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ORIGINAL PAPER

Efficacy and mode of action of kaolin and its interaction with bunch-zone leaf removal against *Lobesia botrana* on grapevines

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Abstract

Lobesia botrana control strategies in vineyards aim to develop environmentally safe tools as an alternative to synthetic insecticides. The activity of kaolin on *L. botrana* performance was studied in laboratory and field bioassays. The efficacy of kaolin and *Bacillus thuringiensis* (Bt) against the moth, with or without bunch-zone leaf removal (LR), was compared in four trials carried out in vineyards in north-eastern Italy. In the laboratory bioassays, kaolin berry coverage reduced the egg-laying preference of *L. botrana* by 53% and decreased female survival and fecundity by 22 and 82%, respectively. Kaolin egg coverage reduced the hatching rate by 14%. The larval settlement preference for berries covered with kaolin was reduced by 72%, but larval survival and development were not affected. In the field bioassay, kaolin reduced the egg-laying preference by 84%. In the field trials, kaolin, Bt and LR reduced *L. botrana* infestation significantly. Although Bt was more effective than kaolin, the efficacy of the two products was similar when combined with LR. Based on the results obtained and its effectiveness also against grapevine leafhoppers, kaolin can play an important role in the context of integrated pest management in vineyards.

Keywords Bunch-zone leaf removal · European grapevine moth · Natural product · Cultural control · Particle film · *Bacillus thuringiensis*

Key Message

- Alternatives to synthetic insecticides against *L. botrana* in vineyards are desirable.
- The hypothesis that kaolin applications reduce *L. botrana* performance and infestation and have a positive interaction with bunch-zone leaf removal (LR) was tested.
- In laboratory bioassays, kaolin reduced egg laying, hatching and larval settlement.
- In a field bioassay, kaolin reduced egg laying.
- Both kaolin and LR reduced moth infestation and, when combined, were as effective as *Bacillus thuringiensis*.

Introduction

The European grapevine moth *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is the most important carpophagous pest in European vineyards and has recently spread to the Nearctic region (Ioriatti et al. 2012). Dependent on the different grape-growing areas and years, the moth may complete two to four generations per year (Martín-Vertedor et al. 2010; Pavan et al. 2013). The larvae from the second generation are carpophagous and can cause yield losses and favour the spread of grey mould (*Botrytis cinerea* Person: Fries) (Fermaud and Giboulot 1992; Pavan et al. 1998, 2014a; Moschos 2006).

The control of *L. botrana* is typically achieved with synthetic insecticides, but concern about environment and health problems, and the insect's resistance to some active ingredients (Civolani et al. 2014; Pavan et al. 2014b) are leading to alternative approaches for the control of this pest (Lucchi and Benelli 2018).

In the context of integrated pest management (IPM), the main environmentally safe tools against *L. botrana* are mating disruption (Ioriatti and Lucchi 2016) and *Bacillus*

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thuringiensis Berliner (Ifoulis and Savopoulou-Soultani 2004). However, mating disruption cannot be as effective when the infestation potential is high or vineyards are small, irregularly shaped or windy (Ioriatti et al. 2011). Moreover, additional costs to control other carpophagous tortricids [e.g. *Eupoecilia ambiguella* (Hübner)], leafhoppers or scales may be necessary. On the other hand, *B. thuringiensis* does not usually meet the needs of farmers due to its lower persistence, which requires two applications per generation, and lower efficacy in comparison with synthetic insecticides (Boselli et al. 2000).

To reduce *L. botrana* infestation and damage, cultivar choice (Fermaud 1998; Moreau et al. 2006, 2008; Pavan et al. 2009, 2018; Sharon et al. 2009) and cultural practices (Vartholomaiou et al. 2008; Pavan et al. 2016) should also be considered. In particular, bunch-zone leaf removal affects larval population levels by increasing the mortality of eggs and newly hatched larvae due to the high temperatures reached by sun-exposed bunches (Pavan et al. 2016; Kiaeian Moosavi et al. 2017, 2018).

Kaolin particle film technology has been widely studied as a tool for arthropod pest control in orchards and field crops. Under laboratory and field conditions kaolin has been demonstrated to be effective in the control of several phytophagous insects and mites (Puterka et al. 2000; Bostanian and Racette 2008; Lo Verde et al. 2011; Nateghi et al. 2013; Silva and Ramalho 2013). Kaolin on plants may prevent pests from identifying a host, and pest activity can also be impaired by the kaolin particles that stick to their bodies (Glenn et al. 1999; Vincent et al. 2003). For these reasons, oviposition and feeding deterrence can occur, with this last effect associated with a lower survival rate and a longer developmental time (Glenn et al. 1999; Barker et al. 2006; Lapointe et al. 2006; Tacoli et al. 2017a, b). Kaolin can also exert a direct toxicity towards motile forms (Bostanian and Racette 2008).

To the best of our knowledge, the effect of kaolin on *L. botrana* has only been studied under laboratory conditions where a reduction in egg laying, hatching and larval survival has been shown to occur (Pease et al. 2016). Concerning other tortricids, kaolin reduced egg laying and larval settlement of *Cydia pomonella* (L.) in the laboratory as well as infestations of *C. pomonella* and *Grapholita molesta* (Busck) in apple and peach orchards, respectively (Unruh et al. 2000; Knight et al. 2001; Lalancette et al. 2005; Bostanian and Racette 2008; Markó et al. 2008). Moreover, kaolin affected field populations of some apple leafrollers (Knight et al. 2001; Lalancette et al. 2005; Markó et al. 2008) for which negative effects on egg laying and larval feeding in the laboratory have also been reported (Knight et al. 2000; Cadogan and Scharbach 2005; Sackett et al. 2005).

In the present study, the efficacy and the mode of action of kaolin against *L. botrana* were tested, extending the scope of the previous laboratory study by Pease et al. (2016). These authors showed a negative effect of kaolin on egg hatching for eggs laid on kaolin-covered berries, but no data were collected for eggs covered with kaolin as it can occur under field conditions. The same authors showed an increase in the mortality of larvae covered with kaolin, which would be a very unlikely event under field conditions, while the influence of kaolin coverage of berries on larval settlement and performance was not evaluated. The aim of this study was to complete knowledge of the activity of kaolin in the laboratory and to assess for the first time the influence of kaolin on larval infestations in field trials. In addition, the possible positive interaction between kaolin and bunch-zone leaf removal was evaluated.

Materials and methods

Lobesia botrana **mass rearing**

Lobesia botrana individuals used in the laboratory and field bioassays were derived from mass rearing of the moth conducted in a climatic chamber at 24 ± 1 °C, 70 ± 5% RH and a 16:8 (L:D) daily light cycle. Larvae were fed on an artificial diet (Rapagnani et al. 1990), and females laid eggs on transparent polyethylene (PE) bags (30 cm × 15 cm). The rearing originated from larvae collected in a north-eastern Italian vineyard (Corona di Mariano del Friuli, Gorizia district, 45°55′30″N, 13°29′44″E, 40 m a.s.l., cultivar Pinot Gris) located in the same grape-growing area as the vineyards used for the field trials.

Laboratory bioassays

In the laboratory bioassays, berries of the cultivars Pinot Gris or Italia (BBCH phenological growth stages 75 and 89, respectively, Lorenz et al. 1995) from organic vineyards were used. Before the bioassays the berries were washed in a 4% methanol–water solution to prevent fungus infections on the berry surface. All bioassays were carried out at the same climatic conditions reported above for *L. botrana* mass rearing.

Kaolin Surround WP (Tessenderlo Kerley Inc., Phoenix, AZ, USA) at the rate of a 2% (W/V) water suspension was used in all bioassays. The product was applied on single berries with a hand-sprayer until runoff, in order to simulate what occurs when the product is distributed in a 1000 L/ha volume of water with a field sprayer.

Influence of kaolin on egg laying

A two-choice bioassay was carried out to assess the influence of kaolin coverage of berries on *L. botrana* egg-laying preferences. For this purpose, 1-day-old adults from the rearing were placed in PE bags (30 cm × 15 cm) to mate for 48 h and then berries sprayed with kaolin (kaolin-on-berries) or water (control) were offered to the females as follows. Four rubber rings (1.5 cm external diameter × 1 cm internal diameter × 0.5 cm height) were fixed on the borders of the lids of glass Petri dishes (8 cm diameter) at the same distance from each other. The lids and rings were covered by tulle, and after spraying, two berries per treatment were arranged in alternate positions on the rings. The lids with berries were placed in transparent polystyrene boxes (16 cm × 9 cm × 8 cm; Caubere, Yebles, France) lined with black felt and closed at the top with tulle and covered (Maher and Thiéry 2004). Felt and tulle were used to deter oviposition on any surface other than the berries. Finally, two females were released into each box. The eggs laid in the kaolin-on-berries, and control treatments were counted after 5 days under a dissecting microscope. The bioassay was replicated eight times.

A no-choice bioassay was carried out to evaluate the influence of kaolin on *L. botrana* fecundity. For this purpose, 1-day-old adults from the rearing were placed in PE bags (30 cm × 15 cm) to mate for 48 h. Then, females were individually confined for a further 24 h inside glass tubes (3 cm diameter × 10 cm height) and only those that had laid eggs were considered for the bioassay. The same transparent polystyrene boxes and glass Petri lids with rubber rings were used as described above. One female was released into each box, which contained four berries all sprayed with either kaolin (kaolin-on-berries) or water (control). Females were randomly subdivided between the two treatments. At 24-h intervals, the eggs laid by each female were counted under a dissecting microscope and all berries replaced until female death. To determine the fecundity of females, the eggs that had been laid inside the tubes before the start of the bioassay were also counted. The bioassay was replicated 14 times for both treatments.

Influence of kaolin on egg hatching

The influence of kaolin on the *L. botrana* egg-hatching rate was evaluated in a bioassay that examined eggs laid on berries not covered with kaolin (control), eggs laid on berries covered with kaolin (kaolin-on-berries) and eggs covered with kaolin after being laid on berries (kaolin-on-eggs-and-berries). For the control and kaolin-on-berries treatments, berries with eggs from the previous no-choice bioassay (see previous paragraph) were used. For the kaolin-on-eggs-and-berries, a number of control berries from the previous no-choice bioassay were sprayed with kaolin within a maximum of 24 h after eggs being laid. Within the three groups being compared, there were 127, 366 and 377 eggs, respectively. Berries were placed into transparent polystyrene boxes (5 cm diameter × 1.8 cm height; Caubere, Yebles, France) and were checked after 10 days for egg hatching under a dissecting microscope.

Influence of kaolin on larval settlement

The occurrence of a feeding-deterrent effect from berries covered with kaolin on newly hatched *L. botrana* larvae was evaluated in a two-choice bioassay. For this purpose, four berries, two sprayed with kaolin (kaolin) and two with water (control), were placed at the corners of transparent polystyrene boxes (9 cm × 6 cm × 1.8 cm; Caubere, Yebles, France) with two eggs at the black-head stage of development placed in the middle of the box. To guarantee air exchange and avoid excessive relative humidity inside each box, a lid with a tulle-covered breathe hole (2.5 cm diameter) was used. Each box was checked after 18 h under a dissecting microscope to see which berries the newly hatched larvae had settled on. This bioassay was replicated 40 times.

Influence of kaolin on larval survival and development

The occurrence of any lethal or sub-lethal effect when *L. botrana* larvae fed on kaolin-covered berries was evaluated in a bioassay. Transparent polystyrene boxes (5 cm diameter × 1.8 cm height; Caubere, Yebles, France), provided with breathe holes in the lid (described in the previous paragraph), were used. One black-head-stage egg laid on a PE bag was placed with two berries in each box. Half of the boxes contained kaolin-treated berries (kaolin), and the other half contained water-treated berries (control). The boxes were checked daily for larval development, without opening them so to avoid any external interference. The presence of excrement around the larval entrance hole into the berry was considered a valid signal of larval feeding activity. The boxes were opened only after pupation or when traces of larval activity were not observed anymore. To evaluate larval performance the following parameters were considered: (1) larval survival, (2) larval development time, (3) size of fifth-instar larvae and (4) pupal size. To estimate larval size, after pupation, the fifth-instar head capsules were mounted on slides in Berlese's liquid and the length of the left mandible was measured under a Zeiss Axioplan microscope at 400 × magnification (Pavan et al. 2013). Pupal weight was measured with a precision balance (Sartorius CP2P: capacity 2.1 g; readability 0.001 mg). In order to attribute all measurements to males or females separately, the sex of each individual was established at the pupal stage under a dissecting microscope (Galet 1982). This bioassay was replicated 40 times per treatment.

Field bioassay on egg-laying preference

The influence of kaolin on *L. botrana* egg-laying preference was evaluated under field conditions in a two-choice bioassay.

The bioassay was carried out in late August 2016 (BBCH 89) in a 10-year-old vineyard (Bicinicco, Udine district, 45°55′59″N, 13°13′60″E, 35 m a.s.l., cultivar Chardonnay) with grapevines growing using the Guyot training system and with distances between and along rows of 2.5 and 0.8 m, respectively. In the vineyard, a standard fungicide programme was followed and no insecticides active against *L. botrana* were applied during the growing season. Buprofezin (Applaud Plus, Sipcam, Milano, Italy) was applied on 17 June for the control of the leafhopper *Scaphoideus titanus* Ball. Shoots holding two bunches of similar size and not in contact with each other were chosen. Bunches were also checked for the absence of *L. botrana* eggs. One bunch of each shoot was sprayed with kaolin using a hand-sprayer until runoff, in order to simulate what occurs when distributing product in a 1000 L/ha volume of water with a field sprayer. The other bunch (control) was protected from the spray by covering it in a PE bag until the kaolin suspension had dried. Then, each shoot was trimmed and inserted into a transparent tulle cage (15 cm diameter × 25 cm length) inside which four 1-day-old mated females were released. After 5 days, the cages were removed and bunches harvested. In the laboratory, all berries were checked under a dissecting microscope to count the eggs. The bioassay was replicated 20 times.

Field trials

During 2015–2017, the influence of kaolin and bunch-zone leaf removal on larval infestation of the *L. botrana* second generation was evaluated in four trials carried out in as many vineyards (A, B, C, D) located in north-eastern Italy (Table 1). In the vineyards, standard fungicide programmes were followed and no insecticides were applied during the growing season.

In all vineyards, kaolin (Surround WP, 2% W/V) and *B. thuringiensis* (Dipel DF, Sumitomo Chemical Agro Europe S.A.S, Saint Didier au Mont d’Or, FR, 1% W/V, Dipel DF/water) were compared with an untreated control. Each product was applied at the occurrence of defined *L. botrana* phenological stages as expected on the basis of male flight monitored with pheromone traps (Traptest, Isagro, Milano, Italy) and average air temperatures (Rapagnani et al. 1988, 1989). Kaolin was applied two or three times per year starting from the beginning of egg laying (18 and 24 June 2015; 10, 24 June and 4 July in 2016; 19, 22 June and 3 July in 2017). The number of applications varied depending on the need to ensure satisfactory berry coverage up to the end of egg hatching. *B. thuringiensis* was applied twice, at the beginning of egg hatching and then a week later (24 June and 1 July in 2015; 27 June and 4 July in 2016; 22 June and 3 July in 2017). The products were applied using a backpack sprayer (Oleo-Mac, Sp-126, Emak S.p.A., Bagnolo in Piano, RE, Italy in 2015 and 2016, and M1200, Cifarelli s.p.a., Voghera, PV, Italy in 2017) at a rate of 1000 L/ha.

In all trials, a randomized block design with four replicates was adopted. Each block (row) was divided into three plots of 28 (vineyard A) or 20 (vineyard B) or 24 (vineyards C and D) grapevines. In order to test the efficacy of kaolin and *B. thuringiensis* combined with bunch-zone leaf removal, plots of all trials were divided into two subplots of 14 (vineyard A) or 10 (vineyard B) or 12 grapevines (vineyards C and D), which were subjected or not to manual removal of all leaves covering the bunches (17 June 2015, 10 June 2016 and 19 June 2017).

The infestation of *L. botrana* was estimated at about 40 days from the beginning of the second flight. In each subplot, 100 bunches were sampled on 10 (vineyard A, C and D) or 8 (vineyard B) grapevines, excluding edge plants. The sampling was based on an a priori scheme (Pavan et al. 1998) to avoid subjective choice of the sampled bunches.

Table 1 North-eastern Italian vineyards in which the efficacy of kaolin, *B. thuringiensis* and bunch-zone leaf removal against *L. botrana* was evaluated

Vineyard	Locality, district, geographical coordinates and altitude	Cultivar (vineyard age)	Row orientation, training system and distance between and along rows	Farming system	Trial year
A	Cormons, Gorizia, 45°57′51″N, 13°26′49″E, 56 m a.s.l.	Pinot Gris (10 years)	N70°E–S20°W, Guyot, 2.5 × 0.8	Conventional	2015
B	Cormons, Gorizia, 45°57′20″N, 13°26′50″E, 50 m a.s.l.	Pinot Gris (30 years)	N20°W–S70°E, double-arched Guyot, 2.8 × 1.0	Organic	2015
C	San Floriano del Collio, Gorizia, 45°58′02″N, 13°31′31″E, 53 m a.s.l.	Pinot Gris (15 years)	N25°W–S65°E, Guyot, 2.2 × 0.7	Organic	2016
D	Cormons, Gorizia, 45°56′32″N, 13°27′23″E, 44 m a.s.l.	Pinot Gris (10 years)	N25°W–S65°E, Guyot, 2.4 × 0.7	Organic	2017

On each bunch the number of larval nests was counted in the field.

Statistical analyses

A repeated *G* test of goodness of fit was used for the two-choice bioassays (number of laid eggs and settled larvae), and a two-sample *t* test was used for the no-choice bioassay (number of laid eggs). For proportions comparison, the Ryan test (rate of egg hatching) and Fisher's exact test (rate of survived larvae and male pupae) were used. A paired-sample *t* test was used for the comparison of eggs laid over time. A log-rank test was used to compare *L. botrana* female survival (Mantel 1966). Abbott's formula for correction of the egg mortality rate was used (Abbott 1925).

To compare field-trial data (number of larval nests), a three-way ANOVA with Bonferroni confidence interval adjustment and Tukey's post hoc test were used, considering treatment, bunch-zone leaf removal and vineyard as effects. Prior to analysis data normality was tested with the Shapiro–Wilk test, homogeneity was tested with Levene's variance test, the presence of outliers was assessed, and the data were $\log(x+1)$ transformed.

Statistical analyses were performed with GraphPad InStat version 3.1 for Macintosh (GraphPad software 2001) and IBM SPSS Statistics 20 (IBM Corporation 2011).

Results

Laboratory bioassays

Influence of kaolin on egg-laying preference, and female survival and fecundity

In the two-choice bioassay, *L. botrana* females laid significantly fewer eggs in the kaolin-on-berries than control treatment (Table 2). On average, the egg-laying preference on berries covered with kaolin was reduced by 53%.

In the no-choice bioassay, *L. botrana* females lived significantly fewer days (7.9 ± 0.9) in the kaolin-on-berries than control (10.1 ± 1.4) ($\chi^2 = 15.37$, $df = 1$, $P < 0.001$) with a 22% reduction in survival. The day before the beginning of the bioassay (T0), the mean (± SE) number of eggs laid inside the glass tubes was not significantly different between the two groups of females used in the kaolin-on-berries (29.2 ± 3.8 eggs/female) and control (26.0 ± 3.5 eggs/female) ($t = 0.62$, $df = 26$, $P = 0.54$). During the bioassay, females laid significantly fewer eggs in the kaolin-on-berries than control (Table 2). On average, kaolin reduced female fecundity by 82%. The egg-laying pattern over time was different between the kaolin-on-berries and control (Fig. 1). As a consequence of the different patterns, the number of eggs laid per female was significantly lower in the kaolin-on-berries than control from T1 [(T1), $t = 3.13$, $df = 26$, $P = 0.0043$; (T2–T4); $t = 4.58$, $df = 26$, $P = 0.0001$; (T5), $t = 3.81$, $df = 26$, $P = 0.0008$]. In the kaolin-on-berries, no females laid eggs from T6, whereas in the control some females laid eggs up to T8. With respect to T0, in the kaolin-on-berries, a significant decrease in the number of eggs laid per female was already observed at T1 ($t = 2.80$, $df = 13$, $P = 0.015$), whereas, in the control, the number of eggs significantly decreased only from T6 [$t = 2.44$, $df = 13$, $P = 0.03$].

Influence of kaolin on egg hatching and larval settlement

The *L. botrana* egg-hatching rate was significantly lower in the kaolin-on-eggs-and-berries than in both the kaolin-on-berries and control treatments (Table 2). The hatching of eggs covered with kaolin was reduced by around 14%.

In the two-choice bioassay, the number of newly hatched larvae settled was significantly lower in the kaolin than in the control treatment (Table 2). The settlement preference for berries covered with kaolin was reduced by 72%.

Influence of kaolin on larval survival and development

No differences in *L. botrana* larval survival were observed between the kaolin and control (Table 3). The percentage of male pupae was not different between the kaolin and control. None of the considered development parameters showed statistically significant differences between larvae reared on the

Table 2 Influence of kaolin on *L. botrana* in the laboratory bioassays

Parameters	Control	Kaolin-on-berries	Kaolin-on-eggs-and-berries	Statistical analyses
Eggs per female in the two-choice assay (mean ± SE)	67.3 ± 12.5	31.5 ± 3.9	–	$G = 105.9$, $df = 1$, $P < 0.0001$
Eggs per female in the no-choice assay (mean ± SE)	146.7 ± 20.7	26.1 ± 5.5	–	$t = 5.6$, $df = 26$, $P < 0.0001$
Egg-hatching rate (%)	99.5 b	98.4 b	85.8 a	Ryan test, $P < 0.05$
Larval settlement in the two-choice assay (%)	77.8	22.2	–	$G = 23.5$, $df = 1$, $P < 0.0001$

For egg hatching, different small letters indicate significant differences ($\alpha = 0.05$). Eggs per female in the no-choice assay were compared with a two-sample *t* test

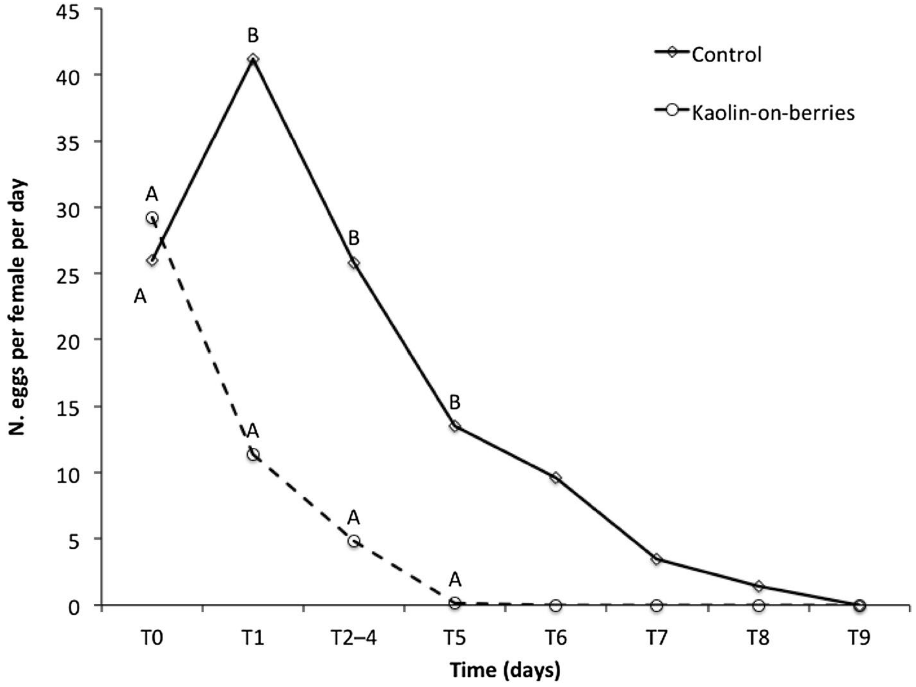

Fig. 1 No-choice laboratory bioassay. Eggs laid over time by *L. botrana* females placed on kaolin-on-berries or control from T1. T0 eggs previously laid on glass tubes by the same females. Different capital letters on the same day indicate significant differences according to a paired-sample *t* test ($\alpha = 0.01$)

Table 3 Influence of kaolin-on-berries on *L. botrana* larval survival and development parameters

Parameters	Kaolin		Control		Statistical results
	No. of individuals	% or mean ± SD	No. of individuals	% or mean ± SD	
Total larvae	40		40		
Survived larvae	29	27.5%	28	30.0%	Fisher's exact test, $P = 0.99$
Male pupae	15	51.7%	17	60.7%	Fisher's exact test, $P = 0.82$
Larval development time (days)					
Total individuals	29	28.2 ± 4.0	28	26.6 ± 4.3	$t = 1.38$, $df = 55$, $P = 0.17$
Females	14	28.1 ± 3.0	11	27.5 ± 3.3	$t = 0.49$, $df = 23$, $P = 0.63$
Males	15	28.3 ± 4.8	17	26.1 ± 5.0	$t = 1.24$, $df = 30$, $P = 0.22$
Larval mandible length (μm)*					
Total individuals	27	23.3 ± 3.3	25	24.1 ± 1.1	$t = 1.19$, $df = 50$, $P = 0.24$
Females	14	23.3 ± 4.5	10	24.9 ± 0.9	$t = 1.11$, $df = 22$, $P = 0.28$
Males	13	23.4 ± 1.3	15	23.7 ± 1.0	$t = 0.65$, $df = 26$, $P = 0.52$
Pupal weight (mg)					
Total individuals	29	8.1 ± 1.8	28	8.4 ± 2.0	$t = 0.66$, $df = 55$, $P = 0.51$
Females	14	9.5 ± 1.6	11	10.0 ± 1.9	$t = 0.76$, $df = 23$, $P = 0.45$
Males	15	6.8 ± 0.8	17	7.4 ± 1.4	$t = 1.45$, $df = 30$, $P = 0.16$

For the comparisons concerning development parameters, a two-sample t test was used

*The length was not measured on three larvae because the mandibles were found to be broken

kaolin or control berries. The results were not different if males were considered separately from females.

Field bioassay on egg laying

In the two-choice bioassay, *L. botrana* females laid significantly fewer eggs in the kaolin (6.7 ± 1.4, mean ± SE eggs per cage) than control (42.1 ± 10.9, mean ± SE eggs per cage) ($G = 344.12$, $df = 1$, $P < 0.0001$). On average, the

Table 4 Efficacy of different treatments and bunch-zone leaf removal on *L. botrana* in field trials

Source of variation	Larval nests		
	F	*df*	*P*
Corrected model	7.706	23, 72	< 0.0001
Treatment	37.460	2, 72	< 0.0001
Leaf removal	30.688	1, 72	< 0.0001
Vineyard	5.690	3, 72	0.001
Treatment * leaf removal	3.269	2, 72	0.044
Treatment * vineyard	4.343	6, 72	0.001
Vineyard * leaf removal	4.866	3, 72	0.004
Vineyard * treatment * leaf removal	1.227	6, 72	0.303

Results of three-way ANOVA performed on the number of larval nests of *L. botrana* recorded in four vineyards (A, B, C and D) on plots subjected to different treatments (kaolin, *B. thuringiensis* and control), with or without bunch-zone leaf removal

egg-laying preference on berries in bunches covered with kaolin was reduced by 84%.

Field trials

In the field trials against *L. botrana*, significant differences were observed for all the three effects, i.e. treatment, bunch-zone leaf removal and vineyard (Table 4; Fig. 2a, b). Among treatments, both kaolin and *B. thuringiensis* significantly reduced the number of *L. botrana* larval nests compared to control, but *B. thuringiensis* showed higher efficacy (around 60%) than kaolin (around 40%) ($P = 0.004$, Tukey's post hoc test) (Fig. 2a).

The interaction treatment * leaf removal was significant, indicating that bunch-zone leaf removal influenced the efficacy of treatments (Table 4). In particular, bunch-zone leaf removal significantly reduced the number of larval nests in the control and kaolin treatments, but not in the *B. thuringiensis* treatment (Fig. 2c). Also the interactions treatment * vineyard and leaf removal * vineyard were significant, indicating that the efficacy of kaolin, *B. thuringiensis* and bunch-zone leaf removal against *L. botrana* was influenced by the vineyard (Table 4).

Discussion

Effect of kaolin on female performance

In the two-choice bioassays, a deterrent effect of kaolin on *L. botrana* egg laying was observed in the laboratory in accordance with other studies on *L. botrana* (Pease et al. 2016) and *C. pomonella* (Unruh et al. 2000). In the present study, an analogous but even more marked effect was observed under field conditions.

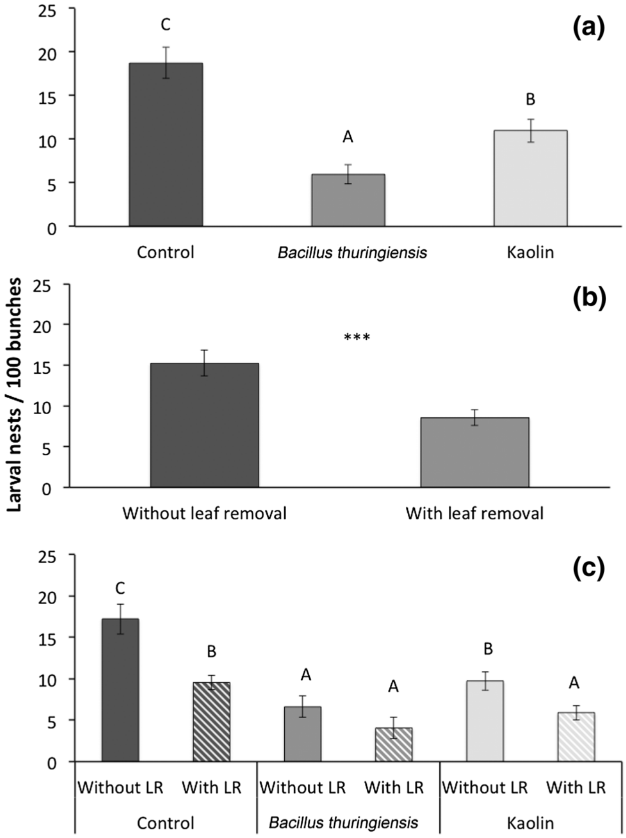

Fig. 2 Field trials on the effect of different treatments (**a**), bunch-zone leaf removal (LR) (**b**) and their interaction (**c**) on *L. botrana* infestations (mean ± SE of larval nests). Different capital letters above columns indicate significant differences according to Tukey's test ($\alpha = 0.01$), and *** indicates significant differences according to ANOVA ($\alpha = 0.0001$)

In the no-choice bioassay, kaolin reduced both survival and fecundity of *L. botrana* females. Because the number of eggs laid per female had already decreased when all females were alive, it could be argued that both egg laying and survival reductions are consequences of stress induced in the females by the presence of kaolin. The negative effect of kaolin on egg laying was recorded as early as the first day that berries covered with kaolin were offered. For *L. botrana* a reduction in oviposition in no-choice bioassays has been reported previously, but female fecundity was not considered (Pease et al. 2016). Other studies on tortricid moths have also evidenced an adverse effect of kaolin on oviposition in no-choice bioassays (Knight et al. 2000; Unruh et al. 2000; Cadogan and Scharbach 2005). Kaolin could impair *L. botrana* ovipositional behaviour by making the host unrecognizable (Glenn et al. 1999; Glenn and Puterka 2005). In agreement with these authors, the change in colour of kaolin-coated berries could affect the moth's visual cues and the physical barrier presented by the kaolin could also alter the insect's tactile and chemical perception of the host. Regarding this, it is known that *L. botrana* females lay eggs on several types of substrates (i.e. fruits of different colour and species, glass marbles and plastic surfaces that have the common feature of being smooth) (Stavridis and Savopoulou-Soultani 1998; Maher and Thiéry 2004; Pavan et al. 2009; Kiaeian Moosavi et al. 2017; Markheiser et al. 2018). Therefore, kaolin could hinder egg laying by changing the berry surface from smooth to dusty and irregular. Kaolin powder could also stick to the chemo- and mechanoreceptors of the tarsi and the ovipositor, disturbing the ability of females to recognize the substrate as suitable for egg laying. In agreement with this hypothesis, when blueberry fruits covered with kaolin were encountered by the braconid *Diachasma alloeum* (Muesebeck), it no longer responded to synomones released by fruits infested by *Rhagoletis mendax* Curran eggs (Stelinski et al. 2006).

Effect of kaolin on egg hatching, and larval behaviour and performance

Pease et al. (2016) observed a strong reduction in the *L. botrana* egg-hatching rate (Abbott-corrected mortality 68%) for eggs laid on kaolin-covered berries. In the present study, this effect was not confirmed (Abbott-corrected mortality 1%) and it was only when the eggs were directly covered with the product that a slight increase in mortality was observed (Abbott-corrected mortality 14%). According to Pease et al. (2016), kaolin contact toxicity may be involved in egg mortality due to absorption of epicuticular lipids (Ebeling 1971) and subsequent egg dehydration. As a consequence, the higher rate of kaolin suspension used in this earlier study (4 vs. 2% here) could have led to the differences recorded in egg mortality. Further, no reduction in the hatching of eggs laid on kaolin-treated leaves has been reported for *C. pomonella*, *C. rosaceana* or *Choristoneura fumiferana* (Clemens) (Knight et al. 2000; Unruh et al. 2000; Cadogan and Scharbach 2005). However, unlike our study, in the case of *C. pomonella* there was also no reduction in hatching when eggs of this species were directly sprayed with kaolin (Unruh et al. 2000).

In the two-choice bioassay on the settlement of newly hatched *L. botrana* larvae, berries covered with kaolin were less preferred. This result is in accordance with a study on *C. pomonella* (Unruh et al. 2000) that highlighted a reduction in larval entry into the fruit due to lower walking speed and fruit discovery rate, and a study on *C. rosaceana* where kaolin negatively affected the larval dispersal behaviour (Sackett et al. 2005). The negative effect of kaolin on *L. botrana* larval settlement is likely to be due to disruptions in orientation caused by the physical barrier covering the berries (Glenn et al. 1999; Vincent et al. 2003), or a feeding-deterrent effect, as reported for sap-feeding pests (Puterka et al. 2005; Sánchez-Ramos 2014; Tacoli et al. 2017a, b). In agreement with these considerations, the presence of kaolin on the berry surface in the field may increase the wandering time of newly hatched *L. botrana* larvae. A delay in penetrating berries by larvae is supported by the longer average development time recorded in the laboratory for larvae on kaolin-covered berries. An increase in wandering time could also increase the risk of mortality due to predation and exposure to adverse environmental conditions.

When *L. botrana* larvae were reared on kaolin-covered berries, survival and development were not affected. In the previous study on *L. botrana*, a high mortality rate was recorded 72 h after kaolin was directly sprayed on newly hatched larvae (Pease et al. 2016). However, under field conditions this effect is negligible because of the very low probability of hitting larvae with the kaolin spray, considering the prolonged egg-hatching period and the short time spent by the newly hatched larvae outside the berries before penetrating them. In contrast to our results, an increase in mortality was observed for *C. rosaceana* larvae reared on kaolin-treated leaves (Knight et al. 2000; Sackett et al. 2005). However, kaolin did not substantially influence mortality or development of *C. rosaceana* larvae when the clay was mixed with artificial diet, suggesting that its negative effect only occurs when kaolin constitutes a physical barrier to feeding (Sackett et al. 2005). Therefore, we may suppose that the effect of kaolin on survival of *C. rosaceana* and *L. botrana* larvae varies in relation to their different feeding behaviours. The larvae of *C. rosaceana*, feeding on leaves, are continuously subjected to the kaolin barrier throughout their development, whereas the feeding deterrence of kaolin for *L. botrana* larvae occurs only when they enter berries.

Influence of kaolin and leaf removal on larval infestation in vineyards

In the study vineyards, kaolin reduced the infestation of *L. botrana* with good efficacy, but it was significantly lower than following *B. thuringiensis* treatment. The efficacy level of kaolin for *L. botrana* in vineyards is in accordance with reports for *C. pomonella* in apple orchards (Unruh et al. 2000; Knight et al. 2001; Markó et al. 2008). Bunch-zone leaf removal was also able to reduce *L. botrana* infestation, as already reported (Pavan et al. 2016). Although kaolin was less effective than *B. thuringiensis* in *L. botrana* control, the differences in efficacy between the two products were no longer significant when combined with bunch-zone leaf removal, suggesting a more positive interaction of this cultural practice with kaolin than with *B. thuringiensis*. Moreover, kaolin could be preferable to *B. thuringiensis* because of its ability to also control cicadellids (Wood and McBride 2001; Puterka et al. 2003; Tubajika et al. 2007; Tacoli et al. 2017a, b). Furthermore, kaolin may provide more consistent results than *B. thuringiensis* due to it not being degraded by

UV radiation and having a high resistance to washing off by rain (Hostetter et al. 1975; Tacoli et al. 2017b). Additionally, when associated with bunch-zone leaf removal, kaolin compensates the possible disadvantages of this cultural practice by reducing sunburn damage of exposed berries and grapevine water stress, without having a negative effect on grape yield and grape qualitative parameters (Glenn et al. 2010; Coniberti et al. 2013; Brillante et al. 2016).

Kaolin could be of profitable for use in IPM strategies in vineyards because of its ability to control *L. botrana* and other grapevine pests and because of its positive interaction with bunch-zone leaf removal.

Further studies should be carried out on the possible positive interaction between kaolin, which inhibits *L. botrana* egg laying, and *B. thuringiensis,* which exerts larvicidal activity, also taking into account the likelihood that kaolin might increase the persistence of *B. thuringiensis* by mitigating the effect of UV radiation, as suggested by the literature (Glenn 2016).

Author contribution statement

FT, EC, PZ, FP conceived and designed the research. FT and FKM conducted the bioassays. FT, EC, PZ, PF conducted field trials. FT and PF analysed the data. All authors wrote, read and approved the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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