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Use of sex pheromone traps to monitor insecticide resistance in European Grape Moth *Lobesia botrana* (Lepidoptera Tortricidae)

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Abstract

The technique of the direct insecticide incorporation in the glue of the pheromone baited trap liners was adopted to monitor the susceptibility of the European Grape Moth (EGM), *Lobesia botrana* (Denis et Schiffermuller) to Azinphos-methyl. Bioassays were carried out on one EGM strain reared in the laboratory and on two EGM field strains from vineyards of the northeast of Italy. It was therefore evaluated the effect on the response with regard to the weight, the age, the sex and the position of the captured EGM males. The laboratory strain and one of the field were also analysed both according to this technique and larvae topical treatment. Weight and moth position did not influence the response to the test. The EGM females resulted less susceptible than the males; this does not invalidate the method since it is based on the use of male specimens only. Concerning the age it was noticed a limited decrease of the lethal concentrations related to the age growing, which indicates the negative effect of the age on the EGM survival. Differences in the susceptibility between field and laboratory strain, whether using adults or whether larvae in the tests were detected. The similarity of the two resistance factors confirms the method's reliability to detect the resistance of the EGM to the Azinphos-methyl.

Key words: pheromone traps, insecticide resistance, *Lobesia botrana,* European grape moth, Azinphos-methyl.

Introduction

Lobesia botrana (Denis et Schiffermuller), the European Grape Moth (EGM) is the key pest attacking Italian vineyards and till mid-nineties EGM populations used to be primarily managed by organo-phosphate applications. Although no evidence of insecticide resistance has been produced so far, the risk should not be underestimated.

The resistance problem is the greatest challenge the applied entomology is to face today. Indeed, the widening of circle of cross and multiple resistances among insect pests limits the number of effective commercial insecticides whereas the costs to develop new ones are increasing exponentially (Metcalf, 1989). The large spread of genes of cross and multiple resistances through the genomes of insect pests has severely limited the weapons of the applied entomology. Moreover, if we acknowledge that insecticide resistance development is a case of evolution, it becomes clear that resistance is inevitable as well as unavoidable as long as the selection pressure is persistent (Brattsten, 1989). Development, implementation and evaluation of new methods to detect and monitor resistance were cited as a major aim by the National Academy of Science in order to develop resistance management strategies (Knight, 1990). Most of the susceptibility tests available today have been designed for laboratory, as it is difficult to control conditions in the field. Performance of susceptible tests in the field would provide the needed data more rapidly allowing to take more aware decision regarding pest management. So far the attracticide susceptibility test represents a new approach, where the insecticide is incorporated in the sticky material of a pheromone trap for the diagnosis, in different insect pests, of insecticide resistance. The incorporation of the insecticide into the adhesive of a sex pheromone trap implies certain advantages such as allowing an easy detection of susceptible and resistant populations and requires limited handling of the insects and traps. On the contrary the test can't be carried out using no-contact insecticide and it is hardly applicable when the insects' response to the toxic is depending on the body weight.

Aim of this work is the set up of this inexpensive, rapid and easy-to-use method to monitor the susceptibility of *L. botrana* in wide areas. Azinphos-methyl was chosen as the test insecticide, because it is the widest used one in a similar study carried out on other lepidoptera, so that comparisons between specific susceptibility shall be possible. Furthermore, resistance is frequently due to increase of enzymatic metabolism of the toxic compound. These types of resistance have low insecticide class specificity and, when selected, cross-resistances to new classes of insecticides may occur.

It has been commonly assumed in pharmacology that a bigger animal requires a higher dose of drug or poison than a smaller one. Whereas this seems fairly obvious, it is not clear how the dose has to be related to size. Some studies are based on insect's body weight (Riedl *et al.*, 1985; Knight *et al.*, 1990).

Moreover, this has to be previously acknowledged for the species object of the study (Robertson *et al.,* 1992). To this purpose, the influence of the moth weight on the response to the treatment was verified.

Materials and methods

Insect populations

Laboratory strain. This EGM strain has

been reared on artificial diet for ten years and periodically refreshed with larvae collected in vineyards.

Giaroni strain. This EGM strain was collected in the vineyard called Giaroni and owned by IASMA in San Michele all'Adige (Italy). The vineyard, of about two ha, is planted with Chardonnay; the grapevines have a mean age of 30 years and are trained in double pergola.

Bagnoli strain. The EGM strain comes from a vineyard set in Bagnoli di Sopra (Venice). The vineyard, of approximately 10 ha, is planted with Chardonnay and Garganega and trained in Sylvoz.

Bioassays

The applied procedure derives from a partial modification of the technique developed by Knight *et al.* (1989-90) on *Platynota idaeusalis* (Walker) and by Varela *et al.* (1993) on *Cydia pomonella* (L.).

Serial dilutions of technical insecticide (Azinphos-methyl 92% purity) in acetone were prepared. One millilitre of each resulting solution and a control of 1 ml of acetone were mixed for 5 minutes into separate aliquots of 7.7 grams of warm entomological adhesive (Tanglefoot, Grand Rapids, Michigan USA). 0.7 cm^3 of this mixture were deposited on a clean trap bottom with a syringe. The adhesive mixture was spread evenly on the surface 14 x 17 cm wide. The ready adhesive supports were then stored in freezer at -16°C.

At least 5 different concentrations and a control were used per each trial. Younger-than-24-hour moths were placed for few minutes at -4°C in order to immobilise them and enable their transfer into plastic boxes. After the moths had resumed their activity, each box was closed with the adhesive support and then turned upside-down to help the adhesion of the adults to the glue.

The supports were placed into 5 litre glass pots and closed hermetically by a paraffin film (Para-film "M"®).

The pots were placed in incubation chamber at 18°C with a photoperiod of 17:7 (L:D) hours. Mortality was assessed at 40 hours. When no movement was discernible after tactile stimulation with a little brush, a moth was considered dead.

Influence of the duration of the tests on mortality. The exposure time is a remarkable parameter, because mortality varies accordingly. Knight *et al.* (1989) pointed out a rapid increment of mortality from 24 to 48 hours of exposure whereas no relevant differences were noticed between 48 and 60 hours. Varela *et al*. (1993) chose a duration of 48 hours for the laboratory tests and a duration of 40 ±1 hours after the sunset for the field tests. In this bioassay two different exposure times were tested: 40 and 64 hours.

The dose-mortality lines were determined using 5 to 6 different concentrations ranging from 500 to 5000 ppm.

Influence of the weight on mortality. A positive correlation between pupae and adult weight was proved by weighing 2 samples of adult specimens, descending respectively from light pupae (< 8 mg) and heavy pupae (> 10.5 mg) (figure 1). According to the weight of the male pupae two classes (< the 8 mg and > 10.5 mg) were distinguished in a sample of 535 specimens. In order to assess the influence of the weight on mortality, bioassays were carried out on the two classes of emerging moths. Moths were placed on the traps, incubated, and mortality checked as described above. The dose-mortality lines were determined using 5 different concentrations ranging from 750 to 3750 ppm.

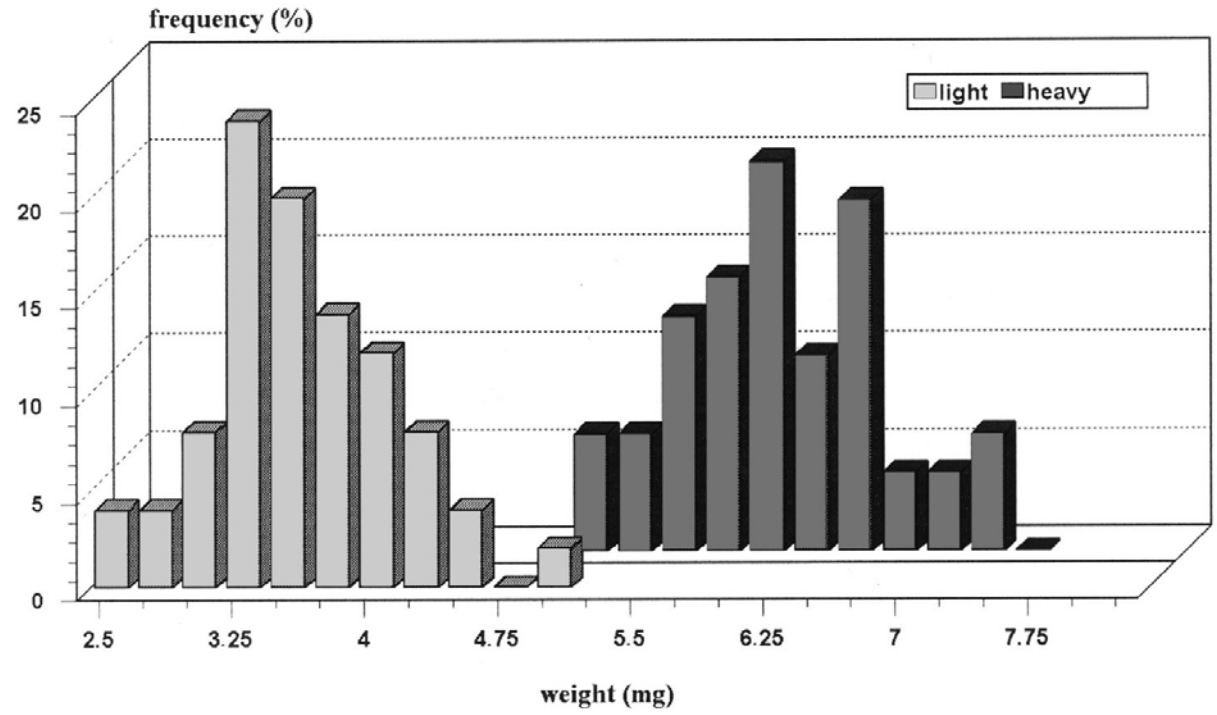

Figure 1. Distribution of the weight of *L. botrana* adult males (laboratory strain) emerged from light pupae (weight < 8.5 mg) and from heavy pupae (weight > 10.5 mg).

Influence of the sex on mortality. Dose-mortality lines refer to coeval (24 hours) males and females from the laboratory colony, which were used to assess the influence of the sex on mortality. Nevertheless, in this study it was not possible to exclude the influence of the female major weight, since the lighter females are heavier than the heaviest males. Moths were placed on the traps, incubated, and mortality checked as described above. The dose-mortality lines were determined using 6 different concentrations ranging from 1000 to 5000 ppm.

Influence of the age on mortality. EGM males reared in the laboratory were collected in three age groups: 0-24 hours (1 day), 48-72 hours (3 days) and 96-120 hours (5 days). The moths were placed in incubation room (18°C, U.R. > 70% and photoperiod 17:7 – L:D) and provided with a 10% sugar water solution added with ascorbic acid (1%). Moths were placed on the traps, incubated, and mortality checked as described above.

Evaluation of the susceptibility of the field populations

Field test. Self-made cylindrical traps (24cm long, diameter of 12cm) were baited with the pheromone cap (Isagro Ricerca S.r.l.) and placed in the field. Adhesive supports with at least 5 different insecticide concentrations and the control were installed in the traps and placed randomly in the field.

The sticky bottoms were collected daily before 8 a.m. and moved into the incubation room at 18°C, the average night temperature recorded in S. Michele all'Adige in July. Portable refrigerators were used to carry the moths from the vineyard to the laboratory. The collection of the males carried on for several consecutive nights. The adhesive supports with no catches were replaced with new ones. In the laboratory, the supports were transferred into the glass pots and placed in climatic room as well as for the laboratory tests. Because most of the moths had been caught at sunset (Balachowsky, 1966), the mortality was checked 40 hours after the sunset i.e. the presumed contact time with the insecticide. The Giaroni strain was tested during the second flight, the Bagnoli strain during the third one. The dose-mortality lines were determined using 7 different concentrations ranging from 750 to 7500 ppm.

Field-life of the treated supports. In order to reuse the adhesive supports, which did not catch any moths during the night exposure, the residual activity of the insecticide was assessed. Non-baited traps with the sticky bottoms treated with 7500 ppm of insecticide, were exposed for periods of 3, 5, 7, 14, 21, 28 and 42 days during the summer time. After the period of exposure, the moth mortality on the aged adhesive supports was evaluated. To this purpose, males from the laboratory strain, younger than 24 hours, were used and mortality was corrected with the control, according to Abbott (1925).

Position of EGM males. EGM males caught in the field traps lie in different positions on the adhesive support (ventrally, laterally and dorsally). In order to evaluate the relevance of this feature, the specimens glued with the ventral part, as in the laboratory bioassays, were distinguished from the others (reverse). The study was carried out in the two vineyards, Giaroni and Bagnoli, respectively with 377 and 687 specimens.

EGM larval bioassay. The technique adopted to monitor the resistance to the organophosphate is applied to the adult stage. In the practice the chemical treatments are used to control the larvae, and it is therefore at this stage that resistance phenomena can be developed. Although it has been already reported for codling moth (Varela, *et al*., 1993) that the resistance observed in the adults is a reliable index of what exists also at the larval stage, it was assumed to compare the efficacy of the insecticide both on the adults and on the larvae of the two strains: laboratory and Giaroni. The Giaroni's larvae were collected from the infested clusters during the first generation of the insects.

EGM larvae of 4th instar having a head diameter of about 560 µm (±80) were used. As the weight of the single larva varies remarkably, it was decided to make use of larvae weighing 2 to 6mg in order to reduce the variability itself. The average weight was similar in the two groups: 3.828 (±1.25) for the laboratory strain and 3.814 (±1.3) for the field one. Both dose-mortality lines were determined applying 5 different concentrations ranging from 100 to 5000 ppm and an acetone treated control. Larvae were treated topically with a microlitre of solution of Azinphos-methyl in acetone. After the treatment, the larvae were transferred on an artificial diet, carried out on small plastic trays (8.5 cm x 8.5 cm; boats-lab-Carlo Erba) by means of a microsyringe (Microliter TM Syringes Hamilton). Mortality was assessed 7 days later.

Results and discussion

Influence of the duration of the tests on mortality. As shown in table 1, the mortality in the control varies between 0.65% and 1.57% according to the exposure duration.

The comparison between the two treatments shows that the two dose-mortality lines are parallel and, as expected, the LC_{50} decreases for longer duration.

Table 1. Probit regressions for Azinphos-methyl at different test length.

Strain	Exposure duration	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope
Laboratory males	40h	601	0.65%	1666 (1519-1828)	4.47
Laboratory males	64h	404	1.57%	1023 (918-1140)	4.68

Table 2. Probit regressions for Azinphos-methyl to different insect weight.

Strain	Weight	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope
Laboratory males	Light	320	0%	1654 (1509-1812)	4.66
Laboratory males	Heavy	305	0%	1717 (1550-1901)	4.15

Table 3. Probit regressions for Azinphos-methyl to different insect sex.

Strain	Sex	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope
Laboratory	Male	601	0.65%	1666 (1519-1828)	4.47
Laboratory	Female	407	0%	2750 (2523-2997)	4.84

Table 4. Probit regressions for Azinphos-methyl to different insect age.

Strain	Age	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope
Laboratory	1 day	601	0.65%	1666 (1519-1828)	4.47
Laboratory	3 days	290	1.85%	1553 (1427-1691)	5.02
Laboratory	5 days	329	1.82%	1056 (921-1210)	4.58

Therefore, it was decided to adopt a 40-hour standard exposure time for the following bioassays since this length is labour saving and provides a good dose-mortality response

Influence of the weight of the insects on mortality. The analysis of the results underlines an evident superposition of the confidence limits (CL) of the lethal concentrations in the two groups together with a parallelism between the two dose-mortality lines (table 2). The weight of insects shows no or little effect on the response.

Influence of the sex of the insects on mortality. The regression lines for the two sexes are parallel. Data (table 3) show a significant difference in the susceptibility of the two sexes: the females result less susceptible as shown by the LC_{50} value (2750 ppm) that is 1.65 time higher than for the male one (1666 ppm).

Influence of the age of the insects on mortality. The three dose-mortality lines determined (figure 2) are parallel. Data in table 4 show that the lethal concentrations decrease at the growing of the age, indicating in this way a negative effect of the age on the surviving. However, this effect is quite limited, since the confidence limits of the LC_{20}, LC_{50} and LC_{90} are almost always overlapping. Notable differences only exist between LC_{50} of 1 day (1666 ppm) and LC_{50} of 5 days (1056 ppm).

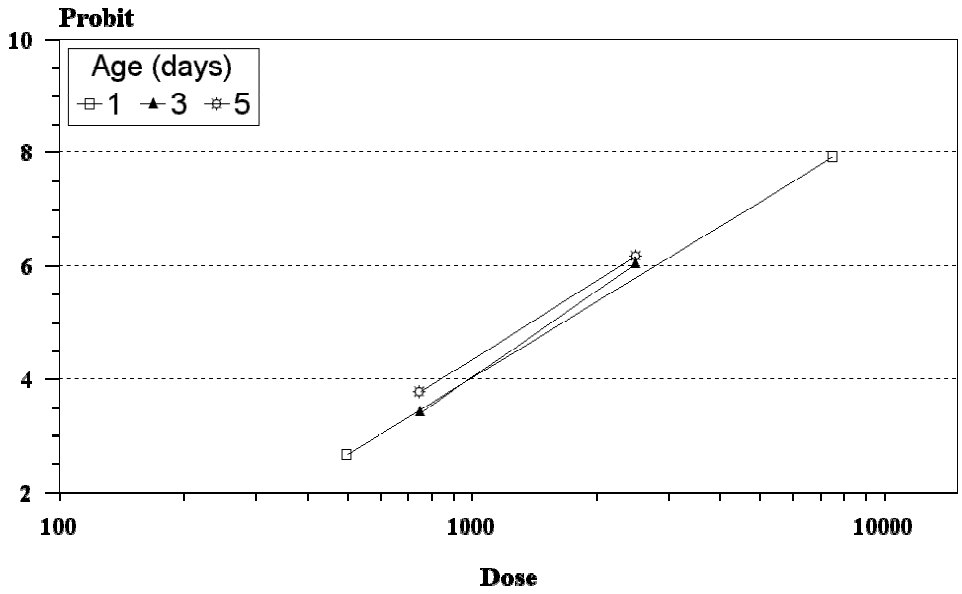

Figure 2. Dose-mortality regression for *L. botrana*: age comparison.

Table 5. Probit regressions for Azinphos-methyl in different insect strain.
RF (Resistant factor) = LC_{50}"Field strain" / LC_{50}"Laboratory strain".

Strain	Position	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope	RF
Giaroni	ventral	626	2.52%	2749 (2509-3013)	3.37	1.65
Bagnoli	ventral	947	14.19%	1375 (1247-1516)	2.92	0.83

Table 6. Lethal concentrations comparison, according to the insect position on the sticky liner.

Strain		Insect position	Mean value	Inferior limit	Superior limit
Bagnoli	LC_{90}	Ventral	3782	3228	4430
		Reverse	4358	3451	5505
		Pooled	4014	3513	4586
	LC_{50}	Ventral	1375	1247	1516
		Reverse	1260	1089	1457
		Pooled	1329	1224	1443
	LC_{20}	Ventral	708	601	833
		Reverse	558	431	722
		Pooled	643	558	740
Giaroni	LC_{90}	Ventral	6603	5520	7900
		Reverse	5659	3500	9151
		Pooled	6181	4441	8604
	LC_{50}	Ventral	2749	2509	3013
		Reverse	2180	1619	2935
		Pooled	2520	2108	3011
	LC_{20}	Ventral	1547	1355	1765
		Reverse	1166	699	1944
		Pooled	1398	1058	1847

Table 7. Probit regressions for Azinphos-methyl at larval stage.

Strain	no. of treated specimens	Control mortality	LC_{50} (CL)	Slope
Laboratory	540	4.44%	394 (301-517)	1.36
Giaroni	352	9.38%	715 (539-949)	1.57

Field-life of the treated supports. The results of this study, carried out on 1488 moths, point out the good persistence of the product in the first days of exposure and its swift reduction for longer exposure times (figure 3).

In the light of these results, it can be suggested to reutilise the adhesive supports, which did not catch any moths, for seven consecutive days after its first installation.

Field populations. The high mortality recorded in the control of the Bagnoli strain is probably due to the greater existing distance between the vineyard and the laboratory and to the possibly faulty cooling in the refrigerant boxes.

Comparison among the different populations. The three regression lines were compared two by two for the parallelism test. They result to be parallel with each other. The slope of the regression line for the laboratory strain is higher than the one obtained for the field populations. This datum, remarked also by Knight *et al*. (1989) on *P. idaeusalis*, is justified by the greater genetic uniformity found in laboratory than that existing in natural conditions.

The resistance factor (RF) shows a minor susceptibility to the Azinphos-methyl of the Giaroni strain compared to the laboratory's one (table 5). This depends on the pressure of selection to which the population was exposed due to the insecticide treatments with organophosphates.

It seems more unusual the resistance factor of the Bagnoli strain, which indicates a major susceptibility in the field population. This value may be due to the immigration into the vineyard of susceptible individuals coming from scarcely sprayed surrounding areas, or to a major susceptibility of the third generation in comparison with the second one as for *C. pomonella* (Riedl *et al*., 1985; Varela *et al*., 1993) and *P. idaeusalis* (Knight *et al*., 1990).

Position of EGM males. For both the field strains the dose-mortality lines determined by reverse specimens are parallel to those obtained by ventrally stuck moths. Moreover, the parallelism previously pointed out between the two strains is confirmed by the bioassay on the reverse moths. Therefore, there is no discrepancy in the responses with regard to the position of the insect.

Though the values of mortality concerning the different catch positions do not vary sensibly (table 6), the use of all the moths in the data processing, with no regard to their position on the trap liner, may cause an enlargement of the confidence limits of the lethal concentrations due to a major heterogeneity of the data, as observed for the Giaroni strain.

E G M l a r v a l b i o a s s a y . Mortality assessed in the control was 4.44% for the laboratory strain and 9.38% for the Giaroni one (table 7). The increase of mortality in the latter may have been a consequence of the major stress of adaptation suffered from the larvae collected in the field.

The two regression lines are parallel. The resistance factor, calculated as the ratio between the LC_{50} of the Giaroni strain and the LC_{50} of the laboratory strain, is equal to 1.81. This value is a confirmation of the value assessed for the adults.

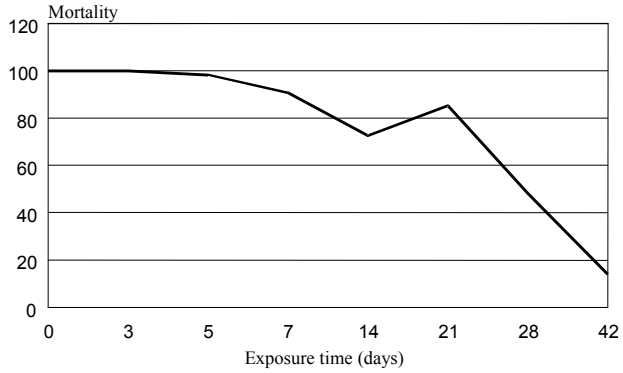

Figure 3. Persistence of the insecticide activity on the trap liners.

Conclusions

The results of this study offer technicians and agricultural operators the opportunity to make use of a new instrument of diagnosis, allowing them to realize when the inefficacy of the treatment is due to a sensible reduction in the susceptibility of the insect or when the failure of the control comes from a wrong pest management.

The use of the pheromone baited traps as a mean of sampling the field population permits to have a large number of specimens to be tested without needing any laboratory multiplication.

The outcomes of this research exclude any significant interference of the weight with the response of the *L. botrana* male moths to Azinphos-methyl. This disagrees with what reported by Knight *et al*. (1989 and 1990) for *P. idaeusalis*: the correction of the data with regard to the corporeal weight, as suggested by these authors, would erroneously lead to consider the light individuals less susceptible than the heavy ones.

Furthermore, the lack of interference of the weight with the response simplifies the application of the method in field, since it does not need the weight check of the captured specimens. The diversity of response depending on the sex agrees with what pointed out by other authors (Busvine, 1971), but it does not invalidate the use of the method based on the capture of the males only. The females result less susceptible and their response could be related to their intrinsic characteristics as observed in other insects (Busvine, 1971).

The influence of the age of the insect on the response is limited, at least concerning the interval considered, and that contributes to make the method of relevant interest for a practical application.

As it was not found any significant difference of mortality depending on the moth position on the sticky liner, the technique can be simplified using all the captured insects with no regard to their position.

For the field tests, the adhesive supports that did not catch any butterflies were always replaced with new ones in order to avoid the risks of a possible degradation of the product. This way to proceed was far too careful and, according to this study, the trap liners without captures can be reused for a few nights.

This study shows parallel values in the susceptibility between Giaroni and laboratory strain, whether using adults or whether larvae in the tests. The similarity of the two resistance factors therefore confirms the method to be a reliable diagnosis instrument of the resistance of the European Grape Moth to the Azinphos-methyl.

Though the susceptibility of the Giaroni strain is significantly less susceptible than the laboratory strain, the RF is low. This could depend on the frequent use of Chlorpyrifos-methyl known for its negative cross-resistance on codling moth (Welter *et al*., 1992).

Finally, it can be asserted that this method can usefully be adopted as a diagnosis instrument of the field resistance to contact insecticides, not only for its experimental effectiveness but also because it is cheap, versatile and extremely easy to use.

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