Bulletin of Insectology ***74** (2): 221-227, 2021*
ISSN 1721-8861 eISSN 2283-0332

Laboratory evaluation of the effects of different concentrations of calcined kaolin applied on orange fruits on the behaviour of *Ceratitis capitata*

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae), is one of the most devastating agricultural pests worldwide. The kaolin particle film has been demonstrated to effectively control *C. capitata* on several crops although several studies have proved that kaolin treatments may alter the agroecosystem entomofauna, and specifically in citrus crops. The aim of this work was to study, under laboratory conditions, the efficacy of different kaolin particle film concentrations (1.5%, 2.5%, 3%, 5% and 6% w/v) applied on orange fruits cv "Valencia Late" to optimize its use against *C. capitata* in citrus groves. We assessed the effect of different kaolin particle film concentrations on the fruit acceptance, repellence, behavioural response, and efficacy fruit infestation. Through all the specific approaches, the 2.5% kaolin treatments applied in two layers successfully reduced the fruit infestation by *C. capitata*. This processed kaolin treatment should offer efficacy in real field conditions as satisfactory as conventional pesticides towards high populations.

Key words: kaolin, *Ceratitis capitata*, medfly, repellence, behaviour, oviposition-deterrence.

Introduction

Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae), is one of the most injurious fruit pests in citrus worldwide, attacking more than 350 different plant species (McQuate and Liquido, 2017). More than 30% of fruits in Spain would be attacked in non-treated orchards (Primo, 2004). In Spanish citrus this pest may causes great losses, mainly in early-season cultivars (Martinez-Ferrer *et al*., 2012).

Currently in Mediterranean citrus crops, organophosphates, pyrethroids, and Spinosad, mixed with protein baits are applied in terrestrial treatments to control medfly populations (Martinez-Ferrer *et al*., 2012). Sterile insect technique, biological control with parasitoids, the mass trapping technique or the combination of several of these methods are being developed (Navarro-Llopis *et al*., 2004; Martínez-Ferrer *et al*., 2012). There is a high risk of pesticide residues on fruit at harvest since *C. capitata* attacks citrus fruits when mature, therefore very close to harvest (Chueca *et al*., 2013). Pesticide treatments during that period can have significant negative effects on the entomofauna of citrus agroecosystem, which can result in secondary outbreaks of other pests (Raga and Sato, 2011), such as Tetranychidae mites (Gerson and Cohen, 1989), cottony cushion scale (Grafton-Cardwell and Gu, 2003). Up to eight sprays can be performed in a grove to control medfly in a season (Chueca *et al*., 2007). However, performing chemical treatments does not normally guarantee that fruit losses will be avoided.

The kaolin particle film is a highly refined kaolinic mineral (kaolinite) Al_2 [$(OH)_2$ Si_2O_5], white, chemically inert and hydrophilic (Glenn *et al*., 1999). Kaolin has been reported as effective against several arthropod pests of different orders and among them it has been proven to control effectively *C. capitata* on peach, persimmon, apple and *Citrus* spp. (Mazor and Erez, 2004; Braham *et al*., 2007). Due to its low toxicity and low environmental impact, it is considered an alternative to synthetic insecticides (Mazor and Erez, 2004; Braham *et al*., 2007; Caleca *et al*., 2010; Lo Verde *et al*., 2011) and one of the few products to control several key pests in organic agriculture where synthetic insecticides are not allowed. In addition, this particle film technology has the advantage that is unlikely to select resistance due to its physical mode of action (Glenn and Puterka, 2005). It also improves plant health by protecting plants from sunburst and heat stress in fruit trees (Glenn *et al*., 2002). When used as a pesticide, kaolin is sprayed as a water suspension on crops, where it forms a particle barrier film that interferes during the host plant location and acceptance process by the insect (Lemoyne *et al*., 2008). Several studies have proved that kaolin treatments may alter the agroecosystem entomofauna in several crops (Knight *et al*., 2001; Markó *et al*., 2008; Pascual *et al*., 2010) and specifically in citrus kaolin use has been related to an increase of *Aonidiella aurantii* (Maskell) (Hemiptera Diaspididae) populations (Grafton-Cardwell *et al*., 2014).

The aim of this work is to study, under laboratory conditions, the efficacy of different kaolin particle film concentrations applied on citrus fruits to optimize its use against *C. capitata* in citrus groves. We assessed the effect of kaolin applications on the fruit acceptance, repellence, behavioural response and efficacy on reducing larvae production.

Materials and methods

Three different laboratory experiments were carried out with mature orange fruits *Citrus sinensis* (cv. Valencia Late) and the kaolin-based product Surround® WP Crop

Protectant (BASF, Barcelona, Spain) (Kaolin 95%). Insects used in all experiments were gravid medfly females from a mass rearing colony at IRTA-Amposta (Tarragona, Spain) reared as described by Albajes and Santiago-Alvarez (1980), maintained at a 18:6 (L:D) photoperiod, 25 ± 1 °C and 60-70% RH. After the emergence, adults were kept for 10 days in similar male:female ratio to ensure mating. Thereafter females were kept in groups of 10 for 10 days inside laboratory cages to become gravid. Adults were supplied continuously with water and sugar:yeast hydrolysate (3:1). All experiments were conducted in laboratory conditions at 22 ± 1 °C, 60 ± 5% RH, and a 16L:8D photoperiod. Six treatments were performed, five with kaolin at 1.5%, 2.5%, 3%, 5%, 6% w/v and one with water (control). Each fruit was rinsed with distilled water and then allowed to dry for 2 hours. After that, each fruit was dipped in a suspension of kaolin in the different concentrations or water for five seconds and left to dry. A second layer of kaolin was applied. Kaolin concentrations and dipped methodology were chosen following Lemoyne *et al*. (2008), Caleca *et al*. (2010), D'Aquino *et al*. (2011), Yee (2012).

In all the experiments, fruits were supported on a 10 × 2 cm plastic ring and adult flies were fed with sugar:yeast hydrolysate (3:1) and water. Prior to testing insects did not had access to host fruits. No adults died during the experiments.

Colour parameters of fruits treated or not with kaolin

Four measurements around the equatorial plane of the fruit and one on the stylar end area of five fruits per treatment were conducted. We measured the L, a and b Hunter lab parameters of the colour system, using a Chroma Meter CR-400 (Minolta, Osaka, Japan). The luminance (L) goes from maximum that is 100 (which would be a perfect reflecting diffuser) to the minimum that would be zero. The other two parameters, a and b, are the chromaticity dimensions: positive a is red and negative a is green. Positive b is yellow and negative b is blue. Hue angle (in degrees) was obtained by the arctangent (b/a) in radians. The saturation of the colour, chroma, was calculated using the square root ($a^2 + b^2$).

Acceptance and repellence experiments

Laboratory choice test for determining first landing (acceptance) were conducted placing six mature oranges, one per treatment, inside a plastic cage (32 × 40 × 30 cm) with a mesh-cover window (24 × 18 cm) on the top. A single gravid female was released inside the cage and the first fruit visited by each fly was recorded. Once the female had landed on a fruit and stayed for 5 seconds, it was removed and replaced by another female. Each female was given 10 minutes to visit some fruit. Eighty-seven observations were conducted.

Laboratory choice test for determining the preference fruits for a long stay (repellence) were carried out with the same experimental setup as the previous assay. Thirty gravid females were released into the cage. We recorded the number of females on each fruit every 15 minutes during 6 hours in four replicates, rotating the position of fruits inside the cage to eliminate any bias. Ninety-six observations were conducted.

Behaviour experiment

One gravid medfly female was released on a citrus fruit inside an experimental arena that consisted of a glass beaker (⌀ = 18 cm; h = 25 cm) turned upside down. During 20 minutes, all behavioural events performed by the insect were recorded. Eight categories were used to define the behaviour of the females. A description of these behaviours is given here: 1) walking: the female moves along the fruit surface by making long or short steps, 2) grooming: self-preening by the insect, which includes cleaning of the antennae, wings, and legs, 3) boring: the female drills the ovipositor inside the fruit, 4) resting: a sedentary phase, during which the insect remains motionless, 5) turning: a shift in direction of the female, 6) landing on fruit: the female arrived on the fruit by flight, 7) landing on glass or ground: the female arrived on the glass or ground by flight, and 8) dragging: the female walked with its ovipositor in contact with the fruit or the vial.

Later, with Audacity(R) recording and editing software (Audacity Team, 2020) we measured the duration of the time spent on the fruit (residence time) and number of the different events. Ten replicates for each of the six treatments were conducted.

Efficacy experiments

In the choice experiment, six mature oranges, one per treatment, were place inside a plastic cage (32 × 40 × 30 cm) with a mesh-covered window (24 × 18 cm) on the top. One hundred and twenty gravid females (20 per fruit) were released into each cage.

In the no choice experiment, for each treatment, two fruits with the same kaolin concentration (or untreated in the control) were placed inside a plastic cage (⌀ = 22 cm; h = 12 cm) with a mesh-covered window (⌀ = 14) on the top. Forty gravid females (20 per fruit) were released into each cage.

In both experiments, the females were allowed to oviposit for 5 days. Then, the fruits were removed from the cages and each fruit was kept separately into small containers and incubated for 2 weeks in a growth chamber at 25 °C to allow larvae to evolve. The number of medfly larvae jumping out of each fruit was recorded and counted daily. Both experiments were replicated 5 times.

Statistical analysis

Statistical analysis was performed by analysis of variance (PROC General Linear Model, SAS Institute, 2020). If necessary, data were arcsine or root-square transformed before the analysis. Means were compared using Tukey's HSD post hoc test at a 95% significance level. The efficacies in choice and no choice experiments were calculated using Abbott's formula (Abbott, 1925).

Results

Colour parameters of fruits treated or not with kaolin

All the measured parameters were significantly different in the control and in the kaolin treated fruits (table 1). The luminance (L) ($F = 140.04$; df = 144, 5; $P < 0.001$) and the hue angle ($F = 21.49$; df = 144, 5; $P < 0.001$)

Table 1. Hunter *L*, *a*, *b*, hue angle and chroma colour parameters (means ± SE) of Valencia Late orange fruits when treated with calcined kaolin at different concentrations.

Treatment	Colour parameters				
	L	*a*	*b*	Hue angle	Chroma
Control	61.7 ± 0.3e	24.1 ± 0.4a	37.0 ± 0.2a	0.58 ± 0.01c	44.2 ± 0.2a
1.5%	65.8 ± 0.3d	20.1 ± 0.3b	28.3 ± 0.5b	0.62 ± 0.02bc	34.8 ± 0.3b
2.5%	66.8 ± 0.3dc	19.1 ± 0.3b	26.6 ± 0.4c	0.62 ± 0.02bc	32.8 ± 0.4bc
3%	67.1 ± 0.2c	19.3 ± 0.3b	24.6 ± 0.4d	0.67 ± 0.01ab	31.3 ± 0.4c
5%	69.6 ± 0.2b	16.3 ± 0.3cd	19.1 ± 0.3e	0.70 ± 0.01a	25.1 ± 0.4d
6%	70.6 ± 0.2ab	15.7 ± 0.3d	18.2 ± 0.4e	0.71 ± 0.01a	24.0 ± 0.4d

Means within each column followed by a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

were significantly higher as the kaolin concentration increased. The *a* and *b* parameters and the colour saturation were significantly lower in the control fruits (F = 78.49; df = 144, 5; P < 0.001, F = 304.28; df = 144, 5; P < 0.001 and F = 402.19; df = 144, 5; P < 0.001, respectively), and both parameters declined as kaolin concentration increased.

Acceptance and repellence

Significant differences were found among treatments for the first female landing on fruits (F = 28.21; df = 35, 5; P < 0.001). More than 50% of the released females landed on the control fruit (figure 1). About 25% of tested females landed on fruits treated with 1.5% kaolin and 10% of the females landed on fruit treated with 2.5% kaolin. Only 10% of the first landings corresponded to kaolin treated fruits over 2.5% concentration. No differences on this value were observed when testing kaolin concentrations of 2.5% or higher.

The percentage and the number of females standing on fruits when they were allowed to land for 6 hours were significantly different among treatments (table 2). More than 85% of the females were counted on control fruits, while from the 2.5% concentration onwards, we found no significant differences among treatments, and the percentage of females on those fruits was below 2.6%.

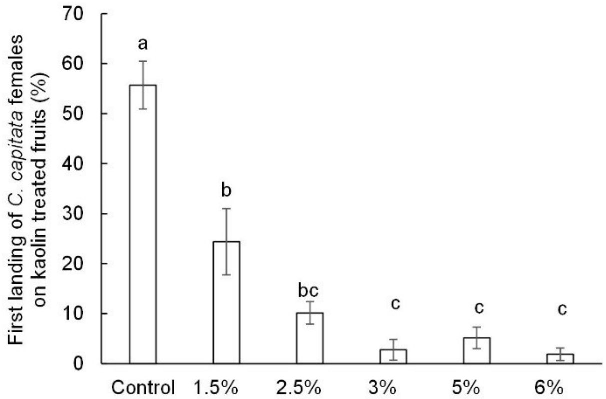

Figure 1. Percentage of first landings by *C. capitata* on kaolin treated Valencia Late orange fruits. Means within each column with a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

For each sampling time the number of females, standing on control fruits was significantly higher than on kaolin treated fruits (table 2). No significant differences were observed in the number of females standing among the kaolin treated fruits concentrations. The number of females on the fruits was stable along the time in all the treatments.

Table 2. Number (means ± SE) of *C. capitata* females on treated Valencia Late orange fruits after the release of 20 females per replicate. Each sampling time corresponds to the average of four observations conducted each 15 minutes.

	Sampling time						Total observations	
	0-1 hours	1-2 hours	2-3 hours	3-4 hours	4-5 hours	5-6 hours	Number per fruit	Percentage
	(F = 21.7; df = 5, 66; P < 0.001)	(F = 32.9; df = 5, 66; P < 0.001)	(F = 90.4; df = 5, 78; P < 0.001)	(F = 82.7; df = 5, 90; P < 0.001)	(F = 79.2; df= 5, 90; P < 0.001)	(F = 121.5; df = 5, 90; P < 0.001)	(F = 442.19; df = 5, 515; P <0.001)	(F = 742.64; df = 5, 515; P < 0.001)
Control	3.7 ± 1.8 a	5.3 ± 1.2 a	5.3 ± 1.8 a	4.3 ± 0.3a	4.7 ± 1.3 a	6.0 ± 0.6 a	5.81 ± 0.29 a	86.8 ± 1.8 a
1.5%	0.3 ± 0.3 b	0.0 ± 0.0 b	0.3 ± 0.3 b	0.7 ± 0.3 b	0.0 ± 0.0 b	1.0 ± 0.6 b	0.49 ± 0.08 b	6.6 ± 1.0 b
2.5%	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.3 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.09 ± 0.03 c	1.8 ± 0.7 c
3%	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.3 b	0.0 ± 0.0 b	0.06 ± 0.04 c	0.7 ± 0.4 c
5%	0.3 ± 0.3 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.06 ± 0.03 c	1.5 ± 0.7 c
6%	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.3 ± 0.3 b	0.3 ± 0.3 b	0.11 ± 0.04 c	2.6 ± 1.3 c

Means within each column followed by a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

Table 3. Behaviour of *C. capitata* females on treated Valencia Late orange fruits. Number (means ± SE) of each event occurred during a 20 minutes observation.

	Number of events					
	Control	1.5%	2.5%	3%	5%	6%
Walking	25.1 ± 7.6	37.0 ± 7.4	28.2 ± 7.1	26.1 ± 4.3 b	54.7 ± 8.8	54.8 ± 13.8
Grooming	14.9 ± 2.1 b	25.7 ± 4.6 ab	19.7 ± 2.6 ab	17.7 ± 2.8 ab	28.7 ± 2.6 a	21.2 ± 3.2 ab
Boring	1.0 ± 0.5 a	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b
Resting	15.3 ± 2.5	14.4 ± 1.9	13.8 ± 3.4	14.5 ± 3.3	12.7 ± 2.2	10.4 ± 1.8
Turn	8.5 ± 1.4	12.0 ± 1.7	10.6 ± 2.3	9.3 ± 2.2	12.2 ± 2.4	12.0 ± 1.7
Landing on fruit	3.8 ± 1.0	7.7 ± 3.1	7.6 ± 2.7	6.4 ± 1.5	13.3 ± 2.4	8.6 ± 2.6
Landing on glass/ground	19.4 ± 8.5 b	26.8 ± 8.4 ab	19.6 ± 5.5 ab	23.7 ± 5.4 ab	52.6 ± 8.8 a	55.1 ± 16.1 ab
Dragging	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Means within each file followed by a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

Behaviour

The numbers of walking (F = 2.57; df = 5, 54; P = 0.04), grooming (F = 2.75; df = 5, 54; P = 0.03), boring (F=3.46; df = 5, 54; P = 0.009) and landing on glass or ground (F = 3.04; df = 5, 54; P = 0.02) events were different among treatments (table 3). The rest of the analysed events (resting, turning, landing on fruit and dragging) were similar among treatments (P > 0.05).

The number of grooming events was higher in the 5% treatment than in the control (table 3). The number of times that females landed on other surface different to the fruit (glass container or ground) was also higher in the 5% kaolin concentration than in the control. No boring behaviour was observed on fruits treated with kaolin, whereas the mean number of boring events on control fruits was 1.0.

The total time spent by females on the fruits was different among treatments (F = 13.91; df = 5, 54; P < 0.001). Females stayed over 60% of the time on the control fruit. By contrast, on fruits treated with kaolin, this percentage ranged from 2.18 to 10.7%, and it was similar for all the kaolin concentrations tested (figure 2).

Efficacy

In both choice and no-choice experiments, all kaolin treatments reduced significantly the number of larvae obtained per fruit respect to the control (F = 13.12; df = 29, 5; P < 0.001 and F = 18.63; df = 59, 5; P < 0.001, respectively) (table 4). In the no-choice experiment, the number of larvae per fruit depended on the kaolin concentration applied until the 2.5% value. From this concentration onwards, when the concentration increased, the number of larvae per fruit was similar.

In the no choice experiment, though the number of larvae obtained per fruit in the 1.5% kaolin treatment was significantly lower than in the control, the efficacy achieved was low (44.6%) compared to the rest of kaolin concentrations tested (table 4).

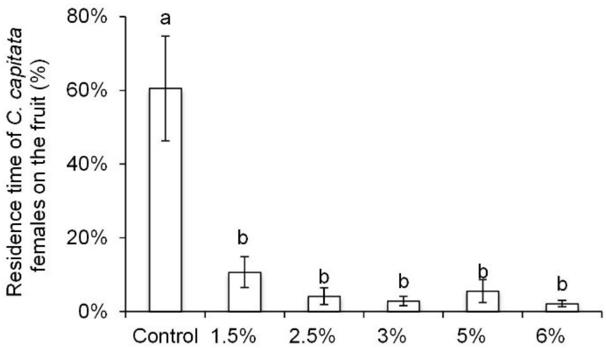

Figure 2. Behaviour of *C. capitata* females on treated Valencia Late orange fruits. Percentage of residence time of *C. capitata* during a 20 minutes observation. Means within each column with a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

Table 4. Number (mean ± SE) of *C. capitata* larvae obtained per Valencia Late orange fruits and Abbott efficacy in choice and no choice experiments. Twenty gravid females of *C. capitata* per fruit were released and allowed to oviposit for 24 hours.

	Choice experiment		No choice experiment	
	Number of larvae	Efficacy %	Number of larvae	Efficacy %
Control	160.2 ± 36.3 a	-	59.9 ± 8.0 a	-
1.5%	43.6 ± 30.8 b	73	33.2 ± 10.3 b	45
2.5%	18.8 ± 11.6 b	88	5.3 ± 5.3 c	91
3%	1.4 ± 1.4 b	99	4.4 ± 3.2 c	93
5%	0.0 ± 0.0 b	100	0.8 ± 0.7 c	99
6%	0.0 ± 0.0 b	100	0.0 ± 0.0 c	100

Means within each column followed by a different letter are significantly different (P < 0.05, Tukey HSD post hoc test).

In the choice experiment, the efficacy of 1.5% kaolin concentration (72.8%) was higher than in the no-choice experiment, and similar to the 2.5% and 3% concentration (88.3% and 99.1% respectively). Efficacy of 5% and 6% kaolin concentrations was 100% (table 4).

Discussion

In our studies, medfly females were able to discriminate the treated fruits, selecting in more than 50% of times the untreated ones to land for the first time. These results agree with laboratory studies that demonstrated that *C. capitata* landed on untreated oranges and stone fruits avoiding kaolin treated ones (Mazor and Erez, 2004; D'Aquino *et al*., 2011). They were also able to discriminate between different concentrations of the treatments, since, among the treated fruits, they preferred to land more often in those treated with kaolin at 1.5% than fruits treated with the higher concentrations. The kaolin treatments significantly modified the colour parameters of the fruits in comparison with the control ones. The luminance increased with the kaolin concentration applied to the fruits. The parameters *a*, *b* and chroma diminished when kaolin concentrations increased, which indicates that females were less attracted by white and more attracted to reddish and yellowish tones. According to that, white was reported to be the least attractive colour for ovipositing medfly females (Katsoyannos, 1987). Medflies are more attracted to yellow-coloured fruit than to fruit of other colours (Katsoyannos, 1987). Once a tephritid female closely approaches or arrives on a host plant, both olfactory and visual cue emanating from potential oviposition sites may be used to locate such sites (Díaz-Fleischer *et al*., 2001). The visual cues that influence the host acceptance at short distance by insect females are altered by the whitish colour of the fruits. Females of specialist tephritids may be attracted to a narrower range of oviposition site colours than are females of generalist tephritids (Díaz-Fleischer *et al*., 2001). Studies on *C. capitata* and other polyphagous tephritids suggest positive response to the odour of a broad range of unrelated compounds emitted by ripening host fruits (Díaz-Fleischer *et al*., 2001). Few species of ripening fruit are white, and females of the generalist species such as *C. capitata* show little or no discrimination between white spheres and spheres of other colours (Díaz-Fleischer *et al*., 2001). Therefore, another possible explanation for the rejection of the first landing of fruits treated with a higher concentration of kaolin could be that the white colour is not attractive for *C. capitata*, since it does not relate it to any ripe fruit. Kaolin had clear influence on the female's choice towards untreated and less concentration treated fruits.

The number of females standing on fruits showed that they avoided to stay on kaolin-treated fruits, and this behaviour was more pronounced on 2.5% kaolin concentration onwards. This is the results of both, the acceptance and repellence effect of the treatments, since we measured the number of females for a 6 hours period, not only the fruit firstly accepted. The kaolin particle film forms a barrier on the fruits changing the texture of the fruit from smooth to dusty and irregular. This surface texture has been proved to repel and irritate the insects and to reduce the adhesion of the insects to the fruit surface (Glenn *et al*., 1999; Salerno *et al*., 2020). Thus, according to our finding, the kaolin concentration effect on *C. capitata* host repellence was alike to host not-acceptance, being higher in kaolin concentrations of 2.5% and higher than that of 1.5% or untreated.

The kaolin particle film treatment affected some of the behavioural events performed by *C. capitata* related to the host acceptance, repellence and oviposition. In addition, females landed more frequently on a different place than the fruit when kaolin concentrations were higher. In both cases, these results were related to the residence time of *C. capitata* on the fruits. This was shorter in kaolin treated fruits than in the control, 6 times lower in the best case (1.5% kaolin). Processed kaolin tactile deterrence effect is well documented (Bostanian and Racette, 2008; Salerno *et al*., 2020). This explains that females accepted the untreated fruits and remained on them during most of the observations, while avoided the kaolin treated fruits and performed more movements (flying or walking) along the experimental arena. It must be considered that all the results of this study were obtained when treating orange fruits with Surround. Surround is calcined kaolin while other kaolin particle films are not and results in a three-dimensional instead of a two-dimensional sheetlike. This three-dimensional structure has been proved in laboratory conditions to significantly reduce the number of insect landings and oviposition compared to other kaolin films and provide a better coating (Yee, 2012).

Kaolin treatments on citrus fruits resulted in a reduction of over 43 to 100% in the number of *C. capitata* larvae obtained per fruit, and this reduction depended on the kaolin concentration tested. Even the 1.5% kaolin film repelled the female, it was able to oviposit in the fruit more than in the other concentrations. However, when using 2.5% or higher concentrations, efficacy was high and similar in all the concentrations tested, ranging from 88% to 100%. Our results proved the effect of kaolin particle film on the reduction of *C. capitata* oviposition. Efficacy was due to repellence and oviposition deterrence. In this study, the reduction estimates for citrus fruits were based on the number of larvae per fruit when using a fruit:female ratio of 1:20 and a five days of exposure time. Oviposition deterrence of kaolin was already demonstrated for other Diptera Tephritidae (Caleca and Rizzo, 2007; Pascual *et al*., 2010; Yee, 2012; Gonzalez-Nuñez *et al*., 2020) and some Lepidoptera Tortricidae (Knight *et al*., 2000; Unruh *et al*., 2000; Cadogan and Scharbach, 2005; Sackett *et al*., 2005; Pease *et al*., 2016; Tacoli *et al*., 2019).

Kaolin was highly effective in controlling *C. capitata* attacking citrus fruits and could be used instead of pesticides (e.g. organophosphates, pyrethroids and spinosyns) in conventional production. The real situation of citrus fruits after a treatment with kaolin in citrus trees resembles the characteristics of the choice experiment since fruits will be perfectly covered with kaolin while other will remain uncoated. Hence, the importance of a correct application of the product in the field to obtain the desired efficacy. In the choice experiment, the efficacy of kaolin was very high, from 72.8% in the case of the 1.5% concentration to 100% in the 5% and 6% concentrations.

Conclusions

In this work we conducted five different experiments under laboratory conditions to study the effect of different kaolin particle film concentrations to protect citrus fruits against *C. capitata*. We demonstrated through all the specific approaches (host acceptance, repellence and ovipositing), that the 2.5% kaolin treatments applied in two layers reduced successfully the fruit infestation by *C. capitata*. This processed kaolin treatment should offer efficacy in real field conditions as satisfactory as conventional pesticides towards high populations and could be a valid tool to incorporate into *C. capitata* control strategies.

Acknowledgements

We are grateful to Rafel Monfort for his assistance in the laboratory. This study was partially funded by Exportadora d'Agris d'Alcanar SCCL, Soldebre SCCL, Amsafresh SL and the Generalitat de Catalunya (CERCA Programme). The authors also thank three anonymous reviewers for their helpful comments on an earlier version of this paper.

Both authors contributed equally to this work.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Received February 2, 2021. Accepted May 19, 2021.