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EFFECTIVENESS OF SPINOSAD AND MINERAL OIL BASED COMMERCIAL PRODUCTS ON OVIPOSITION AND EGG HATCHING OF *GRAPHOLITA FUNEBRANA* TREITSCHKE

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Rizzo R., Caleca V., Lombardo A., Lo Verde G. – Effectiveness of spinosad and mineral oil based commercial products on oviposition and egg hatching of *Grapholita funebrana* Treitschke.

Laboratory trials were performed to evaluate the action of spinosad and mineral oil on eggs of *Grapholita funebrana* Treitschke, the key pest in plum orchards. Fruits of cultivars Angeleno, President and Stanley were used in the trials. The first set of tests was carried out by introducing two mated females of *G. funebrana* into a cage together with fruits of a single cultivar. The second set of trials tested the three cultivars simultaneously. In all trials, one third of the fruits of each cultivar was treated with mineral oil, another third with spinosad and the final third was left untreated. Treatments were carried out before introducing mated females into the cages. The number of fruits with eggs, the number of eggs laid on each fruit and the number of hatched eggs were recorded. The number of eggs per fruit recorded in all trials was the same, indicating that plum moth females, after choosing the fruits for oviposition on the basis of the cultivar or the applied product, tend to use all suitable fruits in the same way. Oviposition in terms of number of fruits was significantly lower in mineral oil and spinosad treatments in all trials, compared to the control. Differences between the two products were found in trials carried out with Angeleno alone and with the three cultivars together. The percentage of egg hatching on the fruits was always significantly lower with spinosad treatment compared to the mineral-oil treatment and the control. In the trials carried out using the three cultivars, the number of infested fruits was significantly higher for Angeleno and no differences in the hatching percentages were found among the cultivars. The ovicidal and antiovioposition activities of commercial products containing mineral oils or spinosad could represent an interesting tool to reduce *G. funebrana* damage, both in organic and conventional plum orchards.

KEY WORDS: organic plum orchards, oviposition, egg hatching, plum moth, Lepidoptera, Tortricidae.

INTRODUCTION

The plum moth, *Grapholita funebrana* Treitschke, is considered the most important pest in plum orchards. The damage is caused by larvae feeding into the fruit, causing changes in terms of the colour, early ripening, rotting and fruit fall. Moreover, infested fruits bear gum-associated penetration holes made by neonate larvae and exit holes made by mature larvae leaving the fruits (ALFORD, 1987).

In conventional farming, synthetic insecticides are commonly used to control plum moth infestations, and the timing of application is often critical (BUTTURINI *et al.*, 2000). In recent years, there has been an increase in the use of the mating disruption method for the control of several pests, among them the plum fruit moth. Nevertheless, in many areas, the use of this method is currently limited because of environmental factors (wind, temperature), orchard characteristics (extent, shape, isolation, etc.) and cost (PFEIFFER and KILLIAN, 1988; RICE and KIRSCH, 1990; THOMSON *et al.*, 1999; GUT *et al.*, 2004).

In organic farming, according to European Union Regulations (EC) No 889/2008, synthetic products are not permitted; synthetic pheromones cannot be sprayed for mating disruption, but they are allowed in traps or dispensers. For organic plum orchards, both mineral oil and spinosad are included in the list of allowed products (Annex II, EC Regulation 889/2008).

Mineral oil has been used to control a large number of pest species on a variety of crops for many years (DAVIDSON *et al.*, 1991; AGNELLO, 2002). Trials carried out on several insect species, among them the tortricids *Grapholita molesta* (Busck) and *Cydia pomonella* (L.), showed that mineral oil has ovicidal activity (SMITH and PEARCE, 1948; RIEDL *et al.*, 1995) and may prevent oviposition (ZWICK and WESTIGARD, 1978; RIEDL *et al.*, 1995; FERNANDEZ *et al.*, 2001). Nevertheless, in both field and laboratory studies, oil treatment failed to reduce damage caused by *C. pomonella* to acceptable commercial levels (FERNANDEZ *et al.*, 2005, 2006; RIEDL *et al.*, 1995). Other laboratory studies on *Choristoneura rosaceana* (Harris) indicated that mineral oil significantly reduces the number of eggs laid as well as the level of egg survival (WINS-PURDYA *et al.*, 2009).

Spinosad is a mixture of spinosyns A and D, which is obtained from the fermentation of the actinomycete bacterium *Saccharopolyspora spinosa* Mertz and Yao. It is considered an effective insecticide against tortricids, like *G. molesta*, and other Lepidoptera (DOERR *et al.*, 2004; SMIRLE *et al.*, 2003), as well as against several species of Diptera, Coleoptera, Thysanoptera and Hymenoptera (BRET *et al.*, 1997; DUTTON *et al.*, 2003; THOMPSON and HUTCHINS, 1999).

The ovicidal and antioviopositional activity of commercial products containing mineral oils or spinosad could represent an interesting tool to control *G. funebrana* in organic plum

orchards. In the present research, laboratory trials were carried out with mineral oil and spinosad based products in order to determine whether they reduce oviposition through repelling *G. funebrana* females, and whether they reduce the hatching percentage of *G. funebrana* eggs.

MATERIALS AND METHODS

Adults of *G. funebrana* used in laboratory trials emerged from pre-imaginal instars that developed in infested fruits, which were collected from untreated trees in an organic plum orchard in San Giuseppe Jato (37°59'50" N, 13°12'45"E, Palermo Province, Italy). The pupae were sexed (BEEKE and DE JONG, 1991) and kept separately in plastic cages (30 × 20 × 30 cm). As the flight performance of *G. molesta* is greater in adults fed before flight (DORN *et al.*, 2001), *G. funebrana* adults were supplied with a 10% honey–water solution (g/ml). Mated females were obtained by placing two males and two females (≤24 h old) in the same cage for 2 days before each test. Rearing was carried out with L16:D8 photoperiod, at constant temperature (26°C) and relative humidity (75%). The moths were not exposed to plant-related odour sources before the trials and were used only once. Moreover, the mated females used in the laboratory trials were removed from the cages after 2 days and then dissected to confirm their mating status, recording the presence of one or more spermatophores in the bursa copulatrix (KNIGHT, 2006).

Uninfested fruits collected in the same San Giuseppe Jato organic orchard were used in all trials. Fruits of the same size were picked 12–24 hours before starting the trials and were handled by their pedicels to prevent contamination of the fruit surface.

In the first set of trials ("Single Cultivar"), two mated females were introduced into a cage (constructed of wood and glass), containing 18 fruits of the Angeleno cultivar. Six of the fruits had been sprayed with a spinosad based product, another six had been sprayed with a mineral oil product and six had not been sprayed. Success, Dow AgroSciences, was the commercial product containing 120 g per litre of spinosad; UFO, Bio-Intrachem, was the commercial product containing 96.9 g per 100 g of mineral oil. Success and UFO were used at concentrations of 120 ml in 100 l of water and 150 ml in 100 l of water, respectively, as suggested by the manufacturers. The entire surface of each fruit was sprayed 5–6 hours before the fruits were randomly located in the cage. The same trial was performed using President and Stanley cultivars. Each "Single Cultivar" trial was repeated eight times.

A second set of trials ("Three Cultivars") tested the three cultivars simultaneously. In these trials, nine fruits of each cultivar were placed in a cage (27 fruits per cage) and two mated females were introduced. Three of the nine fruits of each cultivar were left unsprayed, three were sprayed with Success and three were sprayed with UFO; the commercial products and doses were the same as described for the previous assays. This trial was repeated eight times.

The number of fruits with eggs and the number of eggs laid on each fruit were counted after removal of the females (2 days) and the number of hatched eggs was counted daily over the following 7 days. All trials were carried out under the same rearing conditions described above. Moreover, the females used in the laboratory trials were removed from the cages after 2 days and then dissected to confirm their mating status (KNIGHT, 2006).

STATISTICAL ANALYSIS

As the number of fruits with eggs and the number of hatched eggs are countable data (presence/absence of eggs on fruits and hatched/unhatched eggs), the binary logistic regression was performed. This method of statistical analysis uses the odds ratio (ratio of percentages) to compare one or several treatment percentages (product and cultivar) with the percentage under the reference conditions (control), when one or more of the input factors is changed. For the "Single Cultivar" laboratory trials, *Treatment* was the only factor to be considered. In the "Three Cultivars" laboratory trials, the independent variables were *Cultivar* and *Treatment*. Moreover, data on the number of eggs per infested fruit were normalised by means of a Box–Cox transformation and then analysed using ANOVA and Tukey's post-hoc test.

MINITAB software (Minitab, Inc., State College, PA) was used for all statistical analyses.

RESULTS

The statistical analysis of infested/uninfested fruits in the "Single Cultivar" tests shows that, in all of the cultivars, the number of infested fruits was significantly higher in the control than in UFO and Success treatments (Tables 1–3). The infestation level recorded on fruits treated with UFO and Success resulted significantly different only on the Angeleno cultivar.

With regards to the hatching percentage, in the Angeleno cultivar, none of the eggs hatched in the Success treatment; therefore, it was only possible to compare the control and mineral-oil treatment, and no significant difference was shown between them (Table 1). In the President cultivar, the hatching percentage on Success treated fruits was significantly lower than on the control and mineral oil treated fruits. The latter two treatments did not result in a significant difference (Table 2). In the Stanley cultivar, no statistical differences were found in the hatching percentage recorded in UFO and Success treatments. Both of them showed a hatching level significantly lower than the control (Table 3).

In the second set of trials ("Three Cultivars"), significant differences were found in the percentages of fruits with eggs among both the different treatments and cultivars. The risk of infestation recorded on Angeleno was significantly higher than on President or Stanley, and there was no statistical difference between the latter two (Table 4). The egg hatching percentages recorded on the control as well as the UFO and Success treatments provided significantly different results, but no differences were found among the three cultivars (Table 4).

In all trials, the number of eggs on each infested fruit ranged from 1 to 4, without significant differences among the treatments in the "Single Cultivar" trials or among the cultivars and treatments in the "Three Cultivars" trials (ANOVA, $p < 0.05$).

DISCUSSION

The antiovideopont effect of mineral oil has been demonstrated for several insect species belonging to Homoptera, Coleoptera and Lepidoptera (POWELL *et al.*, 1998; LIANG and LIU, 2002; TREACY, 1991), the latter including *C. pomonella* and *G. molesta* (RIEDL *et al.*, 1995;

Table 1 – Binary logistic regression statistics applied to the infested fruits and the number of hatched eggs, in the “Single Cultivar” trials carried out on the Angeleno cultivar. The reference level is the control.

Angeleno								
Predictor	%	Coef.	Coef. SE	Z	P	Odd Ratio	Confidence Interval	
							Lower	Upper
Fruits with eggs								
Mineral oil	29.2	−1.22	0.43	−2.83	0.01	0.29	0.13	0.69
Spinosad	8.3	−2.73	0.60	−4.57	0.00	0.06	0.02	0.21
Control	58.3							
Log-Likelihood = −75.344 Test that all slopes are zero: $G = 29.730$, $DF = 2$, $P = 0.000$								
Hatched eggs								
	% (No.)							
Mineral oil	79.2 (44)	−0.61	0.64	−0.95	0.34	0.54	0.15	1.92
Spinosad	0 (6)							
Control	87.5 (56)							
Log-Likelihood = −33.381 Test that all slopes are zero: $G = 0.872$, $DF = 1$, $P = 0.350$								

Table 2 – Binary logistic regression statistics applied to the infested fruits and the number of hatched eggs in the “Single Cultivar” trials carried out on the President cultivar. The reference level is the control.

President								
Predictor	%	Coef.	Coef. SE	Z	P	Odd Ratio	Confidence Interval	
							Lower	Upper
Fruit with eggs								
Mineral oil	25	−1.10	0.44	−2.49	0.01	0.33	0.14	0.79
Spinosad	16.7	−1.61	0.48	−3.33	0.00	0.20	0.08	0.52
Control	50							
Log-Likelihood = −81.890 Test that all slopes are zero: $G = 13.483$, $DF = 2$, $P = 0.001$								
Hatched eggs								
	% (No.)							
Mineral oil	64.3 (14)	−1.20	0.71	−1.69	0.09	0.30	0.07	1.21
Spinosad	11.1 (9)	−3.87	1.15	−3.37	0.00	0.02	0.00	0.20
Control	85.7 (42)							
Log-Likelihood = −29.489 Test that all slopes are zero: $G = 19.569$, $DF = 2$, $P = 0.000$								

FERNANDEZ *et al.*, 2001, 2005, 2006). In contrast, research on the antiovideponent action of spinosad products is very limited.

Both the “Single Cultivar” and “Three Cultivars” trials in this study showed that the application of the two products containing spinosad (Success) and mineral oil (UFO) significantly reduced the probability of oviposition by *G. funebrana* in comparison with untreated fruits. Moreover, as the “Three Cultivars” trials show, the antiovideponent action of the two products does not seem to be affected by

the cultivar, despite the clear preference of *G. funebrana* females for Angeleno fruits. The antiovideponent action of Success was higher than UFO, as shown by the significant differences recorded when *G. funebrana* females were exposed to Angeleno alone and to the three cultivars together. Nevertheless, the better performance of Success in the laboratory could be subverted in the field, owing to its shorter persistence in comparison with UFO. In addition, analyses of the number of eggs laid on the infested fruits seem to indicate that plum moth females, after choosing the

Fruit with eggs								
Predictor	%	Coef.	Coef. SE	Z	P	Odd Ratio	Confidence Interval	
							Lower	Upper
Treatment								
Mineral oil	27.8	−1.13	0.39	−2.90	0.00	0.32	0.15	0.69
Spinosad	12.5	−2.23	0.47	−4.79	0.00	0.11	0.04	0.27
Control	50.0							
Cultivar								
President	26.4	−1.22	0.39	−3.09	0.00	0.30	0.14	0.64
Stanley	13.9	−2.11	0.45	−4.65	0.00	0.12	0.05	0.29
Angeleno	50.0							
Log-Likelihood = −106.251				Test that all slopes are zero: $G = 51.730$, $DF = 4$, $P = 0.000$				
Hatched eggs								
Predictor	% (No.)	Coef.	Coef. SE	Z	P	Odd Ratio	Confidence Interval	
							Lower	Upper
Treatment								
Mineral oil	64.0 (25)	−2.87	0.91	−3.15	0.00	0.06	0.01	0.34
Spinosad	9.1 (11)	−5.88	1.37	−4.29	0.00	0.00	0.00	0.04
Control	96.5 (54)							
Cultivar								
President	80.0 (20)	−0.24	0.83	−0.29	0.77	0.78	0.15	4.01
Stanley	86.7 (17)	−0.97	1.08	−0.90	0.37	0.38	0.05	3.15
Angeleno	71.7 (53)							
Log-Likelihood = −27.763				Test that all slopes are zero: $G = 41.187$, $DF = 4$, $P = 0.000$				

fruits for oviposition on the basis of the cultivar or the applied product, tend to use all suitable fruits in the same way, always laying a similar number of eggs per fruit.

In general, the insecticidal effect of mineral oil and spinosad has been widely studied on a large number of insects, both pests and natural enemies (DAVIDSON *et al.*, 1991; WILLIAMS *et al.*, 2003; BIONDI *et al.*, 2012a, 2012b). On the other hand, the egg hatching failure due to the product sprayed before oviposition, has been reported for mineral oil products against the eggs of *Trialeurodes vaporariorum* (Westwood), *Liriomyza trifolii* (Burgess) (ZWICK and WESTIGARD, 1978; LAREW, 1988) and *C. pomonella* (RIEDL *et al.*, 1995), but this effect has not been previously investigated for spinosad based products. Our results indicate that UFO exhibits a clear negative effect on egg hatch in the "Single Cultivar" trials carried out on Stanley fruits as well as in the "Three Cultivar" trials.

Success pre-oviposition sprays showed to significantly reduce the percentage of eggs that hatched on plum fruits in all trials. Given the present results, it is reasonable to infer that control of *G. funebrana* by UFO and Success may result from their combined effects on oviposition and hatching.

Field experiments carried out in an organic plum orchard in Sicily showed that, at the end of July, the mineral-oil treatments on Angeleno caused a reduction in *G. funebrana* infestation by 36% relative to the untreated control (RIZZO *et al.*, 2012b). This result cannot be considered economically sustainable, which is similar to the results obtained on *C. pomonella* by FERNANDEZ *et al.* (2005) and RIEDL *et al.* (1995). In these cases, a significant suppression of tortricid pests by the mineral oil would require more frequent or higher rates of application (FERNANDEZ *et al.*, 2005), but this procedure is inadvisable because of phytotoxicity, cost and regulatory limitations. Nonetheless, mineral oil is frequently recommended to prevent the development of resistance, because it is one of the few pesticide groups for which resistance has never been documented (WILLETT and WESTIGARD, 1988).

Owing to its natural origin, to its very low mammalian toxicity (BRESLIN *et al.*, 2000) and rapid breakdown in the environment (CLEVELAND *et al.*, 2002; ZHAO *et al.*, 2002), spinosad represents an important tool alternative to synthetic pesticides in organic farming as well as in conventional safety, especially in terms of farm workers and consumer safety (WILLIAMS *et al.*, 2003). On the other hand, spinosad has been demonstrated to induce resistance in several insect pests (ZHAO *et al.*, 2002; HSU and FENG, 2006; WANG *et al.*, 2006) and to have harmful side effects on beneficial arthropods such as bees and some natural enemies (WILLIAMS *et al.*, 2003). For these reasons, policies restricting the use of spinosad have been adopted in many countries, and the alternation between spinosad and other products is often suggested. The reliability of alternating the application of spinosad and mineral-oil products was confirmed by field results obtained on *G. funebrana* by RIZZO *et al.* (2012b). Furthermore, a reduced application of spinosad may result in an increase in the abundance and diversity of harmless arthropods, as found in pear and apple orchards, where more selective interventions for major arthropod pests, including mating disruption for codling moth, are adopted (GUT and BRUNNER, 1998; KNIGHT *et al.*, 1997; MILICZKY *et al.*, 2000).

An interesting finding of this study is that the tested cultivars differed in terms of their susceptibility to the plum moth. Susceptibility to the plum moth could depend on the fruit properties, as has been demonstrated for the olive fruit

fly (RIZZO *et al.*, 2012a). Additional research on the effect of different population levels of *G. funebrana* on these cultivars could lead to a more targeted and integrated pest-management strategy and consequently to a further reduction in the use of chemicals for plum moth control.

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