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Effects of Kaolin on *Lobesia botrana* (Lepidoptera: Tortricidae) and its Compatibility with the Natural Enemy, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae)

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ABSTRACT *Lobesia botrana* (Den. & Schiff.) (Lepidoptera: Tortricidae) is an important grapevine pest in Europe recently encountered in America. *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) is amongst the most effective parasitoids for Lepidopteran species. Studies to evaluate the effect of kaolin, an inert, non-toxic mineral on these species were carried out. Efficacy on *L. botrana* neonate larvae, oviposition and egg hatch were evaluated. Effects of kaolin on parasitism and emergence of *T. cacoeciae* from *L. botrana* and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were also evaluated. *L. botrana* egg hatch and oviposition rates were reduced and neonate larvae mortality was significantly greater in kaolin treated arenas and when included in synthetic neonate larvae diet. Kaolin had no effect on *T. cacoeciae* parasitism I n both hosts. There was only a slight but statistically insignificant effect on *T. cacoeciae* progeny emergence from *L. botrana* eggs and no effect from *E. kuehniella*. The reductions in *L. botrana* oviposition, and egg hatch and increase in larval mortality with kaolin contribute to reduction in population densities and can be considered in rational IPM strategies for *L. botrana*. Due to the laboratory results presented on parasitoid emergence, even though field bioassays would give a more exhaustive evaluation, it appears kaolin can be compatible with *T. cacoeciae* in *L. botrana* management.

KEY WORDS European grapevine moth, parasitoid, kaolin particle film, side-effects

Lobesia botrana (Den. and Schiff.) (Lepidoptera: Tortricidae) is a lepidopteran species which historically prefers grape (*Vitis vinifera* L.) causing direct damage to flowers and fruit set (Bovey 1966) which is normally not economically important. However, *L. botrana* physical damage to the berry is associated with the even greater economic loss from pathogen infection and subsequent rotting due especially to *Botrytis cinerea* Pers. (Deueromycotina) (Roehrich and Bollere 1991) and bacterial species. This Lepidoptera is an important pest in European and Mediterranean area vineyards and has been recently detected in Chile, (González 2008) California, (Varela 2010, Gilligan 2011) and Argentina (Gonzalez 2010). Due to the extension of this species range with its associated damage it has become economically important worldwide.

Traditionally, *L. botrana* has been controlled by chemical means. Yet now with the need to avoid agricultural contamination, secondary effects on auxiliary fauna, and resistance development, other environmentally friendly control methods such as *Bacillus thuringiensis* Berliner (Bacillales: Bacillacidae) (Escudero 2006) and mating disruption (Louis 2001) have also been included in IPM strategies. Also, with auxiliary fauna in mind, biological control has retuned to the centre of many management approaches. The use of oophagous parasitoid *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae) species as inundative control agents is one example. Along those lines, *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) is giving promising experimental results, such as high parasitic potential with *L. botrana* (Moreno 2009).

On the other hand, particle film technology including kaolin was initially employed for the control of the 12-spotted cucumber beetle, *Diabrotica duodecimpunctata* Fab. (Coleoptera: Chrysomelidae) in 1932 (Richardson and Glover 1932). This technology has re-emerged for the use of controlling arthropods such as *Thrips tabaci* Lindeman (Larentzaki et al. 2008), *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) (Silva and Ramalho 2013), *Toxoptera aurantii* Boyer de Fonscolombe (Homoptera: Aphididae) and *Aphis spiraecola* Patch (Homoptera: Aphididae) (Smaili et al. 2014). Mitigation of diseases, sunburn, and heat stress in crop plants (Russo and Diaz-Perez 2005, Cantorea et al. 2009) fruit trees, along with increased yield in blueberry (Spiers et al. 2004) are other benefits of kaolin use (Melgarejo et al. 2004, D'Aquino et al. 2011). With its high pH kaolin can prevent the adhesion of pathogen spore and their germination (Walters 2006) on plant tissue.

Due to its mechanisms of action, kaolin should, theoretically, not be toxic to natural enemies. However, slight effects on *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) (Porcel et al. 2011, Bengochea et al. 2014), *Chelonus inanitus* (L.) (Hymenoptera: Braconidae) and on *Scutellista cyanea* Motschulsky (Hymenoptera: Pteromalidae) (Bengochea et al. 2013) were reported. Also, the arthropod assemblage of auxiliary fauna in olive groves was altered (Pascual et al. 2010). In light of the negative effects reported on other natural enemies it is fundamental to examine side-effects on biological control agents employed in each case.

Herein presented is the evaluation of the effect of kaolin treatments on *L. botrana* oviposition, egg hatch, and neonate larvae mortality. To assure compatibility of management strategies, the effects of the particle film on parasitism and emergence of the natural enemy *T. cacoeciae* was also evaluated in both *L. botrana* and an alternative host, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae).

Materials and Methods

Insects. All individuals used for the experiments were taken from laboratory colonies reared and maintained in a growth chamber at the constant, standard conditions of 24 ± 1 ºC, 60 ± 5% RH, and 16:8; (L:D). *L. botrana* was originally collected in an ecological vineyard in 2000 in La Rioja, Spain. The population was maintained in the laboratory following a previously defined protocol (Del Tío 1996) yet with a modification of the semi-synthetic diet (Sáenz de Cabezón 2003). The *T. cacoeciae* individuals originated from an endemic population collected in La Rioja. The population was maintained in the laboratory (Moreno 2007) since its collection from the field in 2003. The *E. kuehniella* eggs were produced with a laboratory population (Marco et al. 1993) which originated in the Polytechnic University of Madrid in 1990. When necessary, individuals from remote populations were added to our laboratory colonies to avoid genetic funnels. Pre-oviposition periods were carried out when necessary before the beginning of each bioassay. The specific rearing details were published by the corresponding authors herein listed.

Kaolin Compound. The product Surround® WP, which consisted of 95% refined kaolin, $Al_4Si_4O_{10}[OH]_8$, from the Engelhard Corporation, hereafter referred to as kaolin, was employed in all bioassays, unless stated otherwise, at 47.5 grams of kaolin per liter, the maximum suggested concentration for vineyard. It was suspended in water with continual mixing.

Bioassay types. Two types of bioassays were carried out: choice and no choice. In the choice bioassays the individual had equal access to both conditions (kaolin treatment or control) and could choose where to reside, lay its eggs or in the case of *T. cacoeciae,* parasitize. In the no choice bioassays the individuals were placed in arenas of only one condition (kaolin treatment or control). The parameters recorded, such as oviposition, percentage of egg hatch, and neonate larvae mortality, also determined the kind of bioassay. Being that kaolin is not a cuticle penetrating pesticide we could not measure fertility and fecundity, only behavioural and mechanical effects on oviposition and egg hatch. Parasitism by *T. cacoeciae*, in *E. kuehniella* and *L. botrana* eggs and survival of its progeny from each species was evaluated in accordance with each specific bioassay.

Treatments. A quantity of 5.5 ml was applied using a Potter Tower at a pressure of 20 kPa which produced a deposit of 0.05 ± 0.005 ml per cm^2 yielding an equivalent field coverage of 50 kg of the product per hectare. All grape clusters employed were prepared by immersion in a solution of kaolin or the water carrier for 5 seconds during continual mixing to avoid settling of the kaolin particles in the treatment solution. The semi-synthetic laboratory diet disks were treated by submersion for 5 seconds in the solution of kaolin with continual mixing or in the water carrier. All materials employed were set to dry before their addition to the bioassay arenas.

Effect of Kaolin on *L. botrana* Oviposition and Egg Hatch on Synthetic Substrate. In all *L. botrana* oviposition assays, after a preoviposition period of 24 hours, one female and two males were placed in each replicate. The arenas consisted of 330 cc plastic cup with hexagonal base, upside down, inside the base of a 9 cm diameter (∅) Petri dish containing one 2.5 cm ∅ Petri hydration dish filled with water soaked cotton. In the choice bioassay, three alternate sections of the chamber were treated with the kaolin solution. In the no choice bioassay, the oviposition chambers in the kaolin condition were completely covered with kaolin at the application specifications stated above. The adults were moved to new arenas every 48 hours for three consecutive time periods. The number of eggs laid was recorded after moving the adults to new oviposition chambers. The previous chambers were maintained in the experimental conditions described above until egg hatch. After the incubation period of 5 days the number of eggs hatched in each condition was also recorded. Forty-eight replicates were evaluated in the choice bioassays, whereas, thirty-one of each, kaolin treated and control replicates, were evaluated in the no choice bioassays.

Effect of Kaolin on *L. botrana* Oviposition and Egg Hatch on Grape. One female and two male moths were used in each replicate. The experimental arenas consisted of one transparent plastic 9 cm ∅ by 21 cm tall cylindrical box containing two 1 cm ∅ respiration holes in the lid, and one hydration container as described above. All plastic surfaces were covered with filter paper to avoid oviposition on the chamber. In all trails, grape clusters (*Vitis vinifera* var. Tempranillo) of 6 ± 1 g, were treated as stated above before introduction in the oviposition chambers. In the no choice bioassays, one kaolin or one control treated grape cluster was placed inside each chamber. In the choice trail, two clusters, kaolin and control were added to each experimental arena. Oviposition was allowed during 48 hours on each set of grape clusters for three consecutive periods. The number of eggs laid on each grape cluster was recorded after removal of the cluster from the oviposition chamber. The grape clusters were thereafter maintained in standard conditions until egg hatch. The number of eggs hatched in each condition was recorded, yet after their incubation period of 5 days. Twenty-five replicates were evaluated in the choice bioassays, whereas twenty and thirteen replicates of control and kaolin treated, respectively, were evaluated in the no choice bioassays.

Effect of Kaolin on *L. botrana* Neonate Larvae Mortality. Five neonate larvae, less than 24 hours old were placed in the centre of each replicate. The arenas consisted of one 9 cm ∅ by 3 cm tall cylindrical plastic box containing two layers of filter paper lining the bottom. Five disks, 1 cm ∅ by 3 mm tall, of semi-synthetic laboratory diet (Del Tío 1996) were added in a circle 2 cm from the edge of each arena. Every container was topped with a lid containing one 2 cm ∅ filter paper covered respiration aperture. All experimental arenas were treated with the kaolin solution or carrier using the Potter tower as described above. The percentage of surviving individuals was recorded 72 hours after the beginning of the trail. Fifteen replicates of each, treatment and control, were evaluated in the statistical analysis.

Effects of Kaolin on the Parasitism of *E. kuehniella* Eggs and *T. cacoeciae* Progeny Emergence. One *T. cacoeciae* female less than 48 hours old was employed in each replicate. The experimental arenas consisted of one 6.5 cm long, 4.5 cm wide, and 2.5 cm tall, rectangular, transparent plastic box. The lids contained one 2 cm ∅ ventilation hole covered with filter paper. On the inner side of the lid two 5 µl drops of honey were placed at opposite edges of the ventilation hole to provide nutrients to the female. Groups of 20 *E. kuehniella* eggs previously sterilized with UV light for 1.5 hours were glued to 1 by 1 cm yellow cards using tragacanth gum adhesive. These alternative host eggs were sterilized to prevent their hatching during the experiment. The yellow cards with eggs were treated with kaolin in the Potter tower and set to dry before introduction into the experimental arena. The choice bioassays employed two cards, one of each condition, whereas the no choice experiments contained one card with one of the two conditions, kaolin treated or control. Cards containing the host eggs were replaced every 24 hours over four consecutive periods. These egg groups were isolated at standard conditions after removing from the oviposition chambers until parasitoid emergence. At the end of the ten day developmental period parasitism and parasitoid emergence was recorded. In the choice bioassays, 27 replications were evaluated. In the no choice bioassays, 40 replications of each treatment were assessed. Due to the fact that emergence data is implicitly unpaired in these bioassays, parasitized replicates from both the choice and no choice bioassays were used in combination to analyse the effect of kaolin on parasitoid offspring survival.

Effects of Kaolin on the Parasitism of *L. botrana* Eggs and *T. cacoeciae* Progeny Emergence. In this set of bioassays the number of females used, all conditions, and arena materials were the same as those in the evaluation of parasitism and parasitoid progeny survival from the *E. kuehniella* host, except the eggs of the *L. botrana* host had been previously laid on plastic substrate. Groups of approximately 20 *L. botrana* eggs were used on their respective sections of their oviposition chambers after sterilization with UV light for 1.5 hours. During four consecutive 24 hour periods, the *L. botrana* egg groups were replaced and those of the previous days isolated until parasitoid emergence. In the choice bioassay two groups of *L. botrana* eggs with their respective treatments were added to the experimental arenas. The percentages of parasitism and parasitoid emergence were recorded after the 10 day parasitoid developmental period. Twenty replications of both conditions in both the choice and no choice bioassays were carried out.

Statistical Analyses. All statistical analyses were performed with Statgraphics (t Test, Statgraphics 2010). The t-Student Test was used at a 95.0% confidence interval for all bioassays. Abbott´s formula for the correction of mortality data was used when necessary (Abbot 1925).

Results

Effects of kaolin on Oviposition and Egg Hatch of *L. botrana*. Females laid less eggs on all kaolin treated surfaces markedly illustrated in the choice bioassays with grape (Tables 1 and 2). In the bioassays with synthetic substrate, when females had a choice, they laid 11.6% less eggs on the kaolin treated surface. Whereas, in the no choice trials, kaolin reduced the number of eggs laid on the kaolin treated surface by 49.4%. In contrast, in the choice trial on grape, females laid 83.6% less eggs due to kaolin´s presence. In the no choice trial on grape kaolin reduced the number of eggs laid on the kaolin treated surface by 93.8%.

Along with lower oviposition, higher egg mortality was also observed in the kaolin treatment in all bioassays (Table 3). A 21.7 and 46.8% reduction in the percentage of eggs hatch due to the kaolin treatments in the synthetic substrate and in grape, respectively, was observed.

Effects of Kaolin on *L. botrana* Neonate Larvae Survival. The average percentage of neonate larvae mortality in the kaolin treatment was 78.7%, compared to 37.1% in the control. There was a significant difference between neonate larvae mortality rates in this bioassay ($t = -5.24$ and P value < 0.001). This difference was illustrated by the Abbot corrected mortality in the treatment of 66.1%.

Effects of kaolin on *T. cacoeciae* parasitism of *E. kuehniella* and *L. botrana* and parasitoid offspring emergence. No significant difference between treatments was found when the parasitism and emergence data sets were evaluated. This held true for both the choice and no choice bioassays involving the two hosts, *E. kuehniella* and *L. botrana* (Tables 4 and 5).

Discussion

For many decades this inert dust, kaolin, has been known to affect arthropods. Excessive time spent grooming (Alexander 1944), and or disruption of movement and feeding (Glenn et al. 2005, Barker et al. 2006) has been suggested to have contributed to lower oviposition rates. This was the case with the black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Homoptera: Aphididae) (Cottrell et al. 2002). Thus, it was logical that *L. botrana* oviposition rate was lower in the kaolin treatments in all four experiments of choice and no choice on both synthetic substrate and grape.

Lower oviposition in our berry laboratory experiments could be a result of the interference of visual cues (Villanueva and Walgenbach 2007) being that the grapes were white as a consequence of the kaolin coating. The masking of natural attractive fruit volatiles when covered with the kaolin solution could have also contributed to our laboratory bioassay outcome. That was the case with the codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in apple and pear (Unruh et al. 2000). The inhibition of oviposition may have also been influenced by disruption of egg anchorage to the kaolin covered surface (Marchal 1912) as with the pear psyllid (Pasqualini et al. 2002).

Lower oviposition with the obliquebanded leafroller, *Choristoneura rosaceana*, (Harris) (Lepidoptera: Tortricidae) within kaolin treated plots in semi-field bioassays with apple seedlings was also found (Knight et al. 2000), however, no significant difference in embryo survival was observed. The difference may lay in the dissimilarity in the surface area to volume ratio in the isolated eggs of *L. botrana*, as opposed to the egg clusters of similar species. Therefore, the eggs should have had a relatively high percentage of chorion in contact with the kaolin treated surface. Kaolin is known to be particularly absorbent, thus influences moisture levels on treated surfaces. It has also been suggested that kaolin physically affects cuticle lipids resulting in eventual dehydration (Korunic 1998). The absorbent quality and the high percent of chorion in contact with the kaolin treated surface could have contributed to the egg hatch results in our bioassays.

As with our finding, lower nymph density within kaolin treated plots was found with the potato psyllid, *Bactericera cockerelli* (Homoptera: Psyllidae) (Peng et al. 2011). The population dynamics of larvae and oviposition of major lepidopteran pests of cotton have been affected by kaolin treatments (Alavo et al. 2010). Larvae of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), were also affected when feeding on kaolin treated leaf surfaces (Showler 2003). The effects on the larvae demonstrated in our bioassay could have attributed to dehydration as the proposed effect of abrasion of the arthropod cuticle (Puterka et al. 2000) and illustrated in *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) larvae (Díaz et al. 2002).

Lack of side effects to many parasitoid species due to kaolin has been established in a variety of crops, as in our laboratory trial. However, altered species composition of generalist predator assemblages along with lower relative abundance of parasitoids in kaolin treated apple orchard plots was encountered (Sackett et al. 2007). Yet, in those same kaolin treated plots the proportion of parasitized obliquebanded leafroller, *C. rosaceana*, controlled by various different parasitoid species was not demonstrated to be perturbed. Thus, the alteration in assemblage could have been a result of lower host species density.

On the other hand, some authors have purposed an interruption of the ability of the parasitoid to recognize host species due to kaolin treatments. The parasitoid *Psyttalia concolor* Szepligeti (Hymenoptera: Braconidae) for example, did not parasitize kaolin treated *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) larvae in laboratory trials (Adán et al. 2007).

The inability to recognise hosts was also found in citrus field studies with two other hymenopteran parasitoids, *Aphytis melinus* (DeBach) (Hymenoptera: Aphelinidae), and *Comperiella bifasciata* (Howard) (Hymenoptera: Encyrtidae) (Rill et al. 2008). In light of previous studies the lack of inhibitory effects on *T. cacoeciae* parasitism of, and parasitoid emergence from *L. botrana* was encouraging to find.

The present results indicate the potential of kaolin particle film as a rational component to be incorporated in the control of *L. botrana* including its combination with *T. cacoeciae*. The potential benefits and lack of negative ecological impacts associated with the use of kaolin not only rest in its effect on the two arthropod species examined in our laboratory bioassays. Kaolin's interaction with the crop to be protected and other innate advantages which come with the compound include, but are surely not limited to, the reduction of heat stress (Shellie and Glenn 2008) or apparent ability to improve yield (Arthurs et al. 2008) and fruit, the lack of toxicity and phytoxicity at recommended concentrations, long duration to protect treated crops, low probability of resistance development due to its physical, instead of toxic mode of action, increased grape cultivar carrying capacity under some conditions (Wand et al 2006), and its ability to protect and even enhance the germination of some microbial pesticides after their application onto the plant surface (Eigenbrode et al. 2006, Glen et al. 2015). Even with all these positive aspects there are some limitations, such as, some risk to certain natural enemies, previously described, and the limited persistence on plant with heavy rains. Consequently, kaolin with its lack of detrimental impacts detected on *T. cacoeciae* in laboratory bioassays and all its benefits, could lead to another rational, sustainable and non-toxic management option for the control of *L. botrana*.

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Table 1. Percentage of *L. botrana* eggs (Means ± SE) laid on synthetic substrate treated with kaolin in choice bioassay, and number of laid egg in no choice bioassay.

Choice		No Choice	
Control	Kaolin	Control	Kaolin
55.8 ± 2.2	44.2 ± 2.2	29.4 ± 4.1	14.9 ± 2.4
t = 2.74; P < 0.001		t = 3.20; P < 0.001	

The t-Student test was employed in all bioassays (α=0.05)

Table 2. Percentage of *L. botrana* eggs (Means ± SE) laid on grape berry treated with kaolin in choice bioassay, and number of laid egg in no choice bioassay.

Choice		No Choice	
Control	Kaolin	Control	Kaolin
91.8 ± 1.6	8.2 ± 1.6	9.7 ± 1.8	0.6 ± 0.2
t = 26.50; P < 0.001		t = 4.78; P < 0.001	

The t-Student test was employed in all bioassays (α=0.05)

Table 3. Percentage of *L. botrana* egg hatch (Mean + SE) on synthetic substrate and grape berry, with and without kaolin.

Synthetic Substrate		Grape	
Control	Kaolin	Control	Kaolin
78.0 ± 2.2	56.3 ± 2.3	87.2 ± 1.9	40.4 ± 7.5
t = 6.81; P < 0.001		t = 8.26; P < 0.0001	

The t-Student test was employed in all bioassays (α=0.05)

Table 4. *T. cacoeciae* parasitism and emergence percentages (Means ± SE) from *E. kuehniella* eggs treated with kaolin in choice and no choice bioassays.

Parasitism Choice		Parasitism No Choice		Parasitoid Emergence	
Control	Kaolin	Control	Kaolin	Control	Kaolin
17.8 ± 2.4	22.1 ± 2.8	49.3 ± 2.7	45.7 ± 2.9	96.8 ± 1.3	99.3 ± 0.3
t = -1.16; P = 0.25		t = 0.93; P = 0.35		t = -1.82; P = 0.07	

The t-Student test was employed in all bioassays (α=0.05)

Table 5. ***T. cacoeciae*** **parasitism and emergence percentages (Means ±SE) from** ***L. botrana*** **eggs treated with kaolin in choice and no choice bioassays.**

Parasitism Choice		Parasitism No Choice		Parasitoid Emergence	
Control	Kaolin	Control	Kaolin	Control	Kaolin
16.1 ± 2.2	14.5 ± 1.9	33.3 ± 2.3	34.4 ± 2.7	55.6 ± 3.5	47.2 ± 3.2
t = 0.44; P = 0.66		t = -0.32; P = 0.75		t = -1.85; P = 0.07	

The t-Student test was employed in all bioassays (α=0.05)