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Evaluation of repellent effects of plant-based essential oils, red dye, and kaolin on Asian citrus psyllid

Romain Exilien[1] | **Jawwad A. Qureshi**[2] | **Xavier Martini**[1]

[1]Department of Entomology and Nematology, North Florida Research and Education Center (NFREC), University of Florida, Quincy, Massachusetts, USA

[2]Department of Entomology and Nematology, Southwest Florida Research & Education Center, University of Florida, Immokalee, Florida, USA

Correspondence
Xavier Martini, Department of Entomology and Nematology, North Florida Research and Education Center (NFREC), University of Florida, 155 Research Rd, Quincy 32351, MA, USA.
Email: xmartini@ufl.edu

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Abstract

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), vector of the pathogen causing Huanglongbing disease, is currently the most devastating pest of citrus. Despite intensive control efforts, the disease continues to spread. *Diaphorina citri* typically relies on tactile, visual, and odor cues for host selection. By combining an irritant (kaolin clay), a visual masking (red or blue dye), and true odor repellents, we aim to repel *D. citri* from citrus trees. In laboratory conditions, we conducted closed-cage bioassays to compare *D. citri* response to the following treatment combinations: (1) kaolin + food colorants (blue or red); (2) kaolin + odor repellents (thyme, lavender, fir, and coriander oil), and (3) kaolin + red food colorant + thyme oil. The red kaolin treatment exerted a greater deterrent effect on *D. citri* adults than the blue and uncolored kaolin treatments. We also observed an additive effect in terms of *D. citri* repellency with the combination of thyme oil and kaolin and, to a lesser extent, coriander oil and kaolin. However, the combination of lavender or fir oils and kaolin did not increase repellency against ACP as compared with kaolin alone. Thyme oil + red kaolin oil combinations induced similar ACP repellency to that of thyme oil + uncolored kaolin. Finally, fecundity was significantly reduced by the kaolin-based treatments compared to the thyme oil treatment and control plants. Our findings suggested the possible use of red kaolin or the combination of thyme and kaolin as a means of reducing *D. citri* infestation in citrus.

KEYWORDS
citrus greening, *Diaphorina citri*, Hemiptera, HLB, huanglongbing, nano clay, olfaction, Psyllidae, thyme oil, thymol

INTRODUCTION

Citrus greening or huanglongbing (HLB) is the world's most important citrus disease, caused by the presumed bacterium *Candidatus* Liberibacter asiaticus (*C*Las) carried and transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). This disease considerably threatens citrus production worldwide and decimated the Florida citrus industry (Bové, 2006; Fan et al., 2016; Halbert & Manjunath, 2004). There is no known cure, and no HLB-resistant genotype, making citrus crops particularly vulnerable to this disease (Grafton-Cardwell et al., 2013). Despite intensive control efforts, the damage caused by the disease, including yield and fruit quality losses, remains disastrous in all citrus-growing regions (Ghosh et al., 2022). The management of HLB primarily focuses on vector control using conventional insecticides with frequent applications that may result in adverse effects on the environment, non-target organisms, humans, and the development of insecticide resistance (Boina & Bloomquist, 2015; Bové, 2006; Fan et al., 2016; Ghosh et al., 2022; Grafton-Cardwell et al., 2013; Qureshi et al., 2014). This fuels the search for environmentally friendly strategies to reduce reliance on conventional chemical pesticides and mitigate their impact on the environment. A better understanding of the interaction mechanisms between *D. citri* and its host is therefore the cornerstone of effective, sustainable population control.

The adult psyllids locate and infest citrus trees via visual and olfactory stimuli from their host (Kalile et al., 2022; Patt & Sétamou, 2010; Wenninger et al., 2009, Santos Silva et al., 2023). Research has shown that kaolin combined with red-colored stickers and binders significantly reduced the number of *D. citri* landings on citrus. It was not clear, however, whether this reduction was due to the red coloration of the kaolin or to the adjuvants themselves (Pierre et al., 2021). Additionally, studies have indicated that psyllids do not respond to blue color contrary to other colors, particularly green, which they associate with potential host plants (Patt et al., 2014; Sétamou et al., 2014; Stockton et al., 2016). These findings suggest that blue color may reduce psyllids' ability to detect and respond to visual stimuli, potentially due to interference with their visual or behavioral cues.

Research has been conducted to manage *D. citri* populations by manipulating the visual, olfactory, or tactile cues used by the psyllids in host plant location (Chuang et al., 2023; Hall et al., 2018; Kuhns et al., 2016; Mann et al., 2012; Martini et al., 2020; Oliveira et al., 2022; Pierre et al., 2021). However, little is known about the potential of interfering with more than one of these cues. We postulate that synergistic or additive effects in terms of *D. citri* response can be obtained by disrupting more than one cue simultaneously. The simultaneous use of products with the potential to interfere with the insect's tactile, visual, and/or olfactory senses may thus provide effective management of the *D. citri* population. Nano clay such as kaolin (Janick, 2010), and plant-derived essential oils (Karr & Coats, 1992) are used in pest management. These products have gained attention for their potential as alternatives or supplements to conventional insecticides (Exilien et al., 2024; Janick, 2010; Oliveira et al., 2022).

Kaolin has multiple uses in the agricultural industry, including crop protection against pests and sun protection (Janick, 2010). It is an effective broad-spectrum deterrent against agricultural pests (Alavo & Abagli, 2011; Campos & Martinez Ferrer, 2021; Costa et al., 2021; Oliveira et al., 2022; Salerno et al., 2020; Silva & Ramalho, 2013). Similarly, kaolin works as a barrier to reduce environmental stress, including heat stress and sunburn without interfering with photosynthesis (Janick, 2010). It is hypothesized that kaolin particle film targets the visual and tactile cues that mediate communication between host and pest by disrupting the wavelengths of light that pests use to locate their hosts. Kaolin-coated plants become less appealing and palatable to targeted pests due to the change in texture and appearance (Liang & Liu, 2002; Oliveira et al., 2022; Pierre et al., 2021; Salerno et al., 2020). Kaolin is also used in combination with dyes to enhance pest control (Pierre et al., 2021). The efficacy of kaolin has been documented both in the laboratory and field against various hemipteran pests within Aphididae, Aleyrodidae, and Psyllidae (Johnston et al., 2022; Liang & Liu, 2002; Oliveira et al., 2022; Pierre et al., 2021; Salerno et al., 2020).

Plant-based essential oils are widely used against various agricultural and household arthropod pests, due to their insecticidal properties (Enan, 2001; Isman & Machial, 2006; Karr & Coats, 1992; Mossa, 2016). They often contain compounds that act as natural plant defense mechanisms and can be used as repellents or natural poisons against a wide range of pests (Karr & Coats, 1992; Deletre et al., 2016). In addition, essential oils are recognized to have multiple modes of action against insect pests and are usually environmentally safe and nontoxic to humans and non-target organisms (Pavela & Benelli, 2016). Several studies have explored the insecticidal properties of essential oils and their potential application in pest management (Regnault-Roger, 1997; Deletre et al., 2016). Some of them have proved effective against *D. citri* (Chuang et al., 2023; Hall et al., 2018; Kuhns et al., 2016; Mann et al., 2012; Rizvi et al., 2018; Silva et al., 2016). However, due to their short residual activity and high volatility, their use has not been adopted.

Research continues to address challenges associated with the use of essential oils as insecticides by improving the stability and efficacy of formulations to enhance their diffusion and persistence on target surfaces (Lv et al., 2017; Pavoni et al., 2020). Studies have also examined the synergistic effects of combining different essential oils with other natural compounds to enhance overall insecticidal activity and provide a broader spectrum of control (Cloyd et al., 2009; Isman, 2020; Isman et al., 2011). However, very few studies have explored the synergistic potential of combining kaolin with essential oils (Johnston et al., 2022; Reitz et al., 2008). Yet, kaolin is a natural mineral that can adsorb other substances such as essential oils and pesticide components (Lagaly, 2001; Nguemtchouin et al., 2009), and allow for a slower release of the active ingredient (Johnston et al., 2022). The aim of this study was to evaluate a series of treatments combining kaolin, dyes, and plant-derived essential oils to disrupt *D. citri* response to host signals (visual, tactile, and olfactory).

MATERIALS AND METHODS

Citrus seedlings

Experiments were performed with Valencia citrus, *Citrus sinensis* (L.) Osbeck (Rutaceae) (SPB 1-14-19 S0033-1-08-012 ID 0868) grafted onto *Swingle* spp. rootstocks supplied by Southern Citrus Nurseries (Dundee, FL, USA). Seedlings were raised in plastic pots with promix soil (Pro-Mix HP Mycorrhizae; Garden Dominion Supply, Hagerstown, MD, USA) and maintained in a greenhouse (25°C) at the North Florida Research and Education Center of the University of Florida (NFREC, Quincy, FL, USA). Citrus plants were watered twice a week and sprayed when needed with M-Pede insecticidal soap (GOWAN, Yuma, AZ, USA) to control mites, mealy bugs, and scales, and ant populations from plants.

Insect rearing

The adult *D. citri* used for the study were obtained from a laboratory colony established at the NFREC, initiated in the summer of 2017 from adults collected in Franklin County (FL, USA). This colony was pooled with a population of

*C*Las-negative *D. citri* from the Citrus Research & Education Center (Lake Alfred, FL, USA), and wild individuals collected in Bristol (Liberty County, FL, USA), to increase genetic variability. Individuals were reared on potted *Murraya koenigii* (L.) Spreng (Rutaceae) and Valencia citrus in 33 × 33 × 61 cm sleeve cages (Raising Butterflies, Salt Lake City, UT, USA). The colony was maintained in a climate chamber under controlled conditions at 21 ± 1°C, 60%–81% r.h., and L14:D10 photoperiod, and 100 lx light intensity. Rearing plants were watered and pruned regularly, and fertigated every 2 weeks with Miracle-Gro (ScottsMiracle-Gro, Marysville, OH, USA) to stimulate new growth and ensure continued reproduction of *D. citri*.

Essential oils, kaolin clay, and food colorants

Based on previous studies evaluating *D. citri* repellency against several essential oils, four essential oils extracted from fir, coriander seed, thyme, and lavender were selected (Isman & Machial, 2006; Kuhns et al., 2016; Mann et al., 2012). These essential oils have all been registered with the Environmental Protection Agency (EPA). The essential oil of fir (100% purity, EPA Reg. Number 25766-18-1) was supplied by Sri Venkatesh Aromas (Orlando, FL, USA); coriander (100% purity, EPA Reg. Number 4758-150; 52991-13) by Plant Guru (Plainfield, NJ, USA); thyme (100% purity, EPA Reg. Number 92331-2) and lavender oils (100% purity, EPA Reg. Number 115-95-7) were obtained from Sigma-Aldrich (St Louis, MO, USA). The kaolin clay (Surround WP) was supplied by BASF (Florham Park, NJ, USA), and the food colorants by McCormick & Company (Hunt Valley, MD, USA).

Dual choice bioassay 1: Masking visual cues with colored kaolin

This experiment aimed to determine whether colored kaolin would repel *D. citri* more than uncolored (white) kaolin. The trial was set up in cages with citrus plants having at least two young emerging shoots because *D. citri* prefers to feed and oviposit on the new shoots (Sétamou et al., 2016; Teck et al., 2011). One day prior to the start of the experiment, plants were pruned, left with 1–2 branches and 8–10 leaves, and kept at 30–40 cm tall. Five trials were conducted in four mesh cages (60 × 60 × 90 cm; Raising Butterflies). Each cage was enclosed in a white box built with foam boards (Elmer's, Atlanta, GA, USA; Ross, Atlanta, GA, USA) to eliminate external visual cues and avoid any choice bias related to light because *D. citri* individuals are sensitive to direct light and tend to orient toward the direction of light (Sétamou et al., 2012). The cage entrances were covered with a removable polyethylene curtain (60 × 90 cm) (Nalgene Versi-Dry, Rochester, NY, USA) that screened the direct light inside the cages. Curtains were removed only for data collection.

Each cage contained two potted citrus plants placed 30 cm apart. Each trial included four treatments: (1) two untreated citrus plants as control (negative control to assess any position bias); (2) kaolin-coated citrus vs. uncoated citrus; (3) kaolin-coated citrus vs. kaolin-coated citrus + red food colorant; and (4) kaolin-coated citrus vs. kaolin-coated citrus + blue food colorant (Figure 1). Kaolin was applied to citrus plants using hand sprayers (Chapen 1420 mL, Batavia, NY, USA) at rates of 75 g L^{-1}. A 53 µL volume of Tween 20 (Sigma Aldrich) was mixed with the kaolin solution to enhance the coating. To obtain colored kaolin, the kaolin solution was diluted in 13 mL of blue or red food colorant (McCormick & Company, Hunt Valley, MD, USA). A neutral solution (H_2O + Tween 20) was applied to control plants to ensure that only the kaolin or colored kaolin varied in the experiment.

For each replicate, *D. citri* were collected from rearing plants, and their sex determined under a microscope. Then, a group of 20 *D. citri* (10 ♂ + 10 ♀), was carefully aspirated and transferred to a sealed plastic vial (7 cm long, 2.5 cm diameter). One vial was placed at an equal distance between plants in each treatment cage and the vial was attached to a steel clamp at approximately plant height. Subsequently, the covers were removed from the flasks, the cages were observed for 15 min, and the first individuals that landed on the plants were recorded.

Data were collected over a 1-week period. The number of *D. citri* that settled on each plant was counted 3× per

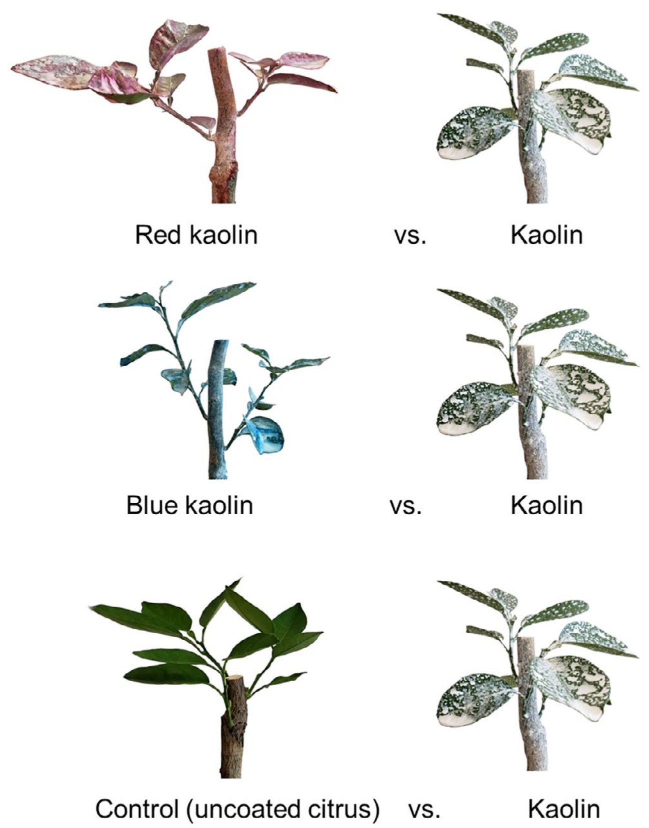

FIGURE 1 Citrus plants collated with colored kaolin-based treatments to assess repellency of Asian citrus psyllids.

day at 4-h intervals from the release of the ACP on Days 1, 2, 3, and 7. Plant placements were changed daily to avoid positional bias. On Day 7, 1–2 young shoots were removed from the plants and observed under the microscope, and subsequently, the number of *D. citri* eggs found was recorded. The experiment was conducted in a climate chamber under controlled conditions at 21 ± 1°C, 60%–81% r.h., L14:D10 photoperiod, and 100 lx light intensity.

Dual choice bioassay 2: Masking visual and olfactory cues with kaolin and repellents

The purpose of this experiment was to investigate the additive effect of essential oils (fir, coriander, thyme, and lavender) as olfactory repellents combined with kaolin clay as a deterrent on *D. citri* behavior. This experiment was carried out for 28 days using a setup similar to that of the previous cage experiment. To evaluate the combined effects of essential oils and kaolin on the psyllid population, four treatment cages with four replicates each were designed: kaolin-coated citrus vs. kaolin-coated citrus +(1) lavender oil; (2) coriander oil; (3) thyme oil; and (4) fir oil. The dissolution and application rate of the kaolin was performed using a similar procedure as described in the previous experiment. Essential oils were diluted with the kaolin solution at a rate of 1.25 mL per application.

Adults of *D. citri* were collected from the rearing cages, then sexed under the microscope. Twenty sexed psyllids (10 ♂ + 10 ♀) were released in each treatment cage from plastic vials, attached to a steel clamp equidistant from the plants and approximately at plant height. Data regarding the number of *D. citri* settled on each plant were collected 3× per day (8:00, 12:00, and 16:00 h) for 7 days per replicate. The placement of the plants was changed every day to avoid position bias. On Day 7, we removed all shoots from each plant and counted the number of eggs of psyllids under the microscope.

Dual choice bioassay 3: Masking visual and olfactory cues with kaolin, red kaolin, and thyme oil

Among the food colorants and olfactory repellents tested, the red dye and thyme oil, respectively, showed the highest repellency against *D. citri* when combined with kaolin compared to uncolored kaolin alone, and compared with the other repellents and blue dye (see Results). Thus, only thyme oil and red dye were used in this experiment. The aim was to assess the possible additive or synergistic effect of the combination of thyme oil and red kaolin compared with other combinations of thyme oil and kaolin-based treatments. We ran every possible combination of thyme oil, red kaolin, and kaolin against a control plant to obtain five treatment cages with four replicates each: (1) red kaolin-coated citrus vs. citrus; (2) kaolin-coated citrus vs. citrus; (3) thyme oil coated citrus vs. citrus; (4) red kaolin-coated citrus + thyme oil vs. citrus + thyme oil; (5) kaolin-coated citrus + thyme oil vs. citrus; and (6) red kaolin-coated citrus + thyme oil vs. kaolin-coated citrus + thyme oil.

Each treatment was prepared and applied at a similar rate to previous experiments. At 4 h after *D. citri* release, we recorded the number of *D. citri* settled on each plant with three daily records (8:00, 12:00, and 16:00 h) for 7 days. Plants were rotated daily to avoid positional bias. All shoots were collected from each plant on Day 7 and the number of psyllid eggs was counted.

Data analysis

Data analyses were performed using the RStudio software (v.2023.09.1) (R Core Team, 2021) to assess the behavioral response of *D. citri* to caged plants. The three daily readings were averaged for each day, and data were squared root transformed for linearity, normality, and homogeneity of variance. For all experiments and all replicates, the number of *D. citri* settled on plants in each cage was compared by two-tailed paired *t*-tests. Comparison between treatments was also performed using a generalized linear model (GLM) with Poisson distribution (Dunn & Smyth, 2018). Oviposition data from bioassay 1 was analyzed with binomial GLM using treatments as a fixed factor and the number of *D. citri* eggs as response variables. The Tukey's honestly significant difference test (HSD) was subsequently run to determine the significance level of treatments. For bioassay 3, oviposition was compared first between plants within cages using two-tailed paired *t*-tests and second between treatment cages using a GLM with gaussian distribution (for all tests: $\alpha = 0.05$). All data presented in the charts are untransformed means.

RESULTS

Masking visual cues with colored kaolin

The choice of individual psyllids varied between the assigned treatments (Figure 2). Significantly fewer psyllids settled on kaolin-coated than on uncoated plants ($t = -7.009$, $df = 4$, $p = 0.002$). When individuals were given a choice between white and red kaolin-coated plants, a significantly higher number of psyllids chose the white over the red kaolin-coated plants ($t = 4.234$, $df = 4$, $p = 0.013$). In contrast, when *D. citri* had to choose between blue and white kaolin, no significant differences were found between individuals that settled on blue and white kaolin-treated plants ($t = -0.283$, $df = 4$, $p = 0.79$). Additionally, no significant variations were found between control groups ($t = -1$, $df = 3$, $p = 0.40$).

Comparing each citrus-coated treatment, red kaolin exerted a greater deterrent effect on adult psyllids than blue kaolin, white kaolin, and the control. We observed a significant reduction in the number of *D. citri* settled on red kaolin-coated plants compared with blue kaolin and

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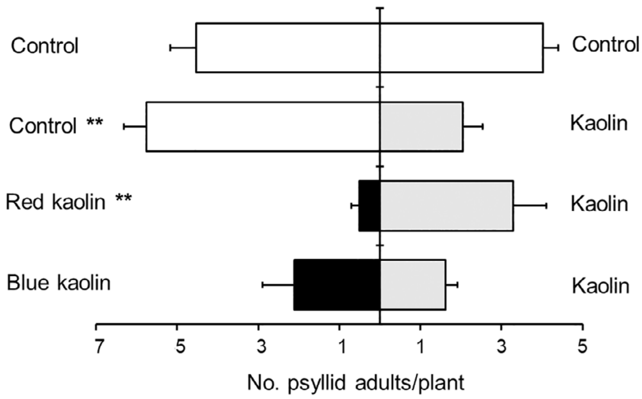

FIGURE 2 Mean (±SEM) number of Asian citrus psyllid adults per citrus plant in dual choice bioassays comparing their responses to colored kaolin treatments. Control = nontreated citrus plants. Kaolin is naturally white and dye was used to achieve red and blue colors. Asterisks indicate significant differences between treatments (*t*-test: **0.001 < *p* < 0.01).

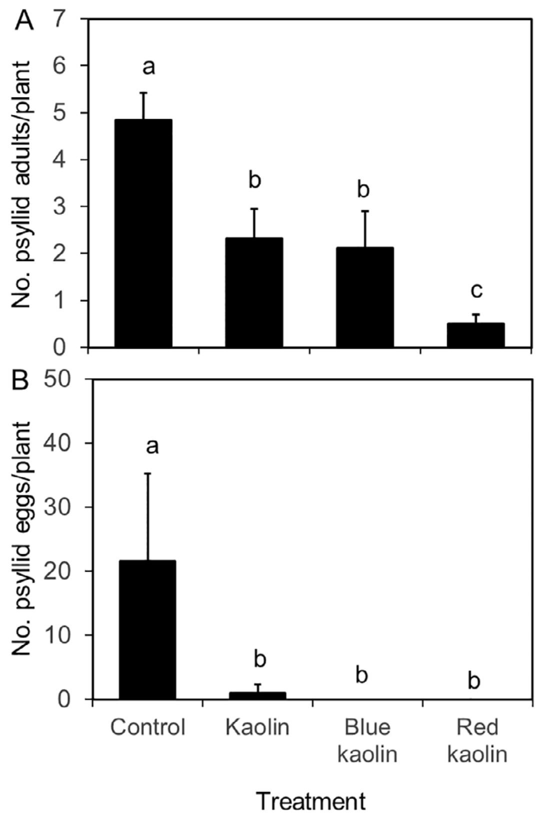

FIGURE 3 Mean (±SEM) number of Asian citrus psyllid (A) adults and (B) eggs per plant in the experiment evaluating psyllid response to colored kaolin. Control = nontreated citrus plants. Kaolin is naturally white, and dye was used to achieve red and blue colors. Asterisks indicate significant differences between treatments (Tukey's HSD test: $p < 0.05$).

kaolin-coated citrus, and the control (GLM, Poisson distribution: $\chi^2 = 249.29$, df = 3, $p < 0.001$). The deterrent effect of blue kaolin and kaolin was intermediate (Figure 3A).

Oviposition was also affected by treatments (GLM, binomial distribution: $\chi^2 = 24.44$, df = 3, $p < 0.001$; Figure 3B), implicating that female psyllid selected the more favorable plants before laying eggs. Oviposition was higher on uncoated plants (control) than on kaolin-coated plants. Colored kaolin exerted a strong influence on female egg-laying, as no eggs were observed on red or blue kaolin-coated plants (Figure 3B).

Masking visual and olfactory cues with kaolin and repellents

Psyllid responses varied between treatment cages. Through Day 5, significantly reduced numbers of psyllids were observed on plants coated with a combination of thyme oil and kaolin compared with those coated with kaolin alone (Day 1: $t = 3.527$, df = 3, $p = 0.038$; Day 2: $t = 6.839$, df = 3, $p = 0.006$; Day 3: $t = 3.905$, df = 3, $p = 0.029$; Day 4: $t = 3.818$, df = 3, $p = 0.031$; Day 5: $t = 4.086$, df = 3, $p = 0.026$; Day 6: $t = 2.529$, df = 3, $p = 0.085$; Day 7: $t = 2.4$, df = 3, $p = 0.090$) (Figure 4A).

Similarly, a significant reduction in the number of psyllids was observed on plants coated with a combination of kaolin and coriander during the first 2 days, compared with those treated with kaolin alone (Day 1: $t = 8.946$, df = 3, $p = 0.002$; Day 2: $t = 3.421$, df = 3, $p = 0.041$). Nevertheless, between Days 3 and 7, psyllid populations showed similar numbers between the two treatments (Day 3: $t = 2.483$, df = 3, $p = 0.089$; Day 4: $t = 2.9053$, df = 3, $p = 0.062$; Day 5: $t = -0.219$, df = 3, $p = 0.84$; Day 6: $t = 0.558$, df = 3, $p = 0.62$, Day 7: $t = -0.138$, df = 3, $p = 0.90$) (Figure 4B).

Throughout the experiment, no statistically significant differences were observed in *D. citri* numbers between the fir + kaolin and kaolin treatments, except on the second day, when psyllid numbers were marginally higher on citrus plants coated with kaolin than on those coated with the kaolin–fir combination (Day 1: $t = 0.325$, df = 3, $p = 0.77$; Day 2: $t = 3.0295$, df = 3, $p = 0.056$; Day 3: $t = 0.302$, df = 3, $p = 0.78$; Day 4: $t = 0.187$, df = 3, $p = 0.86$; Day 5: $t = -0.743$, df = 3, $p = 0.51$; Day 6: $t = -0.522$, df = 3, $p = 0.64$; Day 7: $t = -1.335$, df = 3, $p = 0.27$) (Figure 4C).

Finally, *D. citri* response to the lavender and kaolin combination compared with the kaolin treatment was similar over the initial 6-day period (Day 1: $t = 0.6$, df = 3, $p = 0.59$; Day 2: $t = -0.464$, df = 3, $p = 0.67$; Day 3: $t = -0.052$, df = 3, $p = 0.96$; Day 4: $t = -0.988$, df = 3, $p = 0.40$; Day 5: $t = -2.033$, df = 3, $p = 0.13$; Day 6: $t = -1.78$, df = 3, $p = 0.17$); however, a significant increase in the psyllid population was observed on plants coated with the combination of kaolin and lavender oil on the last day of the experiment ($t = -4.095$, df = 3, $p = 0.026$) (Figure 4D).

Masking visual and olfactory cues with kaolin, red kaolin, and thyme oil

Plant infestation by *D. citri* was significantly affected by the different treatments. Treated plants elicited a reduced response from adult psyllids in contrast to untreated plants (red kaolin: $t = 5.727$, df = 3, $p = 0.01$; kaolin:

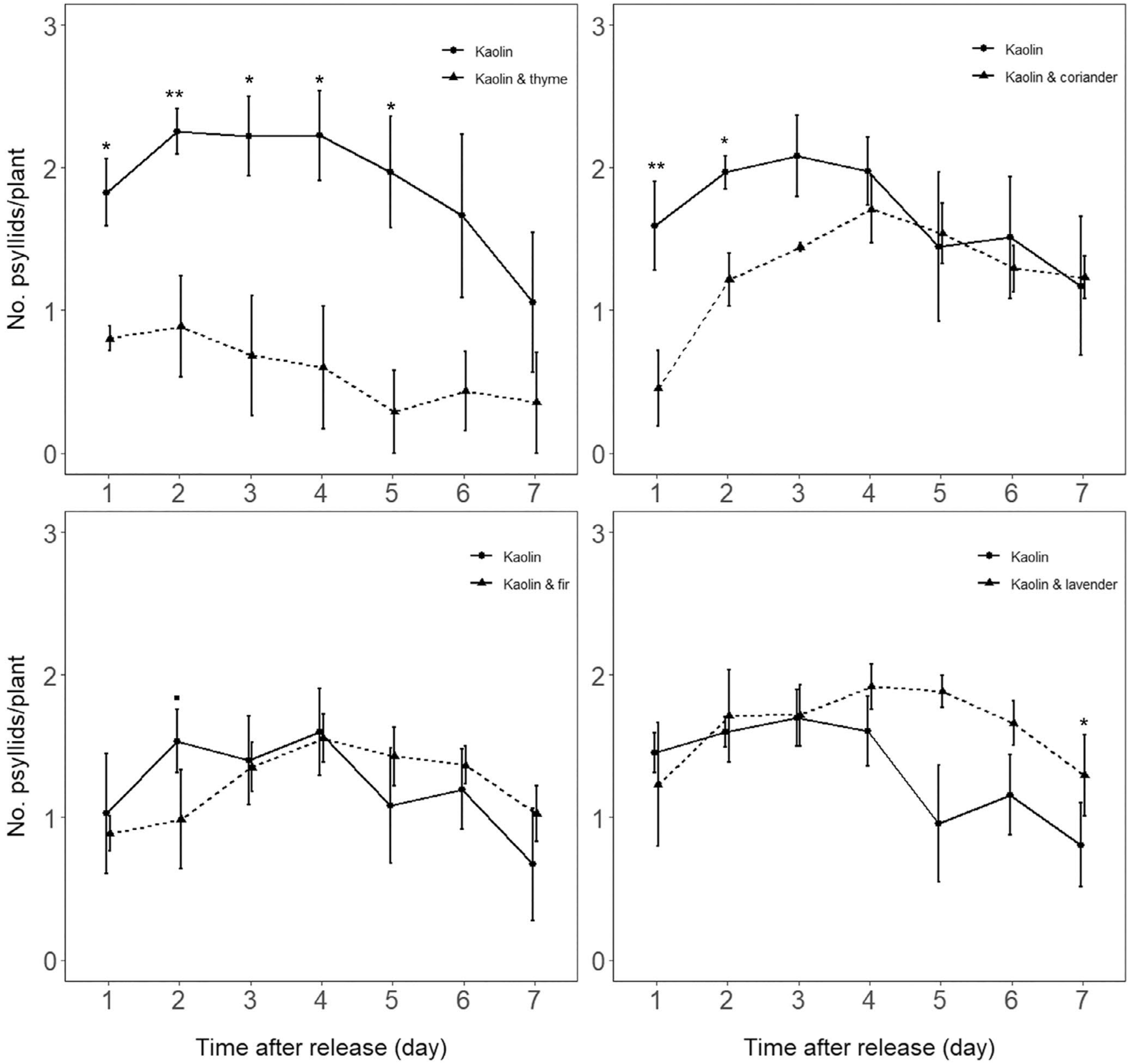

FIGURE 4 Mean (±SEM) number of Asian citrus psyllid adults on citrus plants treated with kaolin + repellents vs. kaolin-coated plants over the 7 day period of the experiment. Repellents: (A) thyme, (B) coriander, (C) fir, and (D) lavender essential oil. Asterisks indicate significant differences between treatments at a given time interval (two-tailed *t*-test: *0.01 < p < 0.05, **0.001 < p < 0.01). A square indicates marginally significant differences (two-tailed *t*-test: 0.05 < p < 0.10).

t = 4.927, df = 3, p = 0.016; red kaolin + thyme: t = 5.036, df = 3, p = 0.015; kaolin + thyme: t = 40.605, df = 3, p < 0.001; thyme: t = 3.446, df = 3, p = 0.041) (Figure 5A). Conversely, no significant differences were observed in the cage comparing plants treated with red kaolin and thyme oil to those with kaolin and thyme oil (t = 1.268, df = 3, p = 0.29) (Figure 5A).

Regarding egg laying, no significant differences were detected in cages that compared uncoated citrus to thyme oil-coated citrus (t = 1.952, df = 3, p = 0.15), uncoated citrus to red kaolin and thyme oil-coated citrus (t = 2.544, df = 3, p = 0.084), and red kaolin and thyme oil to kaolin and thyme oil-coated citrus (t = 1, df = 3, p = 0.39). However, in the later case the number of eggs laid was particularly low. A marginally significant difference was found between uncoated and kaolin-coated citrus (t = 2.955, df = 3, p = 0.059). Conversely, uncoated plants had a higher number of eggs than plants coated with both combined kaolin and thyme oil (t = 8.549, df = 3, p = 0.003), and red kaolin (t = 31.225, df = 3, p < 0.001) (Figure 5B).

In addition, a marginally significant difference was observed in eggs laid per cage during the experiment (GLM, Gaussian distribution: F = 3.036, df = 4, p = 0.05). We found a decrease in the number of eggs laid when the insects

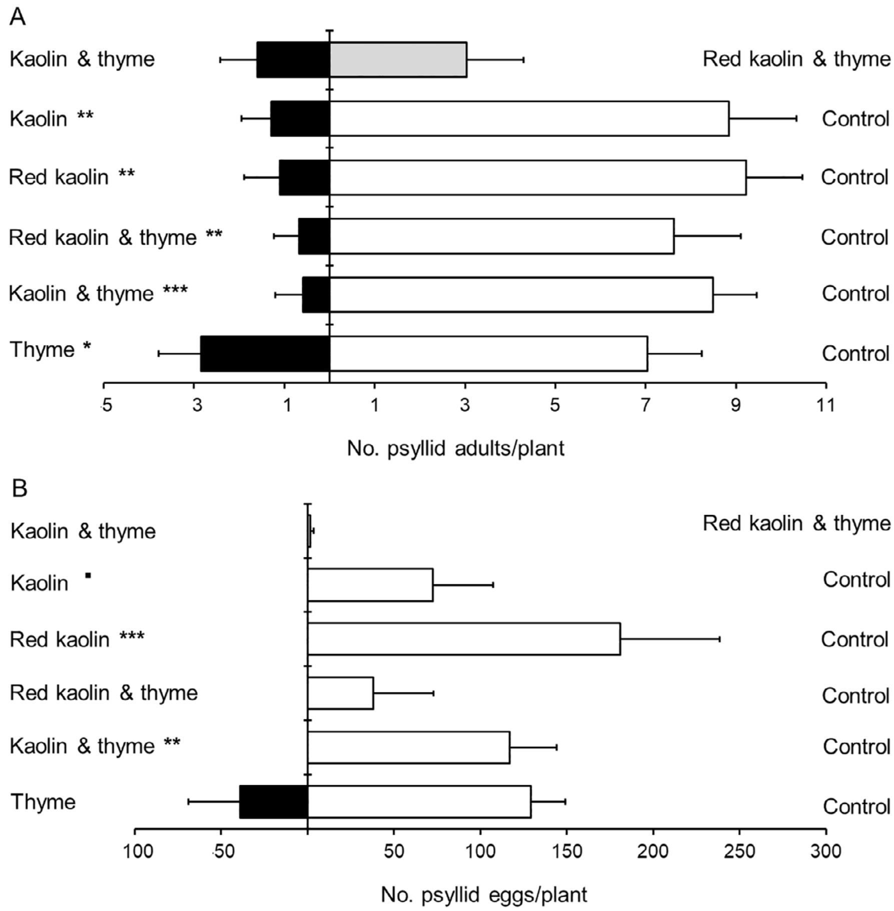

FIGURE 5 Mean (±SEM) number of Asian citrus psyllid (A) adults and (B) eggs per plant in dual-choice bioassays comparing their responses to various combinations of red kaolin and thyme oil. Control = nontreated citrus plants. Kaolin is naturally white and dye was used to achieve the red color. Asterisks indicate significant differences between treatments (*t*-test: *0.01 < *p* < 0.05, **0.001 < *p* < 0.01, ****p* < 0.001). A square indicates marginally significant differences (*t*-test: 0.05 < *p* < 0.10).

were exposed to uncoated citrus and red kaolin + thyme oil-coated citrus, compared to when they were exposed to uncoated citrus and red kaolin-coated citrus (Figure 5B).

DISCUSSION

This work demonstrated that treatments significantly influence *D. citri* settlement and oviposition rate on citrus trees. The combined repellent effect of kaolin and red food colorant was greater than for uncolored or blue kaolin treatments. Both uncolored and blue kaolin treatments reduced the psyllid population by around 50%, whereas the red kaolin treatment achieved an 85.5% reduction.

Several studies have already shown the deterrent properties of kaolin on many insect pests, whether on perennial or annual crops (Alavo & Abagli, 2011; Campos & Martinez Ferrer, 2021; Glenn et al., 1999; Johnston et al., 2022; Oliveira et al., 2022; Silva & Ramalho, 2013). However, this is only the second study to reveal a synergistic effect of red kaolin on *D. citri* behavior (Pierre et al., 2021). Pierre et al. (2021) used a binding agent as an adjuvant to increase treatment efficacy under field conditions. However, the use of the adjuvant did not reveal whether the efficacy of the treatment was exclusively associated with the red dye combined with kaolin, or with the combined effect of all the products. We have thus shown that the simple change of color of kaolin is sufficient to increase its repellency against *D. citri*. Adding red food colorant to kaolin amplifies the visual repellency of the treatment by intensifying the masking of the plant color. In addition, kaolin clay is an irritant for insects, reducing their capacity to cause damage to the crop (Palma et al., 2020; Salerno et al., 2020; Saour & Makee, 2004). Research has shown that the kaolin particles adhere to the insect's body after landing on a kaolin-treated surface. This triggers an excessive grooming response from insects to remove the residue from their bodies, ultimately diverting the pest from its feeding and egg-laying activities (Salerno et al., 2020; Saour & Makee, 2004).

Current results also indicate that plant-based essential oils can further enhance control of *D. citri* when combined with kaolin clay by interfering with its olfactory system in addition to the tactile and visual systems disrupted by kaolin. The detection and selection of host plants by *D. citri* are significantly influenced by a combination of visual and olfactory cues, which operate in tandem. Wenninger et al. (2009) and Sétamou et al. (2012) emphasize the crucial role played by these cues during the process of host plant infestation by *D. citri*. Of all kaolin–essential oils spray combinations, kaolin and thyme oil substantially suppressed the response of *D. citri* to normally attractive citrus odors and colors, compared with kaolin alone. Only 3.22% of the psyllid population installed on citrus when thyme oil and kaolin were combined compared to 20.7% in the kaolin only treatment, and 41.2% on untreated citrus. Repellency against psyllids was found with the combination of coriander oil and kaolin (9.3%) as compared to kaolin alone (16.6%). Nevertheless, we did not find significant repellency of lavender oil and kaolin, and the combination of fir oil and kaolin as compared with kaolin alone. These results differed from other studies demonstrating the repellent effects of lavender and fir oils on psyllid populations (Chuang et al., 2023; Kuhns et al., 2016; Mann et al., 2012). However, in those studies, lavender and fir oils were tested against untreated control and not against kaolin-treated plants. In this study, we also evaluated only a single concentration of each essential oil. Given that *D. citri* is known to be sensitive to varying levels of volatile compounds (Amorós et al., 2019; Patt et al., 2011), exploring a range of concentrations in future research could reveal additional effective levels of these essential oils.

Notwithstanding their higher repellency effect, both combinations of thyme oil and kaolin, and thyme oil and red kaolin induced a similar response from adult psyllids to plant infestation. Thyme oil enhanced the kaolin treatment by repelling *D. citri* from citrus through odor cues. Moreover, adsorption properties of kaolin may reduce the volatility of thyme oil as the active ingredients of essential oils are highly volatile, and increase its persistence (Johnston et al., 2022; Nguemtchouin et al., 2009). Nano clays are widely used in pesticide formulations as adsorbents to minimize pest damage by improving pesticide efficacy and residual activity (Lagaly, 2001; Nguemtchouin et al., 2009). Recently, Johnston et al. (2022) have studied the synergistic effects of kaolin and limonene on silverleaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae). The results demonstrated an additive effect of combining limonene and kaolin in repelling adult whiteflies and subsequently on controlling whitefly development on plants. The combination of limonene and kaolin also led to greater persistence of limonene throughout the experiment, whereas limonene alone became less effective over time. Similarly, the combined effects of four repellents (limonene, geraniol, cumin oil, and citral) and kaolin were evaluated in the management of whitefly populations in squash crops, and all combinations proved more effective in reducing whitefly populations than either product alone (Gonsiorek, 2022). Finally, kaolin-based treatments highly reduced female oviposition activity under stable laboratory conditions, with the number of flushes limited to 2–3, and plants thoroughly covered with sprays. However, these results are probably exacerbated by the laboratory conditions. In field situations, the number of oviposition sites (new emerging leaves) is higher with less kaolin coverage. Specifically, any new emerging buds occurring after kaolin application are not covered by clay.

ACPs and HLB remain the most economically damaging threats to citrus crops. Combining clay, thyme oil, and red pigments may represent a sustainable alternative to conventional insecticides to be validated under field conditions. Our findings open new avenues for further research into the combination of essential oils and nano clays. Repellents have been shown to have low residual activity due to their high volatility properties. Nano clays may help to obtain a slow release of those essential oils and increase their efficacy. This approach has the potential for wide implementation in conventional and organic agriculture farms and in residential habitats.

AUTHOR CONTRIBUTIONS

Romain Exilien: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing – original draft; writing – review and editing. **Jawwad A. Qureshi:** Funding acquisition; project administration; supervision; writing – review and editing. **Xavier Martini:** Conceptualization; formal analysis; funding acquisition; methodology; project administration; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data of this article are available through request to the corresponding author: xmartini@ufl.edu.

ORCID

Jawwad A. Qureshi https://orcid.org/0000-0001-9076-4079
Xavier Martini https://orcid.org/0000-0002-0556-3465

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