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Interference of tritrophic (grape × medfly × parasitoid) interactions by mineral and biomaterial films

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Abstract

Fruit fly infestation is one of the main obstacles to the exportation of fresh agricultural produce. Films of mineral particles and biomaterials have the potential to protect fruits against tephritid fruit fly infestation. The present study evaluated the effects of particle films on the tritrophic interactions of grape (*Vitis vinifera* L.), the fruit fly *Ceratitis capitata* (Wiedemann) and the parasitoid *Diachasmimorpha longicaudata* (Ashmead) under semi-field conditions. Grapes were biometrically characterised (i.e. colour, firmness, mass, length and diameter), treated with mineral particles, biomaterials or distilled water (control), and then used in oviposition and parasitism bioassays. In the oviposition bioassay, the treated grapes were exposed to 50 *C. capitata* pairs in field cages, and after 48 h, the punctures and eggs on each fruit were counted. In the parasitism bioassay, treated grapes were artificially infested with third-instar *C. capitata* larvae (two per fruit), exposed (2 h) to 50 *D. longicaudata* pairs in field cages to determine parasitism index, larval and pupal viabilities and number of flies and parasitoids emerged. Treatment with the mineral film affected fruit colour and reduced *C. capitata* oviposition but failed to significantly affect the parasitism capacity of *D. longicaudata*. The ability of the parasitoid to locate and parasitise *C. capitata* larvae in kaolin-coated fruits suggests that kaolin films could be used in conjunction with biological agents to control fruit flies.

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), is a major quarantine pest across the globe (Silva *et al.*, 2011). In Brazil alone, management costs added to the production and commercialisation losses due to damage by fruit flies are estimated to be around US$120 million per year (Mendes, 2015). Chemical control is the most frequently used management strategy, mainly using insecticides as cover sprays or toxic bait formulations (Baronio *et al.*, 2019). However, the frequent use of insecticides can result in the selection of resistant populations (Kakani *et al.*, 2010; Arouri *et al.*, 2015) and unacceptable chemical residues on fruits. Thus, evaluating alternative fruit fly management strategies is greatly needed (Dias *et al.*, 2018).

Particle film technology is an alternative to conventional insecticides for controlling infestation by *C. capitata* (Palma *et al.*, 2020). It is especially promising for organic fruit production (Sharma *et al.*, 2020) because it neither contaminates the environment nor leaves toxic residues in treated products (D'aquino *et al.*, 2011; Lo Verde *et al.*, 2011). Particle film technology relies on kaolin's properties (Glenn and Puterka, 2005), a mineral mainly composed of aluminium silicate that, when suspended in water, rapidly forms a chemically inert and non-expanding solution with white colour and porous texture (Puterka *et al.*, 2000). Abrasive mineral particles, such as kaolin, change the colour of host plants, thereby repelling pests and disrupting their feeding and oviposition (Showler, 2002). For example, applying mineral particle films with different kaolins to guava and grape significantly reduced the oviposition of fruit fly pests *Anastrepha obliqua* (Macquart, 1835) and *C. capitata* under laboratory conditions (Costa *et al.*, 2021; Da Costa *et al.*, 2021).

Most studies that assess the efficacy of particle films with kaolin focus on bitrophic interactions (Mazor and Erez, 2004; Lemoyne *et al.*, 2008; Leskey *et al.*, 2010; D'aquino *et al.*, 2011, 2021; Yee, 2012), and the extent to which the films affect natural enemies, such as predators and parasitoids, remains poorly understood. However, detailed knowledge of kaolin's lethal and sublethal effects on non-pest arthropods is needed before the mineral can be used in integrated pest management programmes. For example, Bengochea *et al.* (2014) assessed the lethal and non-lethal effects of kaolin on olive trees, the fruit fly *Bactrocera oleae* (Rossi, 1970), and the parasitoid *Psyttalia concolor* (Szèpligeti, 1910).

When searching for hosts, female parasitoids respond to chemical, visual, and mechanical stimuli (Vinson, 1976; Segura *et al.*, 2007; Sharma *et al.*, 2019). When applied to crops, kaolin particle films form a protective barrier that creates a hostile environment for insects, makes plants visually or tactually unrecognisable, and prevents the oviposition of pest insects (Glenn *et al.*, 1999; Bürgel *et al.*, 2005), and may affect the behaviour of predators and parasitoids (Vincent *et al.*, 2003). We hypothesise that films of mineral particles and biomaterials can change the physical characteristics of grapes, and such changes will reduce oviposition by *C. capitata* and the parasitism of *C. capitata* larvae by *D. longicaudata*. This work aimed to assess the effects of mineral particles and biomaterial films on the tritrophic interactions of grape, the fruit fly *C. capitata* and the natural enemy *D. longicaudata*.

Material and methods

Insect rearing

The Mediterranean fruit fly used in our study was obtained from a colony maintained at the Fruit Fly Laboratory of the Universidade Estadual do Sudoeste da Bahia on the campus of Vitória da Conquista, Bahia, Brazil. Routine colony procedures included maintaining adults in wooden cages (50 × 45 × 40 cm) with two sides lined with voile fabric, one inclined for oviposition and the other for insect manipulation. Eggs laid on the side of the cage were collected daily, cleaned, transferred and maintained in plastic containers (500 ml) containing an artificial diet adapted from Zucoloto (1987) for larval development and pupation (~10 days). Pupae were collected, placed in plastic containers (500 ml) with vermiculite, and maintained until the emergence of adults. Emerged adults were then transferred to cages for mating and oviposition with free access to water and an artificial diet (3 sugar:1 yeast extract; Silva Neto *et al.*, 2012). The cages were maintained in a climatised room at 25 ± 2 °C, 70% relative humidity and 12L:12D photoperiod.

The *D. longicaudata* colony used in our study was established from parasitised *C. capitata* pupae obtained from the Laboratório de Entomolgia da Embrapa Mandiocultura Fruticultura Tropical (Embrapa/CNPMF). The parasitoid colony was maintained following the methodology of Carvalho *et al.* (1998). Adult parasitoids were kept in acrylic cages (30 × 30 × 30 cm) with free access to water and an artificial diet made of distilled water, honey, agar-agar, ascorbic acid and nipagin (Carvalho and Nascimento, 2002). Third instar *C. capitata* were offered to adult wasp oviposition in ‘parasitism units’, which included 100 *C. capitata* larvae packed in organza fabric and placed on top of the acrylic cages containing parasitoids. Parasitism units were periodically exposed for 1 h to 5-day-old parasitoids. After this period, the larvae were removed from the parasitism units and maintained on a layer of fine vermiculite in plastic containers (500 ml) for pupation and, subsequently, adult emergence.

The grapes characterisation

The grape (*Vitis vinifera* L. ‘Italia’) used in the bioassays were obtained from fresh fruit markets and, later, selected for uniformity of maturation, size and lack of punctures by fruit flies. The biometrical and physical characteristics of the grapes (i.e. mass, length, diameter and colour) were measured before conducting the bioassays. Both colour and firmness, the latter of which requires destructive sampling, were also measured at 24 h after the initiation of the bioassays. Grape mass was determined using an analytical balance (AUY 220, Shimadzu) with a precision of 0.1 mg. The diameter and length of the grapes were measured using a digital pachymeter (Model MPD-200; Metrotools, São Paulo, Brazil) with a precision of ± 0.02 mm. Fruit firmness for each grape ($n = 20$) was determined after the treatment application using a penetrometer (model WA68; TR, Italy) equipped with an 8 mm-diameter tip, and results were expressed in Newton (N). Luminosity (L^*), a^* and b^* (from white to black, green to red and blue to yellow, respectively) colour coordinates were determined using a Minolta colorimeter CR 400 (Minolta, Osaka, Japan). The colour of the grapes was measured twice, before and after the treatment, always in the same position (opposite sides), using four fruits per treatment. The chroma, $C^* = (a^2 + b^2)^{1/2}$, and the hue angle, arc tangent of (b/a), were also calculated (Lemoyne *et al.*, 2008).

Oviposition bioassay

The oviposition experiments were performed in a completely randomised design with eight treatments and four repetitions. A total of 12 grape per treatment were used, which were distributed across four replicates over 3 days ($N = 96$). The treatments included Surround WP (NovaSource, Phoenix, AZ, USA), kaolin 605 white (BrasilMinas, Guarulhos, SP, Brazil), kaolin 607 cream (BrasilMinas), kaolin 608 white (BrasilMinas), kaolin 611 grey (BrasilMinas), cassava starch, potato starch and control (distilled water). The particles were dispersed in distilled water (200 g l^{-1}) with guar gum (~5 g l^{-1}) to improve formulation viscosity and stability (Campos *et al.*, 2015; Gao *et al.*, 2020; Costa *et al.*, 2021; Da Costa *et al.*, 2021). The kaolin and biomaterial concentrations used in our treatments were based on previous studies (Costa *et al.*, 2021; Da Costa *et al.*, 2021). The biomaterial particles, cassava and potato starches, were obtained from a natural products market in Indianapolis (SP, Brazil). Before starting the bioassays, the grapes were sanitised for 30 min in sodium hypochlorite (0.5%) and then individually immersed for 10 s in a beaker that contained 60 ml of the corresponding treatment solution. After immersion, the grapes were dried at 25 ± 2 °C for 1 h.

The plot comprised a field cage (2 × 2 × 2 m), supported by a metal structure covered by a nylon fabric, containing a potted *Spondias tuberosa* L. plant with ~1.20 m height and radius canopy around 30 cm. Eight treated grapes, corresponding to one grape per treatment, were hung on top of the field cage, spaced 33 cm apart, and then exposed to 50 pairs of 7-day-old *C. capitata* for 48 h. After exposure, each grape was dissected to count the total number of punctures with eggs, punctures without eggs and eggs. During the bioassay, the cage conditions were maintained at a temperature of 27.08 ± 1.5 °C (min and max of 13.6 and 37.4 °C, respectively), relative humidity of 51.6 ± 5.85 (min and max of 29.8 and 78.8%, respectively) and luminosity of 19.894 lux.

Parasitism bioassay

The parasitism of *D. longicaudata* on *C. capitata* larvae was evaluated using choice tests in a completely randomised design with eight treatments and four repetitions. A total of 12 grapes per

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treatment were used, which were distributed across four replicates over 3 days ($N = 96$). The treatments were the same as those used in the oviposition bioassay. Before starting the bioassays, the grapes were sanitised for 30 min in sodium hypochlorite (0.5%) and then individually immersed for 10 s in the suspensions. After drying at room temperature, the treated grapes were artificially infested with *C. capitata* larvae using a methodology adapted from Pires *et al.* (2021). Briefly, the grapes were perforated to a depth of 1.5 cm using a 1.5 mm-diameter needle, and any pulp residue formed during the penetration was removed to prevent orifice obstruction. Two third-instar *C. capitata* larvae were then inserted into the orifice of each grape using a fine-tipped brush tool, and the orifice was closed using a small cotton ball. After 1 h, the grapes were finally exposed to the parasitoids.

Similar to the oviposition bioassay, the parasitism bioassays were performed in field cages (2 × 2 × 2 m), each containing a potted plant. For each bioassay, eight artificially infested grapes were treated with particle suspensions or water and arranged as previously described for the oviposition bioassay. Then, 50 pairs of 5-day-old *D. longicaudata* were released into the field cage. The grapes were removed after 2 h of parasitoid exposure. In the lab, the larvae exposed to the parasitoids were removed from the grapes and kept in plastic containers containing a vermiculite layer until adult emergence. The numbers of emerging parasitoids and flies, larval viability (VL% = no. parasitoid pupae × 100/total fly larvae), pupal viability (VP% = no. emerged parasitoids + no. emerged flies × 100/total fly pupae) and parasitism index (IP% = no. emerged parasitoids × 100/no. emerged flies thinsp;+ no. emerged parasitoids) were determined (Matrangolo *et al.*, 1998).

The bioassays were performed at a temperature of 22 ± 1.5 °C (min and max of 17.1 and 33.9 °C, respectively), relative humidity of 51 ± 8.5% (min and max of 51 and 80%, respectively) and luminosity of 13.586 lux measured during set up of the bioassay (8:00 am).

Statistical analyses

The homoscedasticity and normality of the data for the biometrical and physical characteristics of the grapes and oviposition of *C. capitata* and *D. longicaudata* were evaluated using Bartlett and Shapiro–Wilk tests, respectively. Datasets that violated these assumptions (e.g. the number of punctures with and without eggs, number of eggs and number of parasitoids and flies) were square root-transformed and, subsequently, analysed using a generalised linear model (GLM). The GLMs were established using the *nlme* (Pinheiro *et al.*, 2020) and *lsmeans* (Lenth, 2016) packages in R. Paired *t*-tests were used to compare the mean values of pre- and post-treatment L*, C* and hue angle. All analyses were performed using R software (version 3.6.1; R Development Core Team, 2019).

Results

Effect on grapes characteristics

The grapes used for the treatment groups exhibited no significant differences regarding mass ($F = 0.22303$; df = 7.31; $P = 0.97605$), length ($F = 0.6665$; df = 7.31; $P = 0.70095$), diameter ($F = 0.20034$; df = 7.31; $P = 0.9823$), L* ($F = 1.0555$; df = 7.31; $P = 0.42077$), C* ($F = 1.1042$; df = 7.31; $P = 0.39$) and hue angle ($F = 0.5303$; df = 7.31; $P = 0.80286$; Table 1).

The immersion of grapes in mineral and biomaterial suspensions affected both L* ($t = -11{,}795$; df = 31; $P < 0.0001$) and C* ($t = 7.9406$; df = 31; $P < 0.0001$), and the different immersion treatments resulted in significantly different L* ($F = 1258.1$; df = 7.31; $P < 0.0001$), C* ($F = 183.69$; df = 7.31; $P < 0.0001$) and hue angle ($F = 188.71$; df = 7.31; $P < 0.0001$; Table 2). Kaolin and starch treatments increased grape luminosity, with the highest values observed in grapes treated with Surround WP and kaolin 605 and the lowest for control grapes. In contrast, C* values were consistently decreased by immersion in the suspensions, and the hue angle was lower in the Surround WP- and kaolin 605-treated grapes than in the control group. All the mineral films and starches increased fruit firmness ($F = 28.554$; df = 7.31; $P < 0.0001$).

Effect on *C. capitata* oviposition

Treatment had no effect on the number of punctures without eggs (AIC = 20.63; df = 31) but did significantly affect the number of punctures with eggs (AIC = 29.58; df = 31) and the number of eggs (AIC = 94.31; df = 31; Table 3). Briefly, both kaolin and cassava starch reduced the number of egg punctures, with fewer eggs in fruits treated with Surround WP, kaolin 605 and kaolin 608. In contrast, the treatment with potato starch yielded the highest mean egg number (3.18 ± 0.46).

Table 1. The biometrical and physical characteristics of the grapes before application of the suspensions

Treatments	Weight (g)	Length (mm)	Diameter (mm)	Luminosity (L*)	Chroma (C*)	Hue
Kaolin Surround® WP	11.86 ± 0.47 a	28.16 ± 1.03 a	25.35 ± 1.03 a	36.50 ± 0.88 a	8.18 ± 0.81a	113.20 ± 3.16 a
Kaolin 605 white	11.61 ± 1.11 a	28.12 ± 1.27 a	25.38 ± 1.21 a	36.48 ± 0.20 a	8.57 ± 0.13a	111.21 ± 1.86 a
Kaolin 607 cream	11.54 ± 0.97 a	28.83 ± 1.97 a	25.44 ± 0.67 a	36.91 ± 0.75 a	8.75 ± 0.70 a	114.03 ± 1.97 a
Kaolin 608 white	11.49 ± 1.09 a	29.35 ± 0.97 a	27.71 ± 2.01 a	36.59 ± 0.56 a	8.48 ± 0.33 a	113.63 ± 1.71a
Kaolin 611 grey	11.32 ± 0.45 a	28.30 ± 0.72 a	24.99 ± 0.95 a	36.88 ± 0.58 a	8.97 ± 0.38 a	115.42 ± 3.40 a
Cassava starch	11.48 ± 0.75 a	28.66 ± 1.10 a	25.10 ± 1.34 a	37.26 ± 0.20 a	8.60 ± 0.88 a	113.13 ± 5.91a
Potato starch	11.18 ± 0.86 a	28.12 ± 0.41 a	25.03 ± 0.56 a	37.09 ± 0.4 a	8.38 ± 0.41a	112.73 ± 4.62 a
Distilled water (control)	11.69 ± 1.14 a	28.26 ± 0.30 a	25.40 ± 0.83 a	37.57 ± 0.53 a	9.08 ± 0.46 a	114.24 ± 1.67 a
Coefficient of variation (%)	7.76	3.83	4.59	2.04	6.58	2.97

Means in the same column followed by the same letter do not differ significantly (Tukey test, $P \leq 0.05$).

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Table 2. The physical characteristics of the after application of the suspensions

Treatments	Luminosity (L*)	Chroma (C*)	Hue	Firmness (N)
Kaolin Surround® WP	87.98 ± 1.72 a	1.28 ± 0.27 d	22.47 ± 2.09 d	6.43 ± 0.16 ab
Kaolin 605 white	87.06 ± 0.84 ab	1.47 ± 0.23 d	67.07 ± 9.39 c	6.20 ± 0.11b
Kaolin 607 cream	85.57 ± 0.61 b	9.48 ± 0.31 a	147.90 ± 3.46 a	6.47 ± 0.09 ab
Kaolin 608 white	77.11 ± 1.10 c	2.27 ± 0.31 cd	113.46 ± 11.17 b	6.82 ± 0.19 a
Kaolin 611 grey	76.89 ± 0.51 c	5.46 ± 0.16 b	136.12 ± 4.48 a	6.42 ± 0.41 ab
Cassava starch	71.81 ± 0.58 d	2.75 ± 0.07 c	119.13 ± 3.89 b	6.33 ± 0.20 ab
Potato starch	56.36 ± 1.21 e	5.29 ± 1.05 b	116.05 ± 3.24 b	6.04 ± 0.27 b
Distilled water (control)	37.32 ± 0.74 f	8.55 ± 0.53 a	112.87 ± 1.93 b	5.05 ± 0.07 c
Coefficient of variation (%)	1.37	10.23	5.66	3.44

Means in the same column followed by different letters differ significantly (Tukey test, $P \leq 0.05$).

Effect on D. longicaudata *parasitism*

One hundred and fifty-seven (157) of the 172 puparia yielded adult insects (69 fruit flies, 88 parasitoids), with larval and pupal viabilities of 89.6 and 91.3%, respectively. The parasitism index was 56%, ranging from 30% in the potato starch treatment to 69.6% in the control. Treatments did not affect the numbers of parasitoids (AIC = 42.35, df = 31) or flies (AIC = 35.78; df = 31; Table 4).

Discussion

The evaluation of the grapes before their use in the bioassays indicated excellent fruit uniformity, preventing the potential influence of fruit characteristics on the results, as suggested by Da Costa *et al.* (2021). Applying mineral and biomaterial films to the grapes did not affect the number of punctures without eggs, confirming the laboratory-based findings of Da Costa *et al.* (2021). The mechanical resistance provided by the films might have discouraged flies from ovipositing in the fruit after puncturing it. However, films should ideally inhibit both oviposition and fruit puncturing since puncture injuries in some fruits (e.g. apples) can facilitate the entry of fungi and bacteria (Santos *et al.*, 2008).

The kaolin and cassava starch treatments reduced the number of eggs, with fewer eggs laid in grapes treated with Surround WP, kaolin 605 and kaolin 608, corroborating previous findings (Costa *et al.*, 2021; Da Costa *et al.*, 2021). In previously reported laboratory studies, kaolin reduced fruit fly oviposition in bitrophic interactions of grape × *C. capitata* (Da Costa *et al.*, 2021) and guava (*Psidium guajava* L.) × *A. obliqua* (Costa *et al.*, 2021), and the number of punctures in apple (*Malus domestica* L.) × *C. capitata* (Leskey *et al.*, 2010; Ourique *et al.*, 2017), mango (*Mangifera indica* L.) × *C. capitata* (Ourique *et al.*, 2017) and citrus × *C. capitata* (D'aquino *et al.*, 2011). Kaolin has also been reported to reduce fruit fly oviposition in field studies of citrus × *C. capitata* (Lo Verde *et al.*, 2011), apple × *Rhagoletis pomonella* (Walsh) (Villanueva and Walgenbach, 2007), cactus (*Opuntia ficus-indica* 'Gialla') × *C. capitata* (D'aquino *et al.*, 2021) and the number of punctures in citrus × *C. capitata* (Braham *et al.*, 2007). In contrast, the biomaterials failed to protect the fruits from oviposition, and the potato starch treatment yielded the highest mean egg number, appearing to stimulate oviposition. These findings agreed with previous laboratory-based studies (Da Costa *et al.*, 2021). However, it is important to note that potato starch was reported to preserve guava peel colour and to protect guava fruits from oviposition by *A. obliqua* (Costa *et al.*, 2021).

The reduced oviposition of *C. capitata* in kaolin-coated grapes was likely due to changes in fruit colour and firmness. More specifically, it is possible that the effects of the white mineral particles on the grape peels' natural green colour interfered with host identification by *C. capitata* females. Indeed, some studies have demonstrated that fruits or spheres coated with white substances experience reduced fruit fly oviposition (Cytrynowicz *et al.*, 1982; Katsoyannos *et al.*, 1986; López-Guillén *et al.*, 2009; Costa *et al.*, 2021; Da Costa *et al.*, 2021). The high reflectance of white surfaces is visually less attractive to fruit flies, as demonstrated in *C. capitata* (Nakagawa *et al.*, 1978; Katsoyannos *et al.*, 1986), *Bactrocera dorsalis* (Hendel, 1912) (Wu *et al.*, 2007) and *A. obliqua* (López-Guillén *et al.*, 2009). In addition, the films formed a physical barrier that affected fruit firmness. The epicarp of some fruits provides natural resistance that prevents some species of flies with short aculeus from puncturing and laying eggs (Aluja and Mangan, 2008). For instance, *C. capitata* preferred to oviposit in fruits with an advanced maturation stage and, consequently, lower firmness (Gómez *et al.*, 2019). Mineral particles also roughen the fruit's surface, making it inadequate for oviposition (Saour and Makee, 2004). According to Salerno *et al.* (2020), the kaolin particle film when applied to natural and artificial substrates, it reduces the insect's ability to attach.

The effect of mineral particles and biomaterial films on grape colour did not affect the parasitism capacity of *D. longicaudata*. As such, parasitoid females could locate *C. capitata* larvae in all treatments (Table 4). Messing and Jang (1992) reported that *D. longicaudata* females responded to fewer visual stimuli than males since olfactory stimuli (e.g. larvae kairomones) play a more critical role in host localisation (Carrasco *et al.*, 2005). Benelli and Canale (2012) reported that naive *P. concolor* females show no colour preferences. These findings agree with those of Bengochea *et al.* (2010), who investigated the effectiveness of kaolin against *B. oleae* in olive groves and its effect on the parasitoid *P. concolor*, indicating that the parasitism capacity of *P. concolor* was unaffected by kaolin treatment. Additional laboratory and semi-field studies have also reported that kaolin is harmless to the fruit fly parasitoid *P. concolor* (Adán *et al.*, 2007; Bengochea *et al.*, 2010). According to Bengochea *et al.* (2010), using kaolin in olive crops is promising because it affects beneficial arthropods to a lesser extent than other commonly used compounds, such as dimethoate. However, these findings also contradict those of

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Table 3. Parameter estimates for the number of punctures with and without eggs, and the number of eggs (mean ± SE) laid by *C. capitata* in grapes exposed to *D. longicaudata* in field cage conditions

Treatments	Standard error	*Z*-value	*P*-value	Number of punctures with eggs	Standard error	*Z*-value	*P*-value	Number of punctures without eggs	Standard error	*Z*-value	*P*-value	Number of eggs
Intercept	0.17	8.95	0.00***	-	0.15	4.0	0.00***	-	0.46	5.19	0.00***	-
Kaolin Surround® WP	0.24	−5.9	0.00***	0.08 ± 0.17 a	0.21	−2.8	0.01**	0.0 ± 0.15 c	0.65	−3.13	0.01**	0.35 ± 0.46 c
Kaolin 605 white	0.24	−6.3	0.00***	0 ± 0.17 a	0.21	−2.8	0.01**	0.0 ± 15 c	0.65	−3.67	0.01**	0.0 ± 0.46 c
Kaolin 607 cream	0.24	−4.9	0.00***	0.33 ± 0.18 a	0.21	−2.8	0.01**	0.0 ± 15 c	0.65	−2.02	0.05*	1.07 ± 0.46 bc
Kaolin 608 white	0.24	−6.3	0.00***	0 ± 0.18 a	0.21	−2.8	0.01**	0.0 ± 15 c	0.65	−3.67	0.01**	0.0 ± 0.46 c
Kaolin 611 grey	0.24	−5.6	0.00***	0.17 ± 0.17 a	0.21	−1.6	0.12	0.0 ± 15 c	0.65	−2.49	0.01*	0.77 ± 0.46 bc
Cassava starch	0.24	−3.9	0.00***	0.59 ± 0.17 a	0.21	−1.6	0.12	0.25 ± 15 bc	0.65	−0.89	0.38	1.81 ± 0.46 abc
Potato starch	0.24	−1.0	0.31	1.3 ± 0.17 a	0.21	−1.2	0.24	0.33 ± 15 ab	0.65	1.22	0.24	3.18 ± 0.46 a
Distilled water (control)	-	-	-	1.5 ± 0.17 a	0.21	-	-	0.58 ± 15 a	0.65	-		2.39 ± 0.46 b
AIC				29.58				20.63				94.31

Means in the same column followed by different letters differ significantly (Tukey test, $P \leq 0.05$).

Table 4. Poisson generalised linear model parameter estimates for the numbers of parasitoids and flies (mean ± SE), and the parasitism index in grapes exposed to *D. longicaudata* in field cage conditions

Treatments	Standard error	*Z*-value	*P*-value	Parasitoids	Standard error	*Z*-value	*P*-value	Flies	Parasitism index (%)
Intercept	0.58	2.31	0.021	-	0.38	1.53	0.13	-	-
Kaolin Surround® WP	0.74	−0.68	0.499	0.83 ± 0.46 a	0.60	0.42	0.68	0.84 ± 0.46 a	49.9
Kaolin 605 white	0.76	−0.43	0.665	1.00 ± 0.50 a	0.50	−0.34	0.73	0.42 ± 0.32 a	70.6
Kaolin 607 cream	0.74	−0.68	0.499	0.83 ± 0.46 a	0.60	0.42	0.68	0.84 ± 0.46 a	49.9
Kaolin 608 white	0.76	−0.43	0.665	1.00 ± 0.50 a	0.54	−0.01	0.99	0.59 ± 0.38 a	63.2
Kaolin 611 grey	0.76	−0.21	0.837	1.17 ± 0.54 a	0.52	−0.16	0.87	0.50 ± 0.35 a	70.0
Cassava starch	0.71	−0.94	0.346	0.67 ± 0.41 a	0.60	0.42	0.68	0.83 ± 0.46 a	44.4
Potato starch	0.68	−1.23	0.220	0.50 ± 0.35 a	0.66	0.88	0.38	1.17 ± 0.54 a	30.0
Distilled water (control)	-	-	-	1.33 ± 0.58 a	-	-	-	0.59 ± 0.38 a	69.6
AIC				42.35				35.78	

Means in the same column followed by the same letter do not differ significantly (Tukey test, $P \leq 0.05$).

Bengochea *et al.* (2014), who reported that kaolin treatment reduced the rate of parasitism by *P. concolor*.

Together, our findings support the conclusion that even though mineral films do not entirely prevent damage by fruit flies, they do not interfere with the parasitism of *C. capitata* by *D. longicaudata*. Thus, mineral film application may be considered in integrated pest management schemes with a high potential to be successful. The ability of the parasitoid to locate and parasitise *C. capitata* larvae in kaolin-coated fruits suggests that kaolin films could be used in conjunction with biological agents to control fruit fly pests in organic agriculture operations. Nevertheless, new studies conducted under commercial conditions are needed to support our recommendations fully.

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