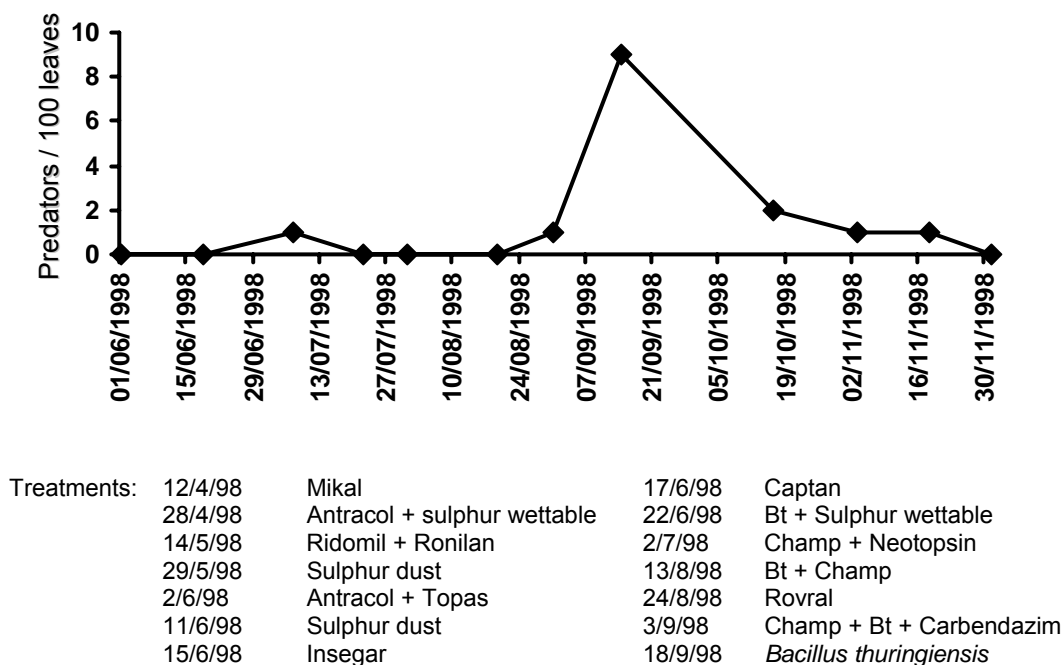


Fig. 5. Effect of fungicides on the population fluctuation of the predator *Phytoseius finitimus* in a experimental vineyard in the grape growing area of Saint Mount (cv. Athiri).

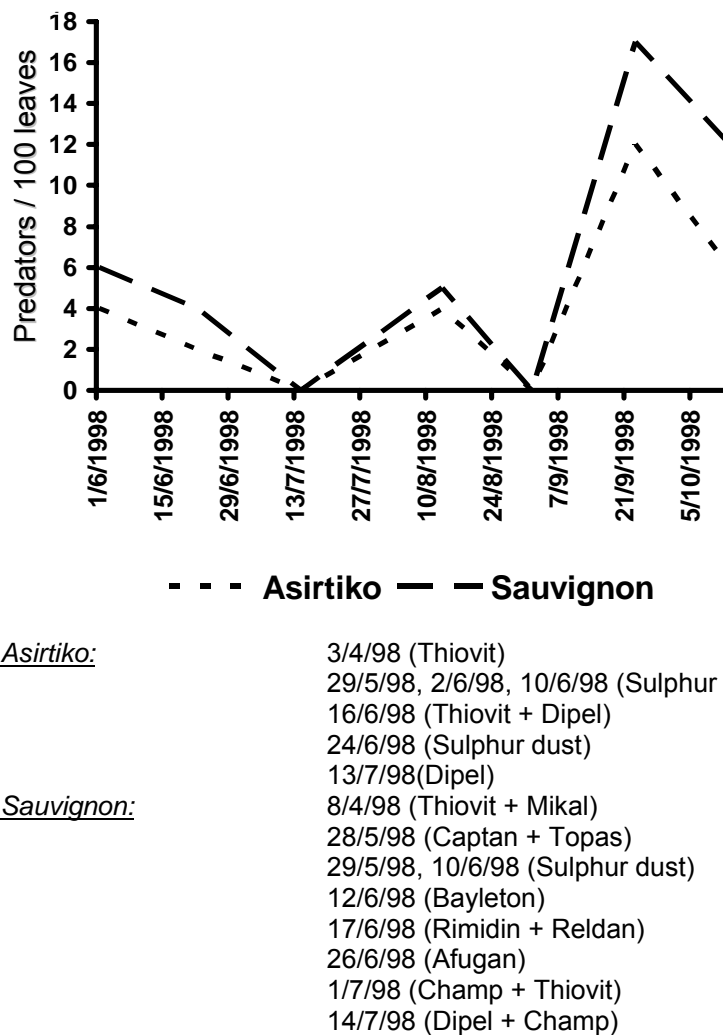


Fig. 6. Effect of spray program on the population of the predator mite *Phytoseius finitimus* in an experimental vineyards in the region of Saint Paulus, Chalkidiki.

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Discussion

The work done in the frame of the project ADAPT II 141/206 was aimed to the 1/ Promotion of a viticulture which respects the environment, is ecologically viable, and sustains the social, cultural and recreational aspects of agriculture. 2/ Securing of a sustainable production of healthy grapes of high quality and with a minimum occurrence of pesticide residues. 3/ Protection of farmer' s health while handling agrochemicals. 4/ Promotion and maintenance of high biological diversity in the ecosystem of the vineyard and in surrounding areas. 5/ Giving priority to the use of natural regulating mechanisms. 6/ Preservation of a long-term soil fertility and 7/ Minimizing pollution of water, soil and air. (Malavolta and Boller, 1999).

To achieve the above targets a collaboration of the Institute of Plant Protection in Volos with several wine producing enterprises in all over Greece was established. Priority was given to the training of agronomists on the principles of IPM which was achieved by carrying out training courses at the Institute of Plant Protection in Volos and local seminars in the different grape growing regions for the grape growers. Furthermore, the creation of a scientific consulting net among Research Centres and enterprises enabled the solution of the existing or appearing problems occurring during the application of the principles of IPM.

Several vineyards of the winery Tsantali in four different grape growing areas of Greece were used as pilot vineyards for the application of IPM.

For the control of pests and diseases the most safe products were used. The heart of all spray programs were sulphur, copper and *B. thuringiensis*. A list of the copper products existing on the Greek market was made according to their percentage in Cu with the recommendation to use those with the lowest content. For each grape region, the most effective and neutral to the populations of the phytoseiids spray program was selected.

The creation in 2000 of the Greek national standard for IPM (AGROSERT) enabled the company Tsantali to obtain the first certification in Greece for grapes.

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“Young grapevine decline” associated with defected propagated material

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Abstract: During the last five years many young vineyards with decline symptoms were observed in different grape growing areas of Greece. Symptoms were frequently similar to those attributed to esca disease. Growers suffered significant economic losses since they had to replant their vineyards. A wide scale investigation was carried out during the spring of 2002 on the whole grape propagated material produced or imported into Greece in order to determine the origin of the problem. Over 20.000 plants were collected and examined for the presence of wood discolorations and pathogenic fungi associated with the esca complex. The material collected came from all: a) the mother rootstock plantations, b) the Greek nurseries producing rooted cuttings and grafted plants and c) the imported material. All samples were transversely cut and observed for wood discolorations. Tissue chips were collected from each plant from the grafting point and the rootstock base and incubated in Petri dishes. Results on the presence of wood discolorations fluctuated between 0,3-3% on the simple cuttings, 2-15% on the rooted cuttings and 15-100% on the grafted plants. The pathogenic fungi isolated were in a low percentage (0,2-2 %). Among them were included *Phaeoconiella chlamydospora*, *Phaeoacremonium* spp., *Botryosphaeria dothidea* and *Cylindrocarpon destructans*. The low incidence of these pathogenic fungi suggested that they could not by themselves be the cause of young grapevine decline. Abiotic causes and lesions occurring in the nurseries, improper storage and transportation conditions of the propagated material might also play a role. A newly launched project by the Ministry of Agriculture will investigate the parameters of the problem.

Key words: grapevine, esca, propagated material.

Introduction

Decline symptoms in young grapevines have been always observed the first years after planting in Greece. In recent years, however, the problem has become more prevalent, since the incidence of decline symptoms in young grapevines has been increased. Several growers had to replant all or part of their young vineyards resulting in significant economic losses from replanting costs. Similar losses are also reported from California and other countries (Mugnai et al., 1999; Whiting et al., 2001).

Declining grapevines show significantly lower vigor, reduced foliage, shortened internodes, smaller leaf size and interveinal chlorosis. In cross section declining rootstocks exhibit dark-brown to black dots and in longitudinal section streaks in the vascular elements, particularly at the base where the roots start, and at the union point of scion and rootstock. The vines exhibit symptoms of graft failure and the graft sometimes is easily broken.

From infected vines the fungi *Phaeoacremonium chlamydospora*, *Phaeoacremonium* spp., *Cylindrocarpon destructans* and *Botryosphaeria dothidea* were isolated (Rumbos, 2000; Rumbos & Rumbou, 2001; Rumbos et al., 2002). It was concluded that in Greek grapevines there are at least two causes of vine decline: a) White rot caused by *Fomitiporia punctata* or other wood destroying fungi in older vineyards (esca proper), and b) poor planting material in newly established vineyards associated with different fungi such as *P. chlamydospora*, *Phaeoacremonium* spp., *Botryosphaeria* spp. and *Cylindrocarpon* spp., which colonize the scion or the rootstock in the nurseries (Rumbos & Rumbou, 2001).

The purpose of this work was to examine the role of fungal pathogens associated with sub-standard grapevine propagated material of cultivars and rootstocks in Greece. “Young Vine Decline” is a term which is used widely in California to describe unexpectedly poor performance of young vines (Stamp, 2001).

Materials and methods

In the period January-May 2002 over 20.000 samples from Greek and foreign grape nurseries were sent to our laboratory for examination. Four groups of grape material were examined: a) Cuttings of rootstocks from Greek mother plantations (number of cuttings 10500) b) Rooted rootstock cuttings from Greece and abroad (number of cuttings 3000) c) Canes of different cultivars used for grafting (number of cuttings 1500) d) Rooted grafted plants ready for planting out (number of cuttings 2500).

All samples were cut lengthways and inspected for wood discolorations. Small fragments of tissue were removed at the graft union and at the rootstock and incubated on Petri dishes containing dextrose-potato-agar medium. In total, 20.000 Petri dishes were used and 80.000 isolations were carried out.

Results

Rootstock and scion cuttings

The percentage of rootstocks from Greek mother plantations (state and private plantations) and the scions from other countries with wood blackening was negligible. Isolations gave only saprophytic fungi (*Alternaria* spp., *Trichothesium roseum*, *Trichoderma* spp, *Penicillium* spp., *Fusarium* spp., *Gliocladium* sp., *Phoma* sp.)

Rooted rootstock cuttings

The percentage of rooted rootstock cuttings with wood discolorations fluctuated between 2-15%. The percentage of the possible pathogenic fungi isolated was even very low (0,5-2%). The pathogenic fungi were *Phaeoacremonium* spp., *Botryosphaeria dothidea* and *Cylindrocarpon destructans*.

Rooted grafted plants

The percentage of rooted grafted plants which exhibited wood discolorations was very high and in several cases reached the 90-100%. However, the percentage of the possible pathogenic fungi isolated was very low (0,5-2%). Only in some cases of cultivars coming from other European Countries the percentage was significant. For example, the fungus *Cylindrocarpon destructans* was isolated from Ugni blanc at 16.7% and from Chardonnay at 8,3%, the fungus *Botryosphaeria dothidea* was isolated from the cultivars Cinsaut and Regina at 12,5%, and the fungus *Phaeoacremonium* spp. from Syrah at 10% and Sauvignon 21%.

Discussion

The results of this work made during 2002 on an enormous number of samples obtained from mother plantations and nurseries, confirm those of a previous work made in our laboratory (Rumbos & Rumbou, 2001). The low incidence of pathogenic fungi isolated suggests that they could not by themselves be the cause of young grapevine decline.

The low incidence of internal blackening and pathogenic fungi colonization in grape cuttings and scions does not support the hypothesis that the grape propagated material is already infected from the mother plants, although possible (Pascoe & Cottral, 2000; Rego et al., 2000). It is quite possible that operations made in the nursery can expose vines to stress agents (Zanzotto et al., 2001). Grafting, disbudding and accidental wounds provide access to colonization by a wide range of fungi. Improper storage and transportation conditions of the propagated material must also play a role and made the decline more acute. Stamp (2001) examined the stress factors associated with young grapevine decline. He demonstrated that nursery stock defects and mechanical and biotic stresses were frequently associated with decline disease. However, many things are not well understood and further research must be done.

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