Resurgence of minor pests following the implementation of mating disruption against *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera, Tortricidae) in Sherry vineyards (Spain)

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Summary

The implementation of the mating disruption technique against the grape berry moth, Lobesia botrana (Denis and Schiffermüller) (Lepidoptera, Tortricidae), in Integrated Pest Management (IPM) Sherry vineyards (Andalusia, South-western Spain) reduced the number of chemical treatments required to manage this serious pest. In order to verify the long term consequences of this type of management on the minor pests of vineyards, a study was carried out over nine years to compare the population levels of four phytophagous insect species (Altica ampelophaga, Planococcus citri, Jacobiasca lybica, Aphis gossypii) in plots where L. botrana was managed with conventional chemical treatments or using mating disruption. Field data on secondary pests were collected on randomly chosen vines at different times on different plant organs such as leaves, shoots and bunches, depending on the type of pest, according to the ATRIA procedure (Andalusian IPM procedure). Results showed a significant increase of grapevine infestation by all four secondary pest species in the plots managed with grape berry moth mating disruption. These findings represented a limitation for the implementation of this technique in Andalusian vineyards.

K e y w o r d s: integrated pest management (IPM); Sherry wine; 'Palomino fino'; grape berry moth.

Introduction

The Sherry Qualified Designation of Origin (DOC) wine region, located in the southwest of Andalusia (Spain) is one of the most famous areas in Europe for its long history of high quality wine production. Sherry, one of the world's oldest wines, enjoys a historical popularity and was cited also by Shakespeare (1598). Nowadays these vineyards are spread out over 7,500 ha, where about 96 % of the total production is due to the 'Palomino fino' cultivar, and the remaining 4 % is cultivated with the varieties 'Pedro Ximénez' and 'Muscat of Alexandria', together with

some table grape varieties cultivated in special greenhouses. In this area, the first detection of the grape berry moth was in 1960, when bunch rot caused by *Botrytis cinerea* Pers. was identified as a side effect due to larval feeding of Lobesia botrana (Denis and Schiffermüller) (Lepidoptera, Tortricidae) (Pemán 1960). Due to favourable eco-climatic conditions indicated by STELLWAAG (1926) and COSCOLLÁ (1980), this lepidopteran became the key pest of vineyards and the main target of insecticide treatments (Gallardo et al. 2009), as occurred also in other European wine producing areas (Stöckel et al. 1997, Pérez 2013). Seven or more treatments were necessary each season to keep this pest below the economic injury level (HIGLEY and PEDIGO 1993). In 1981, following the adoption of Integrated Pest Management (IPM) measures, the first local IPM Team (called "ATRIA") performed a study of the moth's biology. During this time it was shown that L. botrana could perform up to four generations annually (ATRIA 2005). After the discovery, characterization and standardized synthetic production of the moth's sex pheromones, the use of mating disruption technique (MD), which utilizes pheromone dispensers to prevent males from locating and mating with females, has been widely applied in the European wine-growing areas (Ioriatti et al. 2011). It represents an effective and sustainable control technique of the grape berry moth, provided that the initial population density is below the critical level of 4,000 pairs ha-1 (Feldhege et al. 1995), that all pheromone dispensers are installed in the vineyards before the onset of the first seasonal flight and that all the factors that influence concentration, homogeneity, and atmospheric distribution of the synthetic pheromone are satisfied (Ioriatti et al. 2011), including wind speed and hygrometry, plant spacing, training system, plant canopy and leaf density, according to the vine phenological stage. Area-wide application of MD, as performed in Northern Italy, Switzerland, Germany and Spain, guarantees the best results (IORIATTI et al. 2008), by reducing the "border effect" of the lower pheromone concentrations on the vineyard boundaries and counteracting the aggregated distribution of the moth.

In the Sherry area, MD was adopted for the first time in 1994 (Castillo 1995) and between 1997 and 2005,

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about 4,500 ha were managed with this system. As a result, the average number of treatments against L. botrana using conventional chemical control, Insect Growth Regulators or Bacillus thuringiensis, decreased to one or two treatments each year and eventually became unnecessary (ATRIA 2005). However, in the same areas subject to MD, the increasing occurrence of insect species initially considered as minor pests of secondary importance started to become a serious problem (López et al. 1998). These insects are: Altica ampelophaga Guérin-Méneville (Coleoptera, Chrysomelidae), Jacobiasca lybica (Bergevin and Zanon) (Hemiptera, Cicadellidae), Aphis gossypii Glover (Hemiptera, Aphididae) and Planococcus citri (Rossi) (Hemiptera, Pseudococcidae). A. ampelophaga, native of the Iberian Peninsula is considered an age-old pest of Sherry vineyards since at least the 17th century (STELLWAAG 1928). In the past it caused important damage in the majority of Spanish vineyards and also in the Magreb (Northern Africa) (Ruiz 1944, Galet 1982). Its incidence became very low after the use of synthetic organic pesticides (OCETE et al. 1999). Before 1995, in the Sherry area only small populations were detected on plants situated near illuminated countryside houses.

J. lybica, a polyphagous leafhopper affecting cotton, sugar beet, citrus and other fruit trees, as well as many spontaneous indigenous plant species in Mediterranean regions (Ocete et al. 1999), was not an important pest in Spain until the second half of the 90's, when serious damage caused by this species was detected on a large scale, thus requiring specific control (LÓPEZ et al. 1998). This leafhopper, described in Libya, as its specific name indicates, was first found on table-grape varieties from Southeastern Spain by Ruiz Castro (1944). Nowadays, this thermophilic species is becoming the main pest affecting southern Iberian vineyards in Andalusia and Tierra de Barros (Badajoz province) (Ocete et al. 1999). Before harvest, feeding by this hemipteran provokes a leaf abscission and constant "de novo" budding that ultimately causes a reduction of the Baumé degree of the must. After the harvest, a reduction of the vine reserves causes a loss of vigour for the following years (Toledo 2004). A. gossypii is a polyphagous holocyclic hemipteran, mainly considered a pest of cotton, Rutaceae and Cucurbitaceae in Spain, which shows resistance to several pesticides (García and Ferragut 2002). *P. citri* is another polyphagous pest living in different areas in the Mediterranean basin, as well as in tropical and subtropical regions, which affects different plant species such as citrus trees, *Nerium oleander* and several *Ficus, Robinia* and *Geranium* species (Galet 1982, García and Ferragut 2002, Bostanian *et al.*, 2012). It is recognized as a vineyard pest in Brazil (Pacheco Da Silva *et al.* 2014), India (Amala *et al.* 2013) and Tunisia, especially in table grape varieties (Mansour *et al.* 2011). This mealybug can attain up to six generations annually in Andalusia (Ruiz Castro 1944) and it was considered a secondary pest in Sherry vineyards prior to 1995 (López *et al.* 1998).

The aim of this paper was to analyze the long term effects derived from MD management of the grapevine moth on the populations of secondary pests in Sherry vineyards.

Material and Methods

Study area: The study was performed from 1997 to 2005 in Balbaina, a high quality vine producing district in the municipality of Jerez de la Frontera (Cádiz province) spread across a total of 1,600 ha, where about 1000 ha were managed with MD for *L. botrana*. The density of the plantation was 3,675 vines of 'Palomino fino' per ha, grafted on 41B and the local rootstock 13-5 EVEX.

Sampling methods: The study was carried out on two plots of 2 ha each, situated in a relatively flat area in the centre of Balbaina, 2 km away from each other (Fig. 1). The landscape around is hilly (45 m.a.s.l), and the soil is spread over white marls with marine origin. One plot was managed with conventional chemical treatments (Table) against *L. botrana* and the other plot was under MD since 1996, with 300 sex pheromone dispensers per hectare (Rak 2 Plus®, BASF). In this area, the action thresholds for *L. botrana* in conventional management are 10 % of bunches with viable eggs for the 1st generation and 8 % of bunches with viable eggs for the 2nd and 3rd generations;

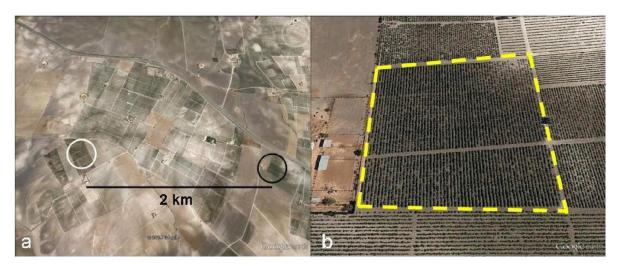


Fig. 1: Aerial view of the study plots. a) The black circle indicates the plot with mating disruption, the white one represents the plot with conventional management. b) Detailed view of the plot with mating disruption.

Table

Treatments performed on mating disruption and conventional chemical management plots

	Mating disrup	otion management ¹			
Active ingredient and employed doses	Pest	Treatment periods	Action threshold	Number of treatments per year	
300 pheromone dispensers ha-1	Lobesia botrana 2 nd week of April		8 % of bunches with variable egg number	0-1	
Flufenoxuron 10 % (50-100 cc·hL ⁻¹)	Altica ampelophaga	1st week of June	Detection of first adults on leaves	1	
Fosalon 30 % (200-250 g·hL ⁻¹)	Jacobiasca lybica	4 th week of June 1 insect per leaf		2	
Malation 4 % (20-25 kg·ha ⁻¹)	Aphis gossypii	1st week of June	une 1 insect per leaf		
Triclorfon 5 % (20-30 kg·ha ⁻¹)	Planococcus citri	1st week of June	Presence in 5 % of vines	1	
	Convention	nal management ¹			
Clorpirifos 48 % (150-200 cc·hL ⁻¹)		1st Generation: 1st week of May	10 % of bunches with variable egg number	1	
Fenitrotion 40 % (150-200 g·hL ⁻¹)	Lobesia botrana	2 nd Generation: 1 st week of June and 4 th week of June	8 % of bunches with variable egg number	2	
Fenoxicarb 25 % (20-40 g·hL ⁻¹)	-	3 rd Generation: 3 rd week of July and 2 nd week of August	5 % of bunches with variable egg number	2	
Clorpirifos 48 % (150-200 cc·hL ⁻¹) or Flufenoxuron 10 % (50-100 cc·hL ⁻¹)	Altica ampelophaga	1st week of June	Detection of first adults on leaves		
	Jacobiasca lybica	1st week of June	1 insect per leaf	0-1	
	Aphis gossypii	1st week of June	1 insect per leaf		
	Planococcus citri	1st week of June	Presence in 5 % of vines		

¹In both types of management, fungicide treatments consisted in copper sulfate and sulphur at label doses, and no herbicide treatments were performed.

in MD management the thresholds are 8 % and 5 % of bunches with viable eggs respectively for the 1st and 2nd- 3rd generations.

Field data on secondary pests were collected on 100 vines randomly chosen along the main diagonals of each plot, using the vine as the primary unit, according to ATRIA procedure (Consejería de Agricultura Pesca y Alimentación 1996). The secondary sampling units were represented by diverse plant organs such as leaves, shoots and bunches, depending on the type of pest at different times of the year. For each pest specific data were collected, such as: (I) the number of A. ampelophaga adults detected on very young shoots, in an established time period (1st week of April); (II) the number of J. lybica nymphs recorded on five leaves per vine during the 2nd week of July and the 2nd week of September (after the harvest), according to the ATRIA procedure. To identify the leafhopper species, a study of the genitalia of periodically captured individuals was carried out following the procedure described by QUARTAU et al. (1989); (III) Percentages of infestation by A. gossypii were calculated according to presence or absence on leaves, whereas for P. citri the number of insect colonies situated under the bark of the main trunk or leaves was recorded. The identification of aphid specimens was made according to ILHARCO and FONSECA (1985). The financial support given by the Autonomous Government for

MD program was suppressed in 2005; therefore this was the upper limit of this study.

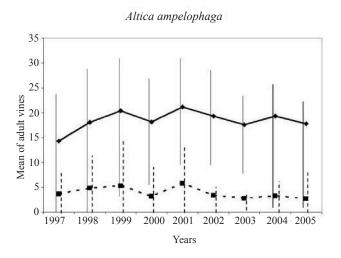
Statistical analysis: Comparisons between conventional system and MD plots were performed by means of cluster analysis using UPGMA (unweighted pair group method using arithmetic averages). Because the data did not comply with the Kolmogorov-Smirnov normality test, the U-Mann Whitney test was used to compare the numbers of the cited pests throughout nine years.

Results

In the plots managed with MD technique for *L. botra-na* the population of this pest was below the action threshold. In contrast, the population of the secondary pests *A. ampelophaga, J. lybica, A. gossypii* and *P. citri* were so high that one or two chemical treatments were required for each of these species, depending on the pest intensity. A summary of the treatments performed under MD and conventional system plots throughout the period of this study is shown in the Table.

In the case of *A. ampelophaga*, main injuries were caused by overwintering adults feeding on new shoots during stages E-F (BAGGIOLINI 1952), between the third

quarter of March and first quarter of April. Statistically significant differences were detected between levels of infestation caused by the overwintering adults between conventional and MD plots since the first year of this study (W = 10,000.0; $p \le 0.01$) (Fig. 2). A very similar trend was observed also for the leafhopper *J. lybica*, considering the number of individuals per leaf, with statistically significant differences between conventional chemical management and MD detected since the first year of observation, especially for pre-harvest sampling in July (W = 10201.0; $p \le 0.01$), but also in post-harvest sampling in September (W = 10201.0; $p \le 0.01$) (Fig. 2).



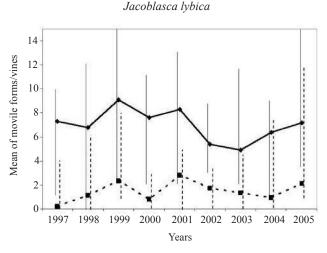
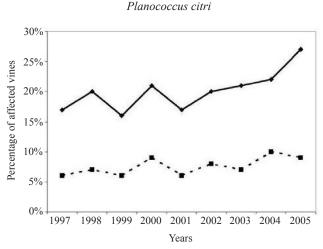


Fig. 2: Average number of adults/mobile forms (± SD) of *A. ampelophaga* or *J. lybica* detected in vines in the study period (data for *J. lybica* refer to post-harvest sampling performed in September).

Considering the percentage of infestation by *A. gossypii*, statistically significant differences in the comparison between the two types of management were detected since $2002 (Z = 30.07; p \le 0.01)$, with an increasing upward trend in the level of aphid infestation in the plot under MD (Fig. 3).

The percentage of vines infested by the mealybug *P. citri* was nearly three times higher in presence of MD than under conventional chemical management during the last five years of this study. These differences started to become statistically significant from 2001 onwards



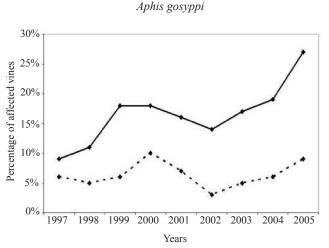


Fig. 3: Percentage of vines affected by *P. citri* or *A. gossypii* in the study period.

(Z = 105.97; $p \le 0.01$), as consequence of a population trend similar to the case of *A. gossypii* (Fig. 3).

Sampling performed from 2005 until present, showed that after the cessation of MD (in 2005) in the plot initially under this type of management, no significant differences were detected in the population of the cited secondary pests in the comparison with the plot that had always been under conventional management (ATRIA, unpublished data).

Discussion

The present study, performed over nine years in the Sherry DOC wine district, compared the population levels of secondary pests in vineyards where management of the key pest grape berry moth *L. botrana* was performed under two management strategies, classical conventional chemicals control and MD. Results indicate a significant increase over time of the population/incidence of all four considered species of non-target pests in the vineyards under MD, and these numbers were so alarming that it was necessary to perform specific chemical treatments to maintain their levels below the action threshold. In particular, incidence of the monophagous beetle *A. ampelophaga* was

always higher in the MD managed vines, and the most serious damage was caused by the overwintering adults feeding on the first shoots (March-April), interfering with the normal development of the plants and causing a reduction of 10-25 % in the number of bunches per vine in our observations. Very similar observations of damage by this beetle on Spanish vineyards are reported in older surveys by Ruiz Castro (1944, 1952), who observed that higher injuries occurred earlier due to the first generation and that the two following generations caused less damage, as the vines had already developed many branches with a greater amount of leaves involved in the photosynthetic process.

In the case of the leafhopper J. lybica, it is interesting to remark that during summer time, under very high temperatures and absence of rain, in the geographical area of this study, vineyards were practically the only green plants in the area, especially after sugar beet and sunflower harvests (González-Andújar et al. 2006). Therefore, vines exerted a strong attraction for these hemipterans and this could explain why the number of mobile pre-imaginal individuals grew up to 18 individuals per leaf in September 2000. In vineyards with MD management the leafhopper infestation was so heavy that it resulted in a precocious leaf abscission, and consequently bud swelling, influencing the typical morphology of the new shoots with very small separation among nodes (Bounaceur and Doumandji-Mitiche 2009). Besides, taking in account climate change, higher average temperatures are going to be expected in this area, thereby favouring thermophilic species such as J. lybica, as it has been observed also for other pests (NYAMUKONDIwa et al. 2013). Due to the important injuries recorded in the area, studies are being performed to assess the possibility to use specific geostatistic procedures to reveal the spatial distribution of this hemipteran and improve its management (Sciarretta and Trematerra 2014). The use of these methods to design maps of infestation will add new insights for the acquisition of predictive tools for Precision Agriculture in the Sherry area (RAMÍREZ 2003).

Considering A. gossypii, this aphid was also found in other vineyards situated in Southern Europe, like Italy (Barbagallo et al. 1994) and Portugal (Ilharco 1996), where its incidence was always below the economic threshold. A significant increase of the percentage of aphid infested vines was observed in the last years of this study in both types of management, but was especially higher under MD. A similar trend was observed also for the mealybug P. citri. In both cases, the increase might have been caused by either favourable climatic conditions and/ or by the development of insecticide resistance phenomena in the Sherry area, as it was observed also for other crops in different regions (HERRON et al. 2001, AHMAD et al. 2003, Carletto et al. 2010). Heavy populations of these hemipterans may decrease plant vigour, cause defoliation and chlorosis. However main damage is likely the one due to transmission of viruses affecting final product quality and vineyard longevity, such as the grapevine leafroll virus (GLRaV-3) that can be transmitted by *P. citri* (CABALEIRO and Segura 1997), and to the excretion of honeydew followed by development of sooty mould, which reduces the marketability of table grapes, as it occurs in Southern Iberian vineyards as well as in Tunisia (Mansour et al. 2011).

Our study showed that in the plots where MD was performed, an increase in the damage by some secondary pests, formerly considered irrelevant in Sherry vineyards, was recorded. A similar phenomenon has been reported also in other crops, such as cotton and pear orchards (Panizzi *et al.* 2000, Greene *et al.* 2001, Varela *et al.* 2011).

Observations on the consequences of MD application in vineyards on non-target arthropods were performed also by Delbac et al. (2013) in Médoc (France) near Bordeaux wine-growing zone. According to their survey, an important diversity in leaf arthropod community was detected under MD, with a high number of the pest mites Panonychus ulmi, representing more than 80 % of the surveyed species, being balanced by an increased population of predatory mites (Phytoseiidae), so that no specific phytosanitary intervention was required in the study area. In pear orchards in Southern Oregon (USA), where MD was used against the codling moth, after curtailing the use of broad spectrum neurotoxic insecticides, resurgence of true bugs was recorded and the European earwig became an increasing concern (Hilton et al. 1998). Application of chemical control against true bugs resulted in a slow decline of their population without a significant reduction in fruit damage at harvest due to their feeding, and in an increased damage by the codling moth. In an apple IPM program in New South Wales (Australia), the combination of MD and the IGR fenoxycarb to control the key pest Cydia pomonella resulted in a reduction of the infestation by the non-target pest woolly apple aphid Eriosoma lanigerum, as a consequence of increased numbers of its natural enemies (NICHOLAS et al. 2005). As a matter of fact, throughout the years of the present study, although no quantitative data are available, a resurgence of natural enemies that could implement the biological control of the secondary pests was not observed (ATRIA, unpublished data). Besides, economic considerations should be taken in account, as the cost of conventional treatments is around 605 €·ha⁻¹, whereas that of MD is about 673 €·ha⁻¹, including the spray costs in both cases, which means a difference of nearly 70 €·ha⁻¹. The higher costs of MD, associated with the observed increase of secondary pests not associated with an increase of natural enemies, constitute a serious handicap for the implementation of IPM in Sherry vineyards, especially as the price of the grape harvest is currently decreasing. The vineyard surface of Sherry area that was 10,500 ha at the beginning of these observations in 1997 is presently reduced to only 7,400 ha, and only 1000 ha are conserved in the district where this study was performed (Balbaina).

These factors, together with the results of our study, underline the difficulties of IPM implementation in some high value grapevine regions, when it is based mostly on MD for the grape berry moth. Similar difficulties in MD implementation are encountered also elsewhere in Europe, where the number of hectares of vineyard under pheromone-mediated MD against grape berry moths is about 140,000 ha, accounting for just 3-4 % of the total grape growing area (IORIATTI *et al.* 2011), On a farmer's point of view, the higher short-term costs and the typical fear of failure elicited by any new technique constitute a heavy con-

straint to MD adoption. However an interesting successful case is the one in occurring the province of Trento (Northern Italy) since 1998, when the local government decided to subsidize the area wide application of MD for *L. botrana* management, so that in four years the pheromone managed area increased about 8 times and further continuously augmented to reach about 90 % of the grape-growing area in the same province (Varner and Ioriatti 1992, Ioriatti *et al.* 2008). The success of the program was attributed to tight interconnection between research scientists, extension entomologists, grower associations, and pheromone supply companies.

The resurgence of secondary pests observed in our study was possibly a consequence of different concomitant factors, that include the geographic position and related pedo-climatic features of the area, which affected the biology and distribution of both autochthonous and allochthonous insects, and possibly also of a pest management strategy that did not consider properly all the elements of the surrounding landscape. A valuable help could be provided by the development of practical instruments of decision support system for vineyard and orchard management, that take in account a holistic approach of the different agro-ecosystem components, including pests, both primary and secondary ones, and their natural enemies. Some examples are those being developed in Italy (Rossi *et al.* 2014) and USA (Jones *et al.* 2010).

The importance and value of a truly integrated pest management approach that combines tactics for a sustainable long-term pest population reduction, such as MD and other semiochemicals based behaviour-manipulating strategies (Khan *et al.* 2008, Witzgall *et al.* 2010), has been fully declared by the recent agriculture legislative actions in the US and EU continents. Respectively, the Food Quality Protection Act and the Directive on Sustainable Use of Pesticides, placed greater restrictions on insecticides used in agriculture. The need is to stimulate the research for solutions that should take in account also the management of secondary pests with the aim to increase the natural populations of their antagonists at the landscape level.

Acknowledgements

We want to thank J. L. JIMÉNEZ GARCÍA, Academia de San Dionisio de Ciencias, Artes y Letras of Jerez de la Frontera; J. ALCÓN GONZÁLEZ, Servicio de Protección de los Vegetales of Jerez de la Frontera and AECOVI JEREZ.

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Received August 11, 2015