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The ISSR useful molecular tools for olive fly studies

<i>M. D. Ochando, S. Rodriguez, S. Hernández & C. Callejas</i>	11-19
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Abstract: Although the need to develop and implement more effective strategies of combating pests and pathogens has always been dire, the urgency of this challenge has increased sharply in recent years due in part to the spread and bioinvasions of many species. There is consensus that an increase in the scientific knowledge of insect pests and their natural enemies will lead to a higher and more effective biological control. In this sense, molecular methodologies can provide us with new characters of study in fields such as taxonomy, identification of biotypes, structure and dynamics of populations of pests, etc.

In the present work the ISSR (Inter-Simple-Sequence-Repeat) technique was applied for the first time to the olive fly, *Bactrocera oleae*, in order to test the suitability of this molecular methodology for population studies on this species, an economically important pest in the Mediterranean region. The ISSR technique amplifies inter-simple sequence repeats i.e. inter-microsatellites, through the use of the Polymerase Chain Reaction (PCR).

Study on the effect of olive fruit fly on some qualitative and quantitative characteristics of olive oil in different storage duration

H. Nouri & J. Shirazi21-27

Abstract: It has been known that the quality and quantity of the olive oil would be changed due to the damages caused to the olive fruits especially by olive fruit fly, *Bactrocera oleae*. However, information on the pest infestation and its duration could facilitate achieving efficient fruit protection measures and optimizing storage programs. Therefore, a study was conducted to investigate the nature and rate of variation in qualitative and quantitative characteristics of olive oil extracted from infested fruits by olive fly at various maturing dates compared to that of healthy fruits. The experiment was designed in a factorial RCBC with fruit (factor A) in 2 levels (infested and healthy fruits) and maturing date (factor B) in six levels (Nov 1-19 and 2-26, Nov 31 to Dec 3, Dec 4-10, 5-17). Treatments were replicated 3 times in a grove with Zard olive cultivar. The oil of a defined sample of each treatment was extracted by centrifuging their fruit fleshes separately after removing stones. The total quantity of each sample was recorded. Afterwards, they were labeled by codes and sent to the Oilseeds Laboratory, Seed and Seedling Improvement Institute to evaluate their quality based on the Standard Olive Oil Quality Protocol. The results revealed significant differences among treatments considering some qualitative characteristics such as oleic acid, acidity and peroxide. In general, healthy fruits had lower acidity and peroxide but higher percent oil and oleic acid compared with those of infested fruits. However, there were not any significant differences among treatments for linoleic acid content. Therefore, it could be concluded that the damage of olive fruit fly would reduce the quality and quantity of olive oil.

Olive diseases and disorders in Australia

V. Sergeeva & R. Spooner-Hart29-32

Abstract: The main aim of our research is to contribute to the knowledge on fungal diseases of olives in Australia. Our study has resulted in several new records of fruit rots and of fungi on leaves, trunk and root diseases. This research has also resulted in new evidence of flower infection by anthracnose pathogen leading to fruit rot. Several fungi, some of pathogenic importance, were observed on olives from different olive growth regions of Australia during a six-year period from 2002-2008. The major diseases of olive in Australia are anthracnose *Colletotrichum gloeosporioides*, *C. acutatum*, cercosporiose *Pseudocercospora cladosporioides* and peacock spot *Fusicladium oleagineum*. Minor diseases of olives are *Phytophthora*, *Rhizoctonia*, Charcoal rot *Macrophomina phaseolina*, and *Neofusicoccum luteum*. Cercosporiose has been considered as a minor disease of olives until now, although fruit is occasionally infected. Fruit damage is equally important as leaf infection. Infection of flowers leading to fruit rot is of economic importance as anthracnose results in significant losses in yield and reduced oil quality.

Olives are susceptible to be damage by heat and sun and other weather conditions, water-logging or a lack of nutrients, and affect the functioning of the plant system and other environmental conditions causes disorders.

Localization of GFP-tagged *Pseudomonas savastanoi* pv. *savastanoi* strains in olive knots

Luis Rodríguez-Moreno, Isabel Pérez-Martínez & Cayo Ramos 33-38

Abstract: We report the construction of *Pseudomonas savastanoi* pv. *savastanoi* strains tagged with either a mini-Tn5-GFP or a mini-Tn7-GFP transposon and evaluate their use for real-time monitoring of bacterial disease development on *in vitro* olive plants. Pathogenicity of *P. savastanoi* strains LRM1-165 (NCPPB 3335 containing a mini-Tn7-GFP) and IPM-71 (ITM317 tagged with a mini-Tn5-GFP transposon) was not affected by GFP expression and allowed localization of *P. savastanoi* cells during infection of *in vitro* olive plants.

Pests that affect the olive groves on Terceira island, Azores

*D. J. Horta Lopes, R. Pimentel, N. Macedo, J. T. Martins, M. Zorman ,
L. B. Ventura, M. H. Aguiar, J. Mumford, & A. M. M. Mexia* 39-46

Abstract: The olive tree is cultivated in the Azores Archipelago only on two islands: Terceira and Pico. In Terceira Island, the area is about 60 hectares and it is confined to the Porto Martins area, in the southeast of Terceira Island. All the production is for table olives.

The main phytosanitary problems identified damaging the olive trees are: olive fruit fly (*Bactrocera oleae* Gmelin.), olive moth (*Prays oleae* Bern.), black scale (*Saissetia oleae* Oliv.) and now the new pest that has emerged in this area at the beginning of June 2008, the olive psylla (*Euphyllura olivina* Costa).

The field methodology used for monitoring and making the risk estimate for these pests was as follows: for the olive moth, one Delta trap with a specific sexual pheromone to capture adults and one yellow chromotropic trap also with a specific sexual pheromone in each one of the five olive orchards selected to be studied. During all the survey period, these five olive orchards were visited every fifteen days. For the fruit damage evaluation caused by the olive fly, 250 fruits were collected from 5 trees (50 fruits in each tree) and for the evaluation of the area affected by olive psylla, 3 inflorescences or branches with leaves were analyzed in each of the 826 trees inside of the production area within a range of 500 meters from the detected infestation focus. All the collected data were then analyzed in Excel and from there exported to ArcGIS 9.1, which enabled the development of olive psylla GIS distribution maps from all the olive production area.

The results allow us to conclude that the olive fruit fly is the main pest of this culture, having it highest population abundance in June, August and October, with October the month with the highest captures. The olive moth reaches high population densities in July. Beyond that, it was possible to get some better knowledge about the olive psylla infestation focus, where it registered two generations (one at the beginnings of June and the other at the endings of October) and the geographic distribution all over the production area, based on the GIS maps analysis.

From the olive fly fruit damage analysis there was an average of 83% infestation over the five surveyed orchards. In some orchards, where there was no treatment, the infestation reached the 98%, meaning that all production was lost.

Contribution to the knowledge of *Euzophera pinguis* Haworth biology in Alentejo (Portugal)

M. I. Patanita 47-50

Abstract: The pest, *Euzophera pinguis* Haworth is an enemy of the olive grove whose attacks have been a great concern for the last few years, in some Portuguese regions, namely in Alentejo. The recent availability of the sexual pheromone of synthesis for this species constitutes a relevant aspect in the protection of the olive grove on account of its potentialities in the identification of the periods of risk. The curve of the flight of the insect was studied aiming at getting information about these periods and it was obtained with the help of funnel traps, in an olive grove in Baixo Alentejo. The study occurred between 2004 and 2006, in a young olive grove located in the "Herdade dos Lameirões". The analysis of the data shows variations in the line of the curve, during the spring until the middle of summer, with peaks of capture of variable intensity in different periods (beginning or end of May, mid June, mid July and beginning of September), suggesting overlaps of distinct generations. However, in any of the analysed situations there was a peak of captures in the beginning of September.

Evaluation of *Calocoris trivialis* damage potential on olive crops

*D. Perdikis, N. Garantonakis, P. Kitsis, A. Giatropoulos,
A. Paraskevopoulos, G. Cassis & S. Panagakis* 51

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Is the fecundity of *Bactrocera oleae* (Rossi) dependent on nitrogenous sources?

M. Marzaro, C. Savio, I. Martinez-Sañudo, L. Mazzon & V. Girolami 52

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The effect of storage time on some characteristics of the oil in olive fruits infested by *Bactrocera oleae*

Hossein Nouri & J. Shirazi 53-58

Abstract: Olive fruit fly is one of the most important olive pests in the world. The pest invaded the olive growing regions of Qazvin (Tarom Sofla), Iran, in 2004 and soon became a major pest damaging economically the olive orchards in all infested regions. The olive fly larvae cause qualitative and quantitative reduction of olive oil by entering to fruits flesh and exposing them to pathogenic fungi. However, the degree of this reduction may vary especially considering the duration of fruits storage from harvest until oil extraction. Therefore, the current research was carried out to compare the effect of storage time of infected from *Bactrocera oleae* olive fruits on the olive oil characteristics. The experiment was designed in a RCBD with six treatments and three replications, on Zard olive cultivar. Treatments included healthy fruits immediately after harvest as control, infected fruits immediately after harvest and infected fruits 1, 2, 3 and 4 weeks after storage. The oil was extracted by centrifuging the fruit flesh samples. Then, the oil samples were coded and sent to Oil Seed laboratory, Seed and Seedling Improvement Institute for further measurements on their quality. The results revealed that acidity of the oil was not significantly different among healthy fruits (control) (0.630%) and infected fruits either immediately (0.547%) or 1 week after storage (0.777%) which had comparatively lower acidity and higher quality. However, the significantly highest oil acidity was observed in the infested fruit treatments 3 and 4 weeks after storage (5.070% and 7.627% respectively) that showed sever reduction in oil quality in these treatments compared with that of others.

A study on the incidence of *Bactrocera oleae* Gmelin (Diptera: Tephritidae) by sampling infested fruits and soil of five selected olive orchards in different times and directions

H. Noori and J. Shirazi 59-64

Abstract: Olive fly, *Bactrocera oleae* Gmelin (Diptera: Tephritidae), was a quarantine pest in Iran before its invasion in 2004. Since then it has been one of the main pests on olive in the country. By having a high population in several generations per year, the pest voracious larvae feed on the fruit flesh and make them fall before harvest. Besides, the harvestable fruits infested by the pest would have degraded quality of oil due to their vulnerability to rotting by pathogenic fungi. Facing with such a dilemma, a study was necessary to obtain the most valid method of pest sampling considering timing and direction for further pest incidence prediction, damage and crop loss assessment and/or control measure decisions. Therefore, a research on the matter was carried out during 2006-07. First, 5 olive groves, 1h each, were selected in Tarom Sofla, Qazvin province. Then, 5 trees were selected randomly in each grove for fruit sampling, weekly. The same number of trees was also selected for soil sampling. The samples were taken from four main directions of each selected olive trees. The results revealed significant differences among treatments. Similarly, the mean number of infested fruits was significantly higher in south (4.11 fruits/sample) and east (3.91 fruits/sample) directions compared with that of north (3.31 fruits/sample) and west (3.21 fruits/sample). Besides, the mean length of fruits proved to be significantly more in south and east directions compared to that for north and west. Thereby, a positive correlation was established between fruit length and infestation incidence through Pearson Correlation Analysis. The similar results were obtained for the population of pupal stage in soil as its mean number was significantly higher in south (1.03) and east (0.96) directions. The various weekly sampling proved that the frequency of pest incidence (infested fruits) increased late in Oct. and Nov. Conspicuously, there were two peaks of pest sampled population, one on Oct. 30, and the other on Nov. 21, 2007.

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Susana Pascual, Ismael Sánchez-Ramos, Manuel González-Núñez 83-88
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- Experiments for the control of olive fly using a “push-pull” method
G. Delrio, S. Deliperi & A. Lentini 89-92
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The importance of application time, for the control of eriophyid mites,
in olive orchard

Zois Zartaloudis, Philippos Ioannidis 93-97

Abstract: Many species of Acari: Eriophyidae have been found in Greek olive orchards. Olive Eriophyid mites are extremely small. They average in length from 0.1 to 0.2mm and are yellow in colour, flattened and pear-shaped. Seven Eriophyid species, make damage the olive trees in Greece, and four of these (*Aceria oleae*, *Oxycenus maxwelli*, *Tegalophus hassani* and *Ditrimacus athiasellus*) are common in almost all olive-growing regions of Greece. It is difficult to estimate the amount and type of injury which is caused by each different species because they occur in mixed populations and have similar feeding habits. In this work it is confirmed that the sulphur, and abamectin are effective means against olive eriophyid mites. Also the effectiveness of these means depends on the time of application (timing). The effectiveness of used plant-protection products was increased in the first application, at the time of the emergence of flower buds than the beginning of the blossoming and in the second application, at the time of the end of blossom than the time of the young fruits (0.5mm of diameter).

Side-effects of based-copper products on *Chrysoperla carnea* (Stephens)
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Susceptibility to *Bactrocera oleae* of advanced selections from a
cross-breeding program

Lorenzo León, Enrique Peinado & Raúl De la Rosa 105-108

Abstract: In the last years, the need for more suitable cultivars promoted the development of olive breeding programs in the main olive-producing countries based in intraspecific cross-breeding between cultivars of known merit aiming at combining the good qualities of the genitors in some of the genotypes of the progenies. Pest and disease resistance are usually cited among the breeding objectives in these programs. However, despite the economic importance of olive fruit fly in the Mediterranean basin, there are not reports about the susceptibility of new genotypes to this parasite. The selections evaluated in this work come from the crosses between 'Arbequina' and 'Picual' olive cultivars carried out in 1997. Initial, middle and end of the ripening period were recorded according to the ripening index of fruits based on colour changes of peel and pulp. Samples of 50 fruits collected at the end of the ripening period were used for determining fruit characters (size, moisture and oil content) and the total infestation percentage by fruit fly. Significant differences between genotypes were obtained for all the evaluated traits. The percentage of infestation varied from 6.5 to

58.4% between selections with five genotypes showing percentage of infestation lower than 15% (including 'Arbequina') and three of them higher than 20% (including 'Picual'). A positive correlation was found between infestation and ripening dates (mainly initial ripening date) and fruit size but not with fruit moisture or oil content. The most susceptible genotypes showed both large fruit size (>3.5g) and late ripening (initial ripening time in October). On the contrary, smaller fruit size, earlier ripening or both were associated with much lower percentages of infestation.

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- Management of *Verticillium* wilt in olive orchards,
using the soil solarization method and soil application of micro-organisms
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- Effectiveness of two attractants against the olive fruit fly (*Bactrocera oleae* Gmel.) and their impact on the arthropods community
Manuel Ruiz Torres 139-153
Abstract: In this study the efficacy of different combinations of traps and attractants against the olive fruit fly, *Bactrocera oleae* (Gmel.), were compared. In 2007 were compared: a mixture of nulture protein (9%) and sodium borate (3%) in two types of traps (Easy-Trap and Tephri-ecological), and diammonium phosphate (4%) in McPhail traps. In 2008, two additional combinations were included: the commercial attractant Tephri-lure (12%) with Easy-Trap and Tephri-ecological trap. Four traps were used for each combination, in both years and traps were placed at random in consecutive trees. The olive grove was in a mountain area, in the municipality of Los Villares (Spain), usually with high olive fruit fly attack. In 2007 the traps were placed from 7 September to 30 November and in 2008 from 30 July to 12 November. Captures of *Bactrocera oleae*, as well as of predators and parasitoids were recorded and the diversity was calculated taking into account data from all the specimens captured, as a measure of selectivity: the higher the diversity the lower the selectivity of traps. The results show that the most effective attractant in the capture of *Bactrocera oleae* is Tephri-lure, regardless of the trap (Easy-trap and Tephri-ecological trap). Tephri-lure is the attractant capturing less predators per olive fruit fly captures, and its impact on parasitoids is negligible. The lowest biodiversity of captures (excluding olive fly) was registered for Easy-trap and therefore this trap has the lowest impact on arthropod community.
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Abstract: *Psytalia lounsburyi* is an African parasitoid of the olive fruit fly *Bactrocera oleae*. Its introduction in France has been made with two different objectives: i) controlling the olive fruit fly in France, and ii) testing how intraspecific hybridization affects the demographic success of small introduced populations. For this, we introduced two parental strains of *P. lounsburyi* originating from either Kenya or South Africa, and a hybrid strain resulting from their admixture. In this paper, we report the first years of research and progress toward our two objectives. In 2006 and 2007, intensive field surveys were carried out to locate 60 suitable release sites in South of France. During fall 2007, before the introduction of *P. lounsburyi*, a first set of olive samples was collected to assess the density of *B. oleae* and the diversity of indigenous natural enemies. The year 2008 was dedicated to parasitoid mass production, release, and a second set of sampling. In summer, a total of about 43,000 individuals *P. lounsburyi* were introduced in the 60 sites. Individuals of the genus *Psytalia* were found in some samples, suggesting the ability of the released parasitoids to locate *B. oleae* in French olive trees and to complete their preimaginal development into these hosts. However, molecular identification of the recaptured individuals needs to be done to confirm these results. More sampling is also necessary to test the ability of African *P. lounsburyi* to overwinter and establish in South of France.

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