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Side effects of kaolin on natural enemies found on olive crops

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Abstract: Protected barriers of kaolin, a natural clay mineral, were tested against olive pests in the last few years with good results on *Bactrocera oleae*, the most serious pest of olive groves. Good control of the carpophagous generation of *Prays oleae*, as well as a minor presence of *Saissetia oleae,* were also reported.

Adults of *Psyttalia concolor* (parasitoid of *B. oleae*), *Chrysoperla carnea* (oophagous predator of *P. oleae*), *Chilocorus nigritus* (predator of diaspidid scales) used in this work as representative of *Chilocorus bipustulatus* and *Anthocoris nemoralis* (predator of *Euphyllura olivina* and *Liothrips oleae*) were selected to be exposed to an inert surface treated with kaolin at the maximum field rate used against *B. oleae*. Dimethoate was used as reference compound. Mortality was recorded at 24, 48 and 72 h. Sublethal effects, as life span in *C. nigritus* and reproductive parameters (fecundity and fertility in case of *C. carnea* and *A. nemoralis,* beneficial capacity in *P. concolor)* were also assessed.

Kaolin was classified as harmless (1) to adults of *C. nigritus.* There were no effects on mortality on *C. carnea* and *P. concolor*, although *C. carnea* fecundity and *P. concolor* progeny were slightly reduced (2). *A. nemoralis* was the most sensitive of the four insect tested, with 44% mortality and 66.6% reduction of eggs production. Dimethoate was very toxic with 100% mortality for each test species after only 24h of exposure. Compared to classical insecticide commonly used in olive crops as dimethoate, Kaolin seems to be a promising compound because of its selectivity. However, because of its uncommon mode of action, other modes of exposure than contact with a treated surface need to be tested to confirm or infirm the apparent harmlessness of this product.

Key words: olive, kaolin, *Anthocoris nemoralis, Chilocorus nigritus, Chrysoperla carnea, Psyttalia concolor*

Introduction

Spain is the first olive oil-producing and exporting country, with the highest surface and number of olive trees in the world. Last year, this crop yielded approximately 1 million tons of olive oil (MARM, 2009). Within all the olive pests, the olive fruit fly *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is the most serious pest of olives in most of the countries of the Mediterranean basin. This fly lays their eggs on the fruit, causing fruit drop and acidity in the olive oil. The olive moth *Prays oleae* (Bern.) (Lepidoptera: Ypomoneutidae) is the second pest in importance. The larvae of each generation of this trivoltine species are feeding on different parts of the olive trees, namely flowers, fruits and leaves. *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), the black scale (Haniotakis, 2005) is also a pest of olive trees of economic importance.

Protected barriers of kaolin, a natural clay mineral, have been tested against olive pests in the last few years with good results on *B. oleae* (De la Roca, 2003; Saour & Makee, 2004). De la Roca (2003) also reported a good control of the carpophagous generation of *P. oleae*, as well as a minor presence of *S. oleae*. Kaolin-based particle was originally employed in fruit production to protect fruits from solar injury by forming a film of reflecting particles on their surface (Glenn et al., 2002). Kaolin sprayed on crops was effective against a range of pests insects such as aphids, fruit flies, Lepidoptera and Coleoptera (Daniel et al., 2005). The inert particle film coating a plant creates a hostile environment for insects and a physical barrier to infestation, impeding insect movement, feeding and egg-laying (Bürgel et al., 2005).

The main goal of this work was the evaluation of possible side effects of kaolin used against *B. oleae* on the auxiliary fauna. *Psyttalia concolor* (Szèpligeti) (Hymenoptera: Braconidae), an endoparasitoid of *B. oleae*, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae), an oophagous predator of *P. oleae, Chilocorus nigritus* (F.) (Coleoptera: Coccinellidae) a predator of diaspidid scales used in this work as representative of *Chilocorus bipustulatus* (L.), (Coleoptera: Coccinellidae) and *Anthocoris nemoralis* (F.) (Hemiptera: Anthocoridae) a predator of the psyllid *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae) and the thrips *Liothrips oleae* Costa (Thysanoptera: Phloeothripidae) were selected to be exposed to kaolin sprayed in an inert substrate as a first step, according to IOBC sequential scheme.

Material and methods

Insect origin and rearing

All experiments were performed on adults. A laboratory colony of *C. carnea* was established from L_1 larvae obtained from Biobest Biological Systems, Spain. Adults of *A. nemoralis* came also from the same company and Entocare Biological Crop Protection (Wageningen, Netherlands) supplied us with *C. nigritus* adults. *P. concolor* has been reared in our laboratory from many years ago on the host *Ceratitis capitata* (Wiedemann). Both rearing and bioassays were performed in a controlled environmental cabinet (25±2ºC, 75±5% R.H., 16:8 (L:D) photoperiod).

Conduct of the trials

To evaluate residual contact activity, glass plates were treated under a Potter precision spray Tower with 1ml of each test solution at a pressure of 55 kPa to obtain a homogenous deposit of 1.5-2mg fluid per cm^2. A systemic insecticide, dimethoate, was used as a commercial standard, and distilled water as control. Concentration for every insecticide used in the bioassay was determined based on the maximum recommended field concentration with a delivery rate of 1000l/ha water. The amount of insecticide applied per hectar was corrected by using the Predicted Initial Environmental Concentration (PIEC), with a correction factor of 0.4 for foliage dwelling predators (Candolfi et al., 2000) (Table 1).

Table 1. Insecticides tested on beneficials.

Active ingredient	Trade name	% a.i. and formulation	Concentration[a]	PIEC (μg/cm^2)
Dimethoate	Danadim Progress®	40 EC	150 ml/hl	6
Kaolin	Surround®	95 WP	5 kg/hl	200

[a]Commercial product

As soon as the plates were dry, the corresponding group of insects per replicate was kept in glass dismountable cages consisting of two treated glass plates and a round metacrilate frame joined by two crossed rubber bands (slightly modified from cages developed by Jacas & Viñuela (1994)). They were provided with food and water when needed. These cages were then transferred to the climatic chamber and connected one by one with an hypodermic needle to a rubber tube provided with a continuous flow of air produced by an aquarium pump to assure forced ventilation. More details on the methodology depending on every insect are given on Table 2.

In preliminary bioassays carried out with *A. nemoralis* following the methodology described by Stäubli & Pasquier (1988), we failed to test the compounds on a inert substrate using only *Ephestia kuehniella* Zeller eggs as food. As such, we also treated under the Potter Tower small pieces of beans by the two sides with the same residue used to treat the glass plates. By addition of beans, we could obtain an acceptable natural mortality in controls as well as recorded oviposition.

All insects were exposed to treated residues for three days. Mortality was scored at 24, 48 and 72 h. Therefore, survivals were moved to a different type of non treated cages to evaluate sublethal effects as life span for *C. nigritus*, reproduction (fecundity and fertility) for *C. carnea* and *A. nemoralis* and parasitism ability for *P. concolor* (% attacked host and progeny).

Table 2. Details on specific methodology used with every natural enemy tested.

Insect	Insects per replicate/ Nº replicates	Adult age	Diet	Water
P. concolor	10 ♀/4	< 24 h	4:1 sugar:yeast	Yes
C. carnea	3♂+3♀/4	< 48 h	Artificial diet	Yes
A. nemoralis	6♂+6♀/5	Unknown	*Ephestia kuehniella* eggs	No[a]
C. nigritus	9 adults[b]/5	Unknown	*Ephestia kuehniella* eggs	No

[a]Green beans were supplied as source of water and oviposition substrate.
[b]Sex was not determined

P. concolor trials

After three days of exposure to a treated surface, 5 surviving females per replicate and control, were isolated for four days in plastic cages. Every day, 30 fully-grown *C. capitata* larvae were offered to each group of females for parasitisation following González-Núñez (1998). One hour later, *C. capitata* larvae exposed were placed into Petri dishes to let them pupate. Parasitism ability was measured as the percentage of attacked host (percentage of puparia without medfly emergence) and progeny size (percentage of parasitoids emerged from parasitized medfly puparia). Data of first day of parasitisation was not considered since females need to learn how to parasitize.

C. carnea trials

Three days after exposure to the treated surfaces, survivors were moved to new cages and both fecundity (mean number of eggs per female laid in a 7-days period) and fertility (percentage of egg hatched) were assessed according to Medina et al. (2001).

A. nemoralis trials

Eggs laid in every piece of treated bean used as substrate for oviposition during the three days of exposure were counted. Then, they were transferred one by one to a plastic cages (9cm in diameter, 3cm in height) to count the emergence of neonates. Eggs laid per day and female and percentage of eggs hatched were recorded.

C. nigritus trials

After being exposed to a contaminated substrate for three days, all survivors from the same replicate and insecticide were moved to ventilated plastic cages (∅: 11cm, h: 5cm) to evaluate life span. Mortality of insects was recorded every week until the death of the last insect. Life span was measured as the average of days that all the insects in each replicate.

Results and discussion

Based on the results obtained in this study, kaolin seems to be not too much toxic to the natural enemies tested when they were in contact with kaolin-treated surfaces. According to IOBC categories, kaolin was classified as harmless (1) or slightly toxic (2), depending on the insect and the parameter studied (Table 3). On the opposite, with the same methods, Dimethoate was very toxic with 100% mortality for each species after only 24h of exposure.

Kaolin was classified as harmless (1) to adults of *C. nigritus.* No deleterious effect was detected. Neither mortality measured after 72 h of exposure nor life span was modified due to the kaolin exposure. Nevertheless, possible negatives effects on fecundity and fertility remained unknown and need to be determined. There were no effects on mortality on *C. carnea* and *P. concolor*, although *C. carnea* fecundity and *P. concolor* progeny were slightly reduced (2) compared to water control. *A. nemoralis* was the most sensitive of the four insect tested. Forty per cent of mortality was recorded after 72 hours of exposure, when mortality was stabilized. The most important observed effect was the strong reduction (66.6%) on the numbers of eggs laid by female and day. Most of them were viable.

Table 3. IOBC toxicity rating after residual treatment in laboratory with kaolin in comparison with the standard commonly applied in olive crops against the olive fly, *B. oleae*.

Compounds	*P. concolor*			*C. carnea*			*A. nemoralis*			*C. nigritus*	
	M (%)	AH (%)	P (%)	M (%)	Fec. (%)	Fert. (%)	M (%)	Fec. (%)	Fert. (%)	M (%)	LS (%)
Kaolin	1	1	2	1	2	1	2	2	1	1	1
Dimethoate	4	-	-	4	-	-	4	-	-	4	-

IOBC toxicity rating for laboratory: 1= harmless (<30%); 2= slightly harmful (30-79%); 3= moderately harmful (80-99%); 4= harmful (>99%).

M=Mortality after 72 h of exposure. AH=attacked host. P=Progeny. Fec= Fecundity. Fert.= Fertility. LS= Life span.

Kaolin mainly acts as a physical barrier against insects, as it has been observed with several pests. When larvae of the obliquebanded leafroller *Choristoneura rosaceana* (Harris), consumed kaolin mixed into an artificial diet, with no continuous physical barrier, effects on mortality were negligible. When larvae fed on apple leaves treated with Kaolin, high mortality were observed, indicating that the effects of kaolin were primarily physical versus a physiological toxin (Sackett et al., 2005). The gypsy moth, *Lymantria dispar* (L.) and the forest tent caterpillar *Malacosoma disstria* Hubner (Cadogan & Scharbach, 2005) showed different feeding behaviour in food choice experiments (significantly less consumption of kaolin treated red oak) and no food choice experiments (no significant differences). Reduction on oviposition was also described for psyllas (Pasqualini et al., 2003) or tephritids as *C. capitata* (Mazor & Erez, 2004) and *B. oleae* (Caleca & Rizzo, 2007). The reported reduction of oviposition can be due to the repellent effect of the compound. According to Glenn & Puterka (2005), repellency can lead to feeding reduction and, as a consequence, to a reduction of oviposition. Anyway, as also observed by Sackett et al. (2005), kaolin might be responsible of some non-determined physiological effects, because slightly more mortality is due to kaolin than to controls.

There are no reasons to believe that those effects reported on pests cannot also occured for natural enemies. However, few studies have been reported on beneficials. Kaolin reduced population of chrysopids on arthropofauna studies carried out on olive groves (Gonzalez-Núñez et al., 2008), probably because prey was not easily available after kaolin treatments. As Kaolin was not toxic for chrysopids in the laboratory, it could be possible that when chrysopids have the choice to move to a different place as it occurs in the fields, they decide to do not land in olive trees or they do it in less numbers. This hypothesis need to be tested because it could explain why their presence in fields treated with Kaolin is lower than in controls even if the chrysopids are not severely affected in laboratory by this product.

To our knowledge, there are no references about kaolin affecting *P. concolor*. The reduction in the progeny cannot be easily explained because females were exposed to a residue of kaolin before parasitisation, unless they contaminated themselves during cleaning. In that case, some kaolin might have been ingested, reducing their feeding ability and, as a result, producing eggs not so healthy.

Several studies have been conducted in pear to evaluate kaolin as an alternative to conventional insecticides on organic orchards, showing a reduction on the oviposition of psyllas (Pasqualini et al., 2003). This author mentions that, as for psyllas, a strong reduction on the reproduction of *A. nemoralis*, its main predator, might also be detected if studies to a bigger scale were performed. Our study showed that *A. nemoralis* females laid significantly less eggs on a substrate treated with kaolin, probably because a repellent reaction.

Although no negative effects could be detected on *C. nigritus* exposed to an inert surface treated with kaolin for three days, more experiments should be done by evaluating other sublethal effects, such as effects on reproduction or increasing the time of exposure to the compound.

Kaolin seems to be a promising compound to be use in olive crops taking into account that kaolin affects beneficial arthropods to a lesser extent than compounds commonly used as dimethoate. However, because of its uncommon mode of action, we think that testing kaolin using only residual tests as proposed by IOBC, could perhaps lead us to non real conclusions and other modes of exposure than contact with a treated surface need to be tested to confirm or infirm the apparent harmlessness of this product. A special attention should be paid in future experiments to sublethal effects, as reproduction and behaviour and to effects to different stages of development.

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