

# Repelling whitefly (*Bemisia tabaci*) using limonene-scented kaolin: A novel pest management strategy

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## ABSTRACT

Despite intensive control efforts, whitefly *Bemisia tabaci*, remains a difficult pest to manage due to its wide host range and ability to easily switch feeding between crop hosts and surrounding weed hosts. To develop more economical management strategies, the combination of kaolin and limonene was tested as a natural organic repellent against whiteflies. Whitefly host selection was compared between kaolin and limonene-coated tomato and control plants using closed cage bioassays. Combining limonene and kaolin had an additive effect on repelling adult whiteflies compared with the control. Field trials were conducted during two fall seasons in Quincy, Florida. In the 2019 field trial, during dry conditions, tomatoes treated with kaolin + limonene (K + L) had a three to four-fold reduction in *B. tabaci* adult populations compared to controls and a two to three-fold reduction compared to kaolin-only and limonene-only treatments. In addition, infection with *Tomato Yellow Leaf Curl Virus* (TYLCV) was at least two times lower on average in K + L treatments compared to the control. This translated to a two-fold increase in marketable yield of tomatoes harvested from K + L treatments. In fall 2020, due to wetter conditions we only observed a decrease in adult populations on plants in the K + L treatment compared to the control, yet no difference in TYLCV incidence between treatments. Limonene-scented kaolin's effectiveness as a whitefly repellent seems to depend largely on rainfall. In dry conditions, more effective control of whiteflies and of TYLCV was achieved.

## 1. Introduction

Silverleaf whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), remains one of the most destructive agricultural pests worldwide due to its ability to feed on a wide variety of economically important crops and its transmission of over 150 different geminiviruses (Lapidot and Polston, 2010). Current integrated pest management programs continue to encounter problems in dealing with the short and long-range dispersal of *B. tabaci*. In Florida, this is due to whitefly populations rapidly exploding due to continuous host availability throughout the growing season with high likelihood of these insects dispersing from non-crop hosts, row crops or areas with less insecticide control such as organic farms (Mazzi and Dorn, 2012). These pest management challenges are further exacerbated by *B. tabaci*'s fast development rate of about two weeks from egg to adult which leads to

many generations within a relatively short time frame that gives rise to population resistance to many widely used insecticides including neonicotinoids (Palumbo et al., 2001; Yao et al., 2017). To mitigate problems associated with insecticide resistance, alternative, non-chemical solutions need to be made available to growers that keep whiteflies from settling on the crop.

Whiteflies are diurnal, phytophagous insects, that rely on a combination of visual and olfactory cues when foraging and selecting a host (Johnston and Martini, 2020). Concerning visual cues, experiments involving Y-tube choice tests have shown certain wavelengths of light such as those corresponding to the color yellow are more attractive to *B. tabaci*, influencing movement and host choice. Pest management practices have taken advantage of this behavioral aspect by using methods such as yellow sticky card traps to monitor and suppress whitefly populations (Gu et al., 2008). Volatile chemical cues also play

; K+L, Kaolin with limonene (also referred as limonene-scented kaolin); TYLCV, Tomato Yellow Leaf Curl Virus; HS-SPME, headspace-solid phase microextraction.

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important roles in whitefly host selection or avoidance as evidenced by how surfactants, oils, kaolin, and other products can repel or reduce *B. tabaci* settlement on a host (Baldin et al., 2015; Liang and Liu, 2009; Liu and Stansly, 2000).

Pest management strategies can take advantage of visual and volatile host selection cues by employing substances that reduce whitefly attraction to target crops. Since *B. tabaci* became increasingly problematic across the world in the 1980s (De Barro et al., 2011), many studies have investigated alternatives to conventional insecticides or programs that limit direct insecticide use by combining with non-chemical methods (Aslan et al., 2004; Naranjo et al., 2004; Riley and Srinivasan, 2019; Shun-xiang et al., 2001). An example is the use of pyrethroid-treated nets in Kenya field trials that resulted in significant *B. tabaci* population reductions on protected tomato plants and a 2-fold increase in mortality (Martin et al., 2014). Additional non-chemical methods that have met varying degrees of success include cultural control, biological control, temporal control, and genetically resistant cultivars, however, growers still require novel solutions for effective whitefly management (Horowitz et al., 2011).

One such solution that shows much promise is kaolin, a mineral clay that reduces whitefly oviposition rates and number of adults settling when sprayed on vegetable leaves (Liang and Liu, 2009). One study has shown kaolin applications can reduce up to 91% of adult whitefly populations in bean crops compared to untreated controls, and much higher than synthetic insecticide treatments (Núñez-López et al., 2015). Kaolin has also shown success with controlling other agricultural pests as well, such as thrips and Asian citrus psyllids (Larentzaki et al., 2008; McKenzie et al., 2002; Spiers et al., 2004).

In lieu of traditional insecticides, kaolin may also work as an effective management tool when combined with semiochemical repellents. Many compounds in plant-derived essential oils repel whitefly adults and reduce oviposition (Deletre et al., 2016). Even semiochemicals such as the sesquiterpenes, 7-epizingiberene or curcumen, from host species such as tomato reduce whitefly host selection when these semiochemicals are isolated, purified, and applied topically to the tomato host leaves (Bleeker et al., 2011). Surprisingly, few attempts have been made to combine kaolin and essential oils. This is despite the fact that kaolin has been described as a good adsorbent for essential oil components, including sabinene,  $\beta$ -pinene,  $\alpha$ -pinene and  $\beta$ -phellandrene (Nguemtchouin et al., 2009). However, the release rate of essential oil components from the impregnated kaolin clay has never been tested. To the authors' knowledge, only a single study has been conducted combining kaolin and single plant-derived semiochemicals or essential oils (lemongrass, and tea tree oils) in a single treatment, but the experiment was conducted to control thrips, *Frankliniella occidentalis*, and *Tomato spotted wilt virus* (TSWV), and the treatments were not tank-mixed but applied separately. When kaolin and essential oils were associated it resulted in the reduction of up to 50% of TSWV incidence compared with the control, whereas essential oil and kaolin alone were less efficient in reducing TSWV (Reitz et al., 2008). We postulate that the combination of kaolin and limonene in a single tank mix has the potential to provide better synergistic control than either kaolin or limonene alone, in addition to providing better pest management alternatives to insecticides.

We selected limonene, a cyclic monoterpene derived from citrus essential oil, to be used in conjunction with kaolin clay in order to reduce the number of *B. tabaci* on tomato. Several past studies and preliminary work have shown that limonene exhibits mild to moderate repellency to adult whiteflies (Deletre et al., 2016; Tu and Qin, 2017). In addition, compared to other repellents efficient against whiteflies, limonene has a moderate price and has been registered as a pesticide by the United States Environmental Protection Agency since 1958 (Case 3083). We hypothesized that combining both limonene and kaolin would have synergistic or additive effects towards whitefly control. To test this theory, we evaluated the effects of both kaolin and limonene on whitefly behavior using cage bioassays under laboratory conditions and

field trials for two consecutive years. We also investigated the rate of limonene release by kaolin over time using gas chromatography-mass spectrometry (GC-MS) in order to examine how the adsorbent properties of kaolin extend limonene's window of efficacy, offering insights into the synergistic properties between these two compounds.

## 2. Materials and methods

### 2.1. Closed cage assays

*Bemisia tabaci* biotype B were originally collected from infested straightneck squash, *Cucurbita pepo* L. cv. Recticollis, in Quincy, Florida in Fall (2018). Whitefly biotype was determined through PCR testing (McKenzie et al., 2009). Whiteflies were reared on TYLCV-infected tomato, *Solanum lycopersicum*, in whitefly-proof screen cages in a rearing chamber ( $26 \pm 2^\circ\text{C}$ ) for at least five generations. All closed-cage assays used unaged adults taken from these laboratory-maintained colonies.

Each cage-assay was set up with two tomato plants approximately 2–3 months old (30–38 cm tall) on either side to act as an open choice test for all introduced whiteflies. A single trial consisted of three medium mesh cages (33 x 33 x 61 cm), each cage containing an untreated control on one side and a treated plant on the other with a distance of 30 cm between both plants. The three different treatments consisted of 1) tomato plant coated in kaolin clay (Surround® WP, BASF Florham Park, NJ, USA) vs an untreated tomato plant, 2) tomato plant coated with DL-limonene (>95% purity, Fischer Scientific Hampton, NH, USA) vs an untreated tomato plant, and 3) tomato plant coated with a mixture of limonene-scented kaolin (K + L) vs an untreated tomato plant. Kaolin and limonene were applied to tomato plants at rates of 30 g/L and 5 mL/L (0.5% concentration), respectively. Total spray volume for each treatment application was 100 mL. At the start of each trial, 50 unsexed and unaged whiteflies were aspirated into a small, cylindrical plastic vial and released in the center of each mesh cage. After one, three, seven, 10, and 14 days, both plants were visually inspected, and the total number of adult whiteflies counted. Each plant was vigorously shaken separately after each count to force whiteflies off their host plant and to make a new host selection at the start of the next time interval. This process was randomized and conducted to avoid host selection bias since adult whiteflies are less likely to move to a new host once an acceptable host has been selected. In addition, the act of shaking the plants did not affect adhesion of kaolin to the plant itself. To maintain a continuous distribution of whiteflies in the cage assays over time, an additional 50 whiteflies were introduced into each cage after seven days to account for population decline due to age-related mortality and escaped whiteflies.

Overall, there were four closed cage assay replicates for each treatment combination: kaolin vs control, limonene vs control, and K + L vs control. These replicates were conducted at different times over a period of several months and plants were rotated each replicate to avoid positional bias. All replicates were separated by black, corrugated plastic barriers (0.5 cm thick) and conducted in stable laboratory conditions ( $22^\circ\text{C}$ , RH = 40%) with uniform light diffusion.

### 2.2. Tomato field trials

Field trials were conducted in fall 2019 and 2020 at the North Florida Research and Education Center in Quincy, Florida. The field trials were organized in a randomized block design of two rows with eight plots per row, and plots consisted of 20 tomatoes, *S. lycopersicum* L. (47 R Quincy cultivar) separated with two plants on both ends to act as buffer. All plot beds were covered with standard black plastic mulch, and tomatoes were planted in the last week of August and harvested in the first week of November. The first treatments applications were made approximately one week after transplant of three-week old seedlings and every subsequent week throughout the duration of the field trial. Four separate treatments were included: 1) untreated control, 2) kaolin clay (Surround® WP), 3) limonene oil, and 4) mixture of kaolin and limonene (K

+ L). Each treatment was replicated four times in the field for a total of 16 plots. Tomatoes were not sprayed with any insecticide during the whole experiment. Standard cultural practices employed by tomato growers in north Florida were used for planting, fertilizing, application of herbicides, fungicides, and irrigation.

Kaolin was applied to plants at half the industrial rate at ¼ lbs. kaolin per gallon of water or 30 g/L with a spray rate of approximately 4.7 L/ha. Half rates of kaolin were used since it was sufficient to induce whitefly repellency in the cage assays (see results), and the full industrial rate led to clogging machinery of the handheld backpack tank mix. Limonene only treatments were applied at 0.5% oil concentration rate using a formulation of 5 mL of limonene per liter of distilled water. To improve the solubility of limonene only treatments, 0.26 mL/L of Tween-20 (Tween®20, Fisher Scientific Hampton, NH) was used as an emulsifier. In K + L treatments, the same concentrations of kaolin and limonene were used for each liter in combination with Tween-20. Kaolin and limonene were mixed with a food mixer for 20 min. Water and Tween-20 were added to the kaolin and limonene mixture just before being sprayed in the field. During field applications, a handheld backpack sprayer with a double headed nozzle and cones on the wand was used to apply kaolin/limonene treatments equally to the top and bottom of tomato leaves where applications were applied on top of the plant during the first pass and on bottom during the second.

### 2.3. Whitefly collection and TYLCV assessment

Adult whitefly populations were assessed in the field by selecting a tomato leaf at mid-plant height and visually counting the total number of whiteflies present on the leaf. This process was repeated on nine additional plants for a total of 10 plants assessed in each treatment. Once baseline adult populations were established of at least two to three whiteflies per leaflet, nymph data was additionally collected about three weeks after the start of each field trial. For each treatment, one terminal or lateral leaflet was taken from the middle section from each of the 10 tomato plants which were relatively uniform in size. Each leaflet was then examined under a Zeiss Stemi DV4 stereomicroscope (Zeiss, Jena, Germany) and the total number of whitefly nymphs was counted regardless of instar stage.

In field trials, the infection stage of TYLCV in each tomato plot was evaluated using a visual rating of 0–4, where 0 indicated an absence of TYLCV and 4 indicated that most or all plants in a treatment exhibited severe or fatal symptoms due to the virus. Due to the inherent subjectivity of visual rating methods, two separate observers conducted visual ratings for different plots in the field each week. Visual ratings 1 through 4 can be qualitatively described as follows where: 1) plants exhibit mild TYLCV symptoms, 2) plants exhibit mild to moderate symptoms, leaf curling is present, 3) plants exhibit moderate symptoms, curling/yellowing/stunting readily apparent throughout plant, and 4) plants exhibit late-stage/severe TYLCV symptoms, necrosis may be present (S.1). Final plot ratings were determined by taking the average visual rating of all 10 plants in a treatment; rt-qPCR was conducted on 10 leaf samples taken from treatments with a rating of 1 and 2 to confirm the presence of TYLCV, and from rating 0 to confirm the absence of TYLCV. All rt-qPCR procedures were conducted at the North Florida Research and Education Center Plant Diagnostic Clinic as described in (Johnston and Martini, 2020).

### 2.4. Limonene release measurement

Volatile organic compounds (VOCs) were collected *in vitro* by headspace-solid phase microextraction (HS-SPME) from polystyrene petri dishes (FB0875712, Fisherbrand®) sprayed with one of three solutions using a pump sprayer (10030, Chapin®). The polystyrene petri dish Solution 1 consisted of limonene (0.5%: DL-Limonene); solution 2 consisted of kaolin (30%) + limonene (0.5%); solution 3 consisted of kaolin (30%) + limonene (0.5%) + Tween-20 (0.03%) (K + L + T). The

VOCs from each treatment were collected in a metal collection chamber at one, three, five, seven, and 15 days from application of one of the three solutions under laboratory conditions (S.2). For each treatment there were three different petri dishes as replicates. Prior to volatile collection treated petri dishes in the collection chamber were sealed with aluminum foil for 10 min to create a volatile headspace. The headspace volatiles were collected by 50/30 µm DVD/CAR/PDMS, Stableflex 24 Ga, fiber assembly (57328-U, Supelco, Bellefonte, PA) housed in a SPME manual holder (57330-U, Supelco, Bellefonte, PA). A collection blank was run before exposing the fibers to petri dishes with one of the treatments. After 10 min of exposure to the VOCs, the fibers were removed and placed in the GC-MS. Before starting sampling, fibers were preconditioned at 270 °C for 10 min. The VOCs collected by HS-SPME were manually injected and desorbed at 270 °C for approximately 15 min into the inlet port (splitless mode) of a Thermofisher 1310 GC-MS, equipped with Trace GOLD TG-5MS GC Column 30 m × 0.25 mm, 0.25 µm (26098–1420, Thermofisher, Waltham, MA). Helium was used as the carrier gas at a linear flow velocity of 1 mL/min. The GC oven programmed temperature ramp began with the low temperature of 50 °C for 2 min, then the temperature was increased at 50 °C/min to 89 °C, 0.5 °C/min to 91 °C, 50 °C/min to 260 °C then at 50 °C/min to 270 °C, and finally held at that temperature for 2 min. Data collection and analysis was undertaken using the Thermo Scientific™ Dionex™ Chromeleon™7 Chromatography Data System Version 7.2.6.

To calculate the quantity of limonene evaporating from the treated petri dishes, a serial dilution was conducted using HS-SPME. Limonene was diluted in dichloromethane, CH<sub>2</sub>Cl<sub>2</sub> (270997, Sigma Aldrich) at the initial concentration of a 1:19 limonene-dichloromethane. Thereafter, the solution was diluted by one-third, seven times. Each dilution was repeated three times. The samples from the serial dilution were collected by HS-SPME as previously described. The same protocol was followed as described in the paragraph above for collecting the data with the Thermofisher® 1310 gas chromatograph-mass spectrometer. Data collection and analysis was undertaken using the Thermo Scientific™ Dionex™ Chromeleon™7 Chromatography Data System Version 7.2.6.

### 2.5. Statistical analysis

All statistical analyses were conducted with the statistical software R (R 3.5.1, RStudio, Boston, MA). For each caged bioassay, a paired *t*-test with a two-tailed distribution was used to evaluate the difference in mean whitefly populations between two treatments for each time point of the 14-day period ( $\alpha = 0.05$ ). Due to unequal distribution between sample sizes, data were log transformed before paired *t*-test analysis. To compare the efficacy of kaolin, limonene and K + L treatments in the laboratory assay, we conducted a generalized linear model with binomial distribution with the distribution of whiteflies between treated plants and control plants as the response and the treatment as the factor.

For all field trials, data were averaged per plot to avoid pseudo-replication. After testing for normal heteroscedastic data distribution, a two-way ANOVA for repeated measures with time and treatments as fixed variables and blocks as a random factor was used. As nymph data of 2019 were not normally distributed, a generalized linear model with Poisson distribution was used to calculate the differences across treatments. Differences in means were compared using a Tukey's HSD *post hoc* analysis with  $\alpha = 0.05$ . Two-way ANOVA was also conducted on visual data ratings for both years where treatment and trial week were treated as factors. To determine if there was a synergistical effect or an additive effect between limonene and kaolin, we conducted multilinear analyses with time, presence/absence of kaolin (1 or 0), and presence/absence of limonene (1 or 0) and there interaction as factors.

For analysis of limonene volatile release, we used a single, two-parameter decay model:

$$y = y_0 e^{-kt}$$

where  $y$  is the quantity of VOCs at time ( $t$ ),  $y_0$  is the initial quantity of limonene VOCs recorded and  $k$  is the constant at which limonene evaporates. VOCs were fit to exponential decay model using the R packages 'drc' (Ritz et al., 2015) and 'aomisc' (Onofri, 2020). The quantity of VOCs was analyzed with the 'glm' function using the 'MASS' (Venables and Ripley, 2002) package and a quasi-Poisson distribution.

### 3. Results

#### 3.1. Closed cage assays

Overall whitefly populations remained significantly lower on limonene, kaolin, and K + L treated plants than on control plants across the 14-day trial period (Fig. 1). For limonene treatments, whitefly counts were lower on day 3 ( $t = 9.105$ ,  $P = 0.003$ ), day 7 ( $t = 5.359$ ,  $P = 0.013$ ), and day 10 ( $t = 3.809$ ,  $P = 0.032$ ), but not day 1 ( $t = 2.563$ ,  $P = 0.087$ ) or day 14 ( $t = 0.505$ ,  $P = 0.648$ ). For kaolin treatments, whitefly counts

were lower on day 1 ( $t = 3.038$ ,  $P = 0.040$ ), day 7 ( $t = 3.013$ ,  $P = 0.048$ ), day 10 ( $t = 8.788$ ,  $P = 0.003$ ), and day 14 ( $t = 4.774$ ,  $P = 0.017$ ), but not on day 3 ( $t = 1.162$ ,  $P = 0.329$ ). For K + L treatments, whitefly counts were lower on all time intervals including day 1 ( $t = 9.356$ ,  $P = 0.002$ ), day 3 ( $t = 4.584$ ,  $P = 0.019$ ), day 7 ( $t = 5.007$ ,  $P = 0.0053$ ), day 10 ( $t = 4.290$ ,  $P = 0.023$ ), and day 14 ( $t = 14.491$ ,  $P < 0.001$ ). While all three treatments had lower numbers of whitefly host selection compared to controls, K + L exhibited higher repellency than either kaolin or limonene alone (GLM with binomial distribution:  $\chi^2 = 173.62$ ,  $df = 3$ ,  $P < 0.001$ ). While kaolin and K + L treatments maintained effective repellency throughout the course of the experiment, there was no significant difference between limonene and control treatments after 14 days.

#### 3.2. Weather data

The 2019 and 2020 fall seasons in Quincy, Florida differed dramatically in weather-related abiotic conditions, particularly in the amount of daily average precipitation (Fig. 2). For the month of September, the primary month of field trials, daily average precipitation was 0.0094 cm in 2019 and 1.3 cm in 2020. In addition, September 2019 was much warmer with the average daily temperature of 26.4 °C compared to September 2020 with a temperature of 24.2 °C. All weather data were collected by the Florida Automated Weather Network (FAWN), station ID #140, less than 500 m away from the site of both field trials.

#### 3.3. Tomato field trials

In fall 2019, we found a significant difference across all treatments for the number of *B. tabaci* adults found on the plants ( $F = 29.693$ ;  $df = 3, 63$ ;  $P < 0.001$ ) (Fig. 3A). Throughout the entire trial period, K + L, limonene and kaolin had significant lower number of adults as compared to control plots ( $P < 0.001$ ,  $P = 0.003$ ,  $P = 0.017$ , respectively) and K + L treatment had lower adults than kaolin and limonene treatments ( $P = 0.002$ ,  $P = 0.009$ , respectively); however, there was no difference between kaolin and limonene treatments ( $P = 0.643$ ). Time was also a significant factor ( $F = 38.729$ ;  $df = 7, 63$ ;  $P < 0.001$ ) and there was a significant interaction between time and treatment ( $F = 4.462$ ;  $df = 21, 63$ ;  $P < 0.001$ ). Specifically, K + L had significantly lower number of adults as compared to control for weeks 2–8, and lower number of adults as compared to kaolin for weeks 4–8, and lower number of adults as compared to limonene for weeks 2, and 4 to 8 (Fig. 3a).

Regarding nymphs counts in 2019, we found a significant difference between treatments during the whole season ( $\chi = 1491.83$ ;  $df = 3, 88$ ;  $P < 0.001$ ). On the overall field trials K + L, limonene and kaolin had significant lower number of nymphs as compared to control plots ( $P < 0.001$ ,  $P < 0.001$ ,  $P = 0.009$ , respectively). K + L treatment also had lower average nymph counts than kaolin only ( $P < 0.001$ ) and limonene only ( $P = 0.027$ ) treatments. Nymph populations were significantly lower on kaolin only treatments compared to limonene only treatments ( $P = 0.031$ ). Time was a significant factor ( $\chi = 115.46$ ;  $df = 1, 88$ ;  $P < 0.001$ ), but there was not interaction between time and treatments. For each individual week, except for counts on week 3 there was significant differences among treatments, notably with K + L having significant ( $\alpha < 0.05$ ) lower number of nymphs from week 3 to week 8 as compared to control plots (Fig. 4a).

Fall 2020 field trials revealed similar albeit less dramatic trends than 2019 field trials (Figs. 3B and 4B). During the whole duration of the study, we found a significant difference between treatments for adult counts ( $F = 14.450$ ;  $df = 3, 96$ ;  $P < 0.001$ ) (Fig. 3B), and K + L and kaolin treatments had lower adult populations than untreated controls ( $P < 0.001$ ,  $P = 0.003$ , respectively). Limonene alone was not significantly different from control ( $P = 0.183$ ), while K + L treatments performed better than limonene in reducing whitefly population ( $P = 0.010$ ), but not than kaolin ( $P = 0.501$ ). Time was a significant factor ( $F = 39.079$ ;  $df = 3, 96$ ;  $P < 0.001$ ), and there was a significant interaction between treatments and time ( $F = 2.951$ ;  $df = 24, 96$ ;  $P < 0.001$ ). K + L

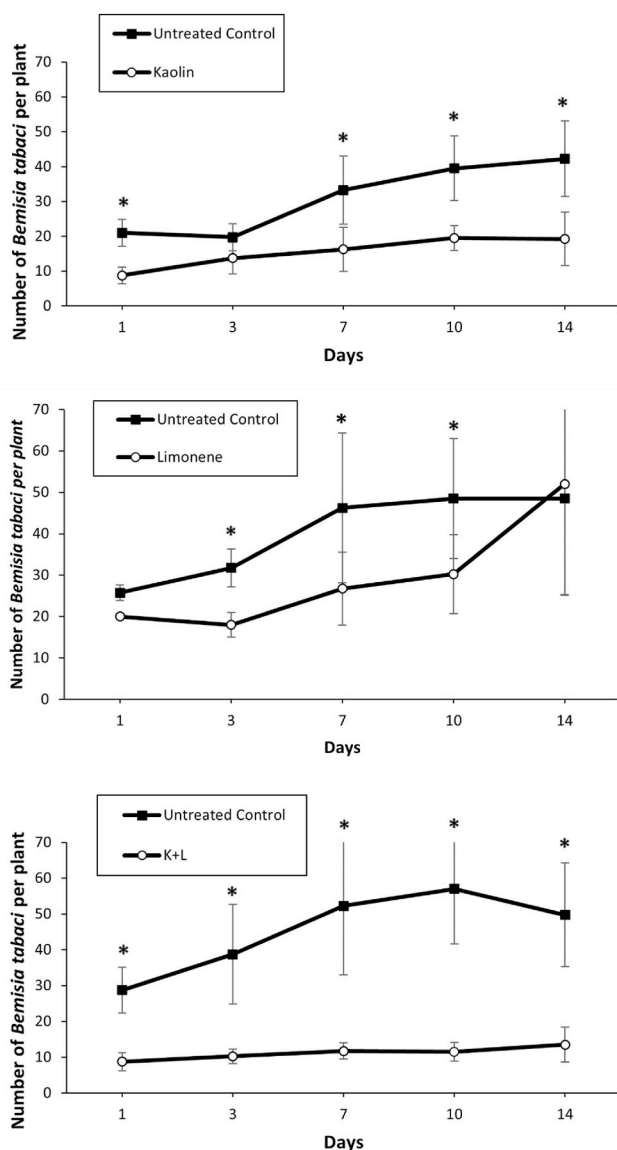
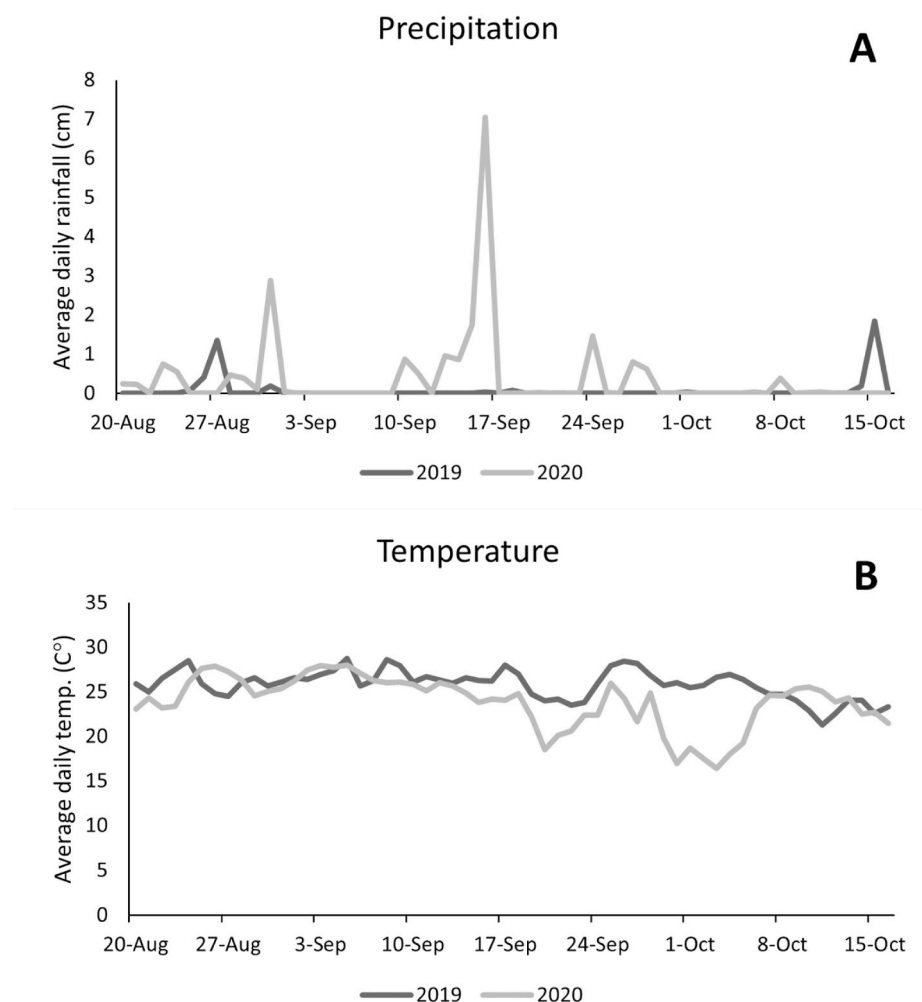


Fig. 1. Average number ( $\pm$ SE) of *Bemisia tabaci* adults choosing treated tomato plants vs. untreated controls over a 14-day period ( $n = 50$ ). (A) Kaolin clay vs untreated control, (B) limonene only vs untreated control, (C) kaolin + limonene (K + L) vs untreated control \*Indicates significant difference between treatments at a given time interval based on two-tailed binomial test ( $P < 0.05$ ).





**Fig. 2.** Average daily precipitation (cm) (A), and temperature (°C) (B) recorded in Quincy, FL during fall field trials, 2019 and 2020. All data was collected by Florida Automated Weather Network (FAWN), station ID #140.

had significantly less whitefly adults than control for weeks 2–5, and 7 to 8; while kaolin had significantly less whitefly adults than control for weeks 4, 6 and 7; limonene less adults than control for week 7 only (Fig. 3b).

Regarding *B. tabaci* nymph data collected in 2020, treatments had a significant effect ( $F = 5.032$ ;  $df: 3, 48$ ;  $P = 0.017$ ) but only kaolin treatment was significantly different as compared to control ( $P = 0.030$ ) throughout the season. Limonene ( $P = 0.791$ ) and K + L ( $P = 0.998$ ) did not perform better compared to controls. On individual week Kaolin was significantly lower than control for weeks 4 and 5 and for weeks 7 and 8 (Fig. 4b).

Finally, the multi linear regressions did not found any significant positive interactions ( $\alpha < 0.05$ ) between limonene and kaolin for adults and nymphs for both 2019 and 2020, indicating that the effects of kaolin and limonene found in the field seem mostly additive.

### 3.4. TYLCV visual ratings

In 2019 field trials, we found a significant difference among treatments regarding visual rating ( $F = 9.262$ ;  $df = 3, 96$ ;  $P < 0.001$ ). It was found that limonene treatments displayed the most apparent TYLCV symptoms towards the beginning of the trial while control and kaolin treatments had the most severe visual symptoms towards the end (Fig. 5). K + L treatments alone, displayed a lower average visual rating than control treatments thorough the season ( $P < 0.001$ ) while kaolin only ( $P = 0.831$ ) and limonene only ( $P = 0.172$ ) showed no significant

difference. Additionally, there was no difference between kaolin only and limonene only treatments based on visual ratings ( $P = 0.162$ ). In 2020, treatment was a significant factor for TYLCV ratings overall ( $F = 4.82$ ,  $df = 3$ ,  $P = 0.002$ ), yet pairwise post-hoc analysis revealed no significant difference in visual ratings between any two individual treatments.

### 3.5. Yield data

In 2019 (Fig. 6), K + L treatments yielded twice as many large, graded tomatoes compared to control and kaolin only treatments ( $F = 4.805$ ;  $df = 3, 12$ ;  $P = 0.020$ ). In addition, K + L yielded higher total weight ( $F = 2.954$ ;  $df = 3, 12$ ;  $P = 0.075$ ) than other treatments by a factor of nearly 2-fold while maintaining a lower percentage of tomatoes culled. In 2020, a combination of bacterial, and fungal blight due to wet conditions yielded no harvest due to a lack of tomatoes and no yield data are reported for any of the treatments.

### 3.6. Volatile collection

Calibration curve resulting from one-third serial dilution yielded an r-squared value of 0.98. The quantity of limonene VOCs collected in the treatment headspace was significantly different among the three treatments ( $F = 150.94$ ,  $df = 2, 43$ ,  $P < 0.0001$ ) and across time ( $F = 7.89$ ,  $df = 2, 41$ ,  $P = 0.001$ ) (Table 1, S.3). The addition of kaolin and Tween-20 to limonene solutions delayed the release of limonene VOCs (Solution K

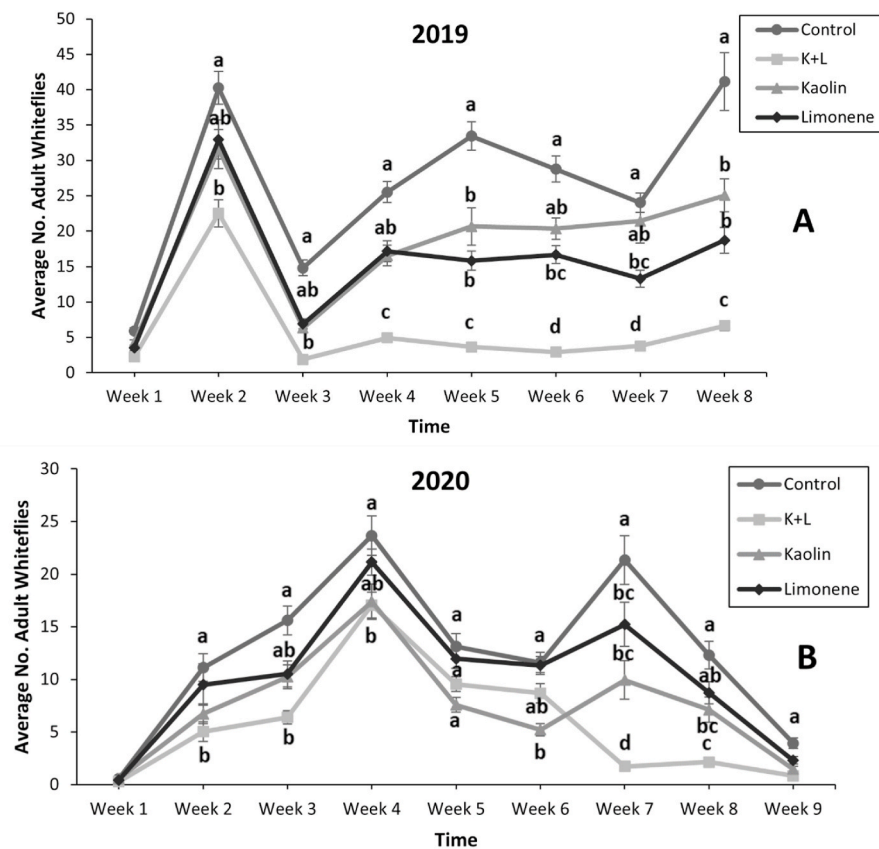


Fig. 3. Average number of *Bemisia tabaci* adults ( $\pm$ SE) per tomato plant by treatment over time 2019 (A), 2020 (B). Each graph shows the same experiment repeated in the fall season of different years. Differences between treatments at individual weeks are denoted by different letters ( $\alpha = 0.05$ ).

+ L:  $k = 0.87 \text{ d}^{-1}$  and Solution K + L + T:  $k = 0.74 \text{ d}^{-1}$ ) compared to limonene alone (Solution L:  $k = 0.56 \text{ d}^{-1}$ ). The interaction between day and treatment was not significant ( $F = 0.40$ ,  $df = 2, 39$ ,  $P = 0.67$ ). Solutions of K + L and K + L + T had more limonene captured in their headspace across time compared to limonene alone (Table 1, S.3). The solution of K + L + T had the highest quantity limonene on the final day of data collection (Table 1).

#### 4. Discussion

As *B. tabaci* has become one of the most problematic pests to manage in row crop vegetables, widespread and overabundant use of insecticides has been one of the most common practices from growers (Horowitz et al., 2020). This, in turn, has led to an increasing rise of insecticide resistance (Palumbo et al., 2001), particularly neonicotinoids (Schuster et al., 2010), which has led to the growing need for alternative non-insecticidal solutions in whitefly pest management.

One potential solution is the application of kaolin clay which acts as a natural contact repellent, making vegetable crops less favorable as a selected host (Spiers et al., 2004). When searching for a suitable host, whiteflies respond differently to environmental cues where visual stimuli influence host selection strongly at first, and both olfactory and gustatory stimuli act as secondary influencers once generalized host searching is completed (van Lenteren and Noldus, 1990). Since *B. tabaci* are shown to be visually attracted to yellow and green wavelengths of light reflected by certain vegetable host plants (Hasanuzzaman et al., 2016; Johnston and Martini, 2020), spraying leaves with kaolin clay that covers the plant in a white film may have the potential to disrupt regular visual cues. In addition to whiteflies, kaolin also acts as a feeding deterrent and interferes with settling behavior in other agricultural pests such as the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) (Miranda et al., 2018). Kaolin has the added benefit of

reducing heat stress on the plant without photosynthesis interference or reduction in the number of visiting pollinators since it is a non-toxic, inorganic mineral (Spiers et al., 2004). Using botanic or plant-derived oils that are naturally repellent to *B. tabaci* such as limonene can further enhance pest management by targeting the olfactory system in addition to the visual system targeted by kaolin. Both kaolin and repellent botanic oils combined show strong potential to reduce the arriving numbers of whitefly adults settling on a host and can subsequently lower the oviposition rate, nymph population, and disease burden of the host as well.

Closed cage assays evaluated whitefly host selection under stable laboratory conditions. Over a 14-day period, closed-cage kaolin treatments maintained significantly higher repellency compared to the control, a trend that was not seen in closed-cage limonene treatments where limonene-coated plants exhibited no difference compared to the control by the end of the trial. Despite limonene losing its efficacy, combining this repellent with kaolin in the K + L treatments led to higher repellency than either component alone compared to controls and this trend stayed consistent to the end of the trial period. These results can be explained by both the stable environment of closed cage assays and the properties of kaolin acting as an adsorbent for limonene in the same way kaolin can act as an adsorbent for other essential oil components (Nguentchouin et al., 2009).

Since kaolin is a clay-based deterrent, it is highly susceptible to being washed away by rainfall and its efficacy is heavily dependent on weather conditions as seen in the contrast between 2019 and 2020 field trials. In the 2019 fall season, the research field received a lack of rain which led to ideal conditions for the application of kaolin and limonene. During this year, both kaolin and limonene treatments saw reduced infestations of both adults and nymphs compared to the control plots, while limonene-scented kaolin treatments had additive effects compared to kaolin or limonene alone. In addition, the lower whitefly populations in

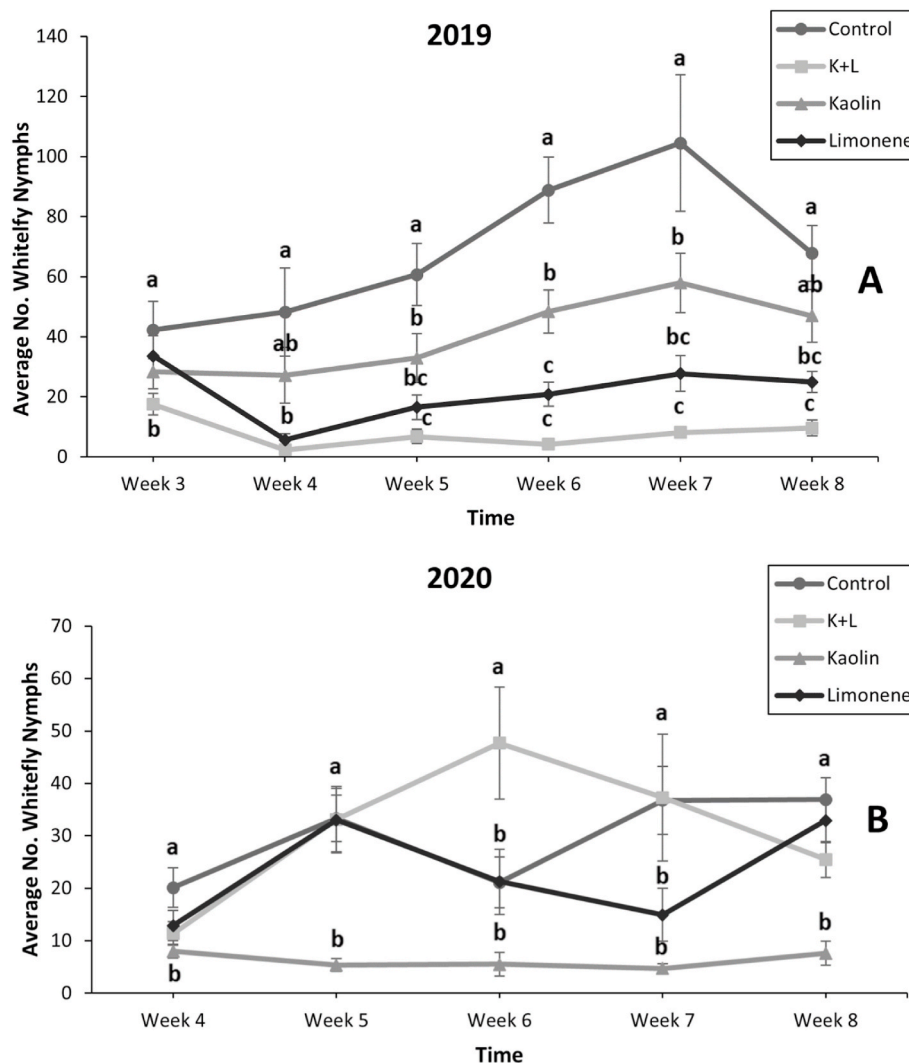


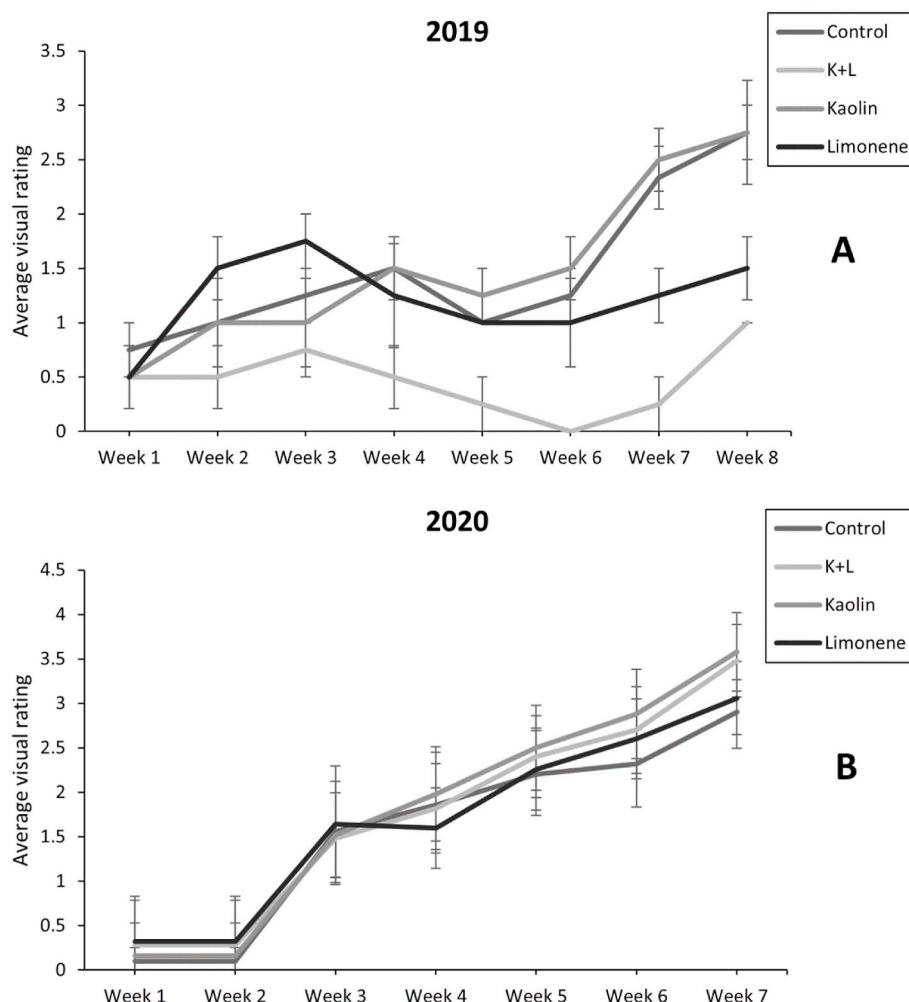
Fig. 4. Average number of *Bemisia tabaci* nymphs ( $\pm$ SE) per tomato plant by treatment over time 2019 (A), 2020 (B). Each graph shows the same experiment repeated in the fall season of different years. Differences between treatments at individual weeks are denoted by different letters ( $\alpha = 0.05$ ).

limonene-scented kaolin treatments correlated with a two-fold increase in quality tomato weight harvested and a 25% reduction in culled tomatoes at the end of the trial period. These results also corresponded to the same trend seen in visual TYLCV ratings where limonene-scented kaolin treatments experienced a two-fold lower average disease rating overall than control and kaolin only treatments. These results are particularly encouraging for areas with low rainfall that suffer high whitefly pressure such as Arizona or California, where limonene-scented kaolin is predicted to be highly efficient.

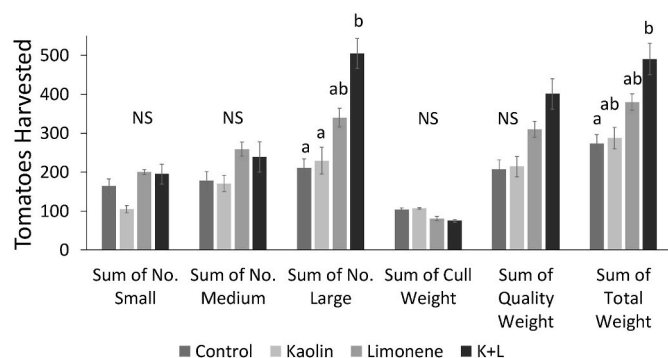
The field data collected in fall 2020; however, was far less remarkable as the efficacy of kaolin was likely reduced by rainfall about 135 times greater than the previous year. A recent study which shows that kaolin can be washed away up to 40% by precipitation supports this conclusion (İTMEÇ et al., 2020). Despite lower efficacy in 2020, both kaolin and K + L treatments still had significantly lower adult whitefly populations compared to untreated controls. However, these numbers were not low enough to prevent oviposition and increase of nymph populations in their respective treatments. This translated to a lack of difference in TYLCV incidence. Additional compounding factors that detracted from the field trial results also arose from biotic factors such as an increased incidence of bacterial and fungal infections due to the wet conditions. Similar effects of how rainfall leads to a decrease in kaolin efficacy can be seen in another study that examined kaolin's ability to prevent aphids from transmitting blueberry scorch virus (BlScV) on

blueberry, *Vaccinium corymbosum* (Raworth et al., 2007). The study found that kaolin's performance was greatly inhibited by rainfall and failed to prevent virus transmission during wet field conditions compared to dry periods where kaolin applications met with success in controlling both the number of aphids settling and plants infected with BlScV. Further evaluation of kaolin during seasons of high precipitation is required for successful implementation under these conditions.

As seen from both lab assays and field trials, limonene-scented kaolin offers considerable protection against *B. tabaci* populations and subsequent disease severity compared to untreated controls during dry weather. During weeks where fields experience heavy rainfall, limonene-scented kaolin may prove ineffective for controlling *B. tabaci*; therefore, alternative treatments during periods of heavy rainfall should be considered. In addition, we found through experimental observation that spraying kaolin and limonene on the underside of tomato leaves proved important as well since this method targets the whitefly's primary feeding location and is less vulnerable to getting washed away (Martini, unpublished data). This is in accordance with previous study finding that kaolin clay is more efficient in repelling *B. tabaci* whiteflies when it is applied on the lower side of the leaf (Liang and Liu, 2009). Application with a tractor-mounted sprayer with higher horsepower facilitates coverage of limonene-scented kaolin on the underside of tomato leaves. Timing of spray applications should also be concurrent with growth of nearby host plants since limonene-scented kaolin is most



**Fig. 5.** Average visual rating of *Tomato yellow leaf curl virus* (TYLCV) symptoms ( $\pm$ SE) per treatment in tomato, 2019 (A), 2020 (B). Treatments with a rating of 1 or higher tested TYLCV positive after qPCR analysis. An average rating of zero indicates no TYLCV was detected from visual symptoms or molecular testing.



**Fig. 6.** Tomato harvest data from Fall 2019, field trial in Quincy, FL. Tomato size was graded using industry standards, and blemished tomatoes were culled. All data is reported ( $\pm$ SD) where quality weight = total weight – cull weight. Different letters indicate significant difference at  $\alpha = 0.10$ . NS: Non significant.

effective as a preventative measure against adult introduction on the crop. Alternative hosts nearby can lead to higher whitefly populations in the target crop as greater numbers of migrating adults are drawn to the field, and migration increases between host crops once one becomes unsuitable (Hilje et al., 2001).

The use of limonene-scented kaolin as part of a whitefly management program still has many potential improvements to consider. Future

**Table 1**

The quantity of limonene release by different treatments with or without kaolin measured using *in vitro* headspace-solid phase microextraction (HS-SPME) across 15 d in laboratory conditions. L = limonene, K + L = kaolin + limonene, K + L + T = kaolin + limonene + Tween-20, SE=Standard Error.

Time from Application (days)	L ( $\mu$ g) $\pm$ SE (n = 3)	K + L ( $\mu$ g) $\pm$ SE (n = 3)	K + L + T ( $\mu$ g) $\pm$ SE (n = 3)
1	738.26 $\pm$ 307.7	2358.47 $\pm$ 547.96	1714.83 $\pm$ 496.94
3	196.97 $\pm$ 23.72	491.75 $\pm$ 229.02	231.31 $\pm$ 47.16
5	143.62 $\pm$ 33.05	193.11 $\pm$ 48.41	231.80 $\pm$ 94.76
7	23.26 $\pm$ 9.55	99.38 $\pm$ 27.13	47.24 $\pm$ 3.89
15	11.31 $\pm$ 6.01	2.80 $\pm$ 1.16	17.84 $\pm$ 7.29

studies would benefit by investigating other compounds/oils that have been identified as *B. tabaci* repellents such as geraniol and citronellol (Deletre et al., 2016), which can also be mixed with kaolin. As *B. tabaci*'s hierarchy of host preference has already demonstrated to be a wide spectrum of attraction (Chang-Chi et al., 1995), it is reasonable to conclude that this insect also has an equally wide spectrum of repellency, where some repellents are likely to display more efficacy than others, including limonene. The clay component of the system can also be improved by researching the development of nanoparticle clays that exhibit higher repellency and better adsorption of botanic oils. Some



examples of promising alternatives to kaolin include bentonite (Constanski et al., 2016) and zinc oxide (Sarhozaki et al., 2020). Since kaolin and limonene exhibit additive repellency effects when mixed, it is also important to ensure a homogenous mixture that does not separate in grower's tanks, which may be achieved by the addition of a better adjuvant into the mixture that improves adsorption. In this study, the persistence of limonene odors on petri dishes was improved by the addition of kaolin and the emulsifier Tween. Further study on different formulations of emulsifiers and kaolin is warranted to improve the persistence of limonene in the field.

## 5. Conclusion

Using novel pest management control methods for whitefly, *Bemisia tabaci*, has become crucial to maintain sustainable agricultural practices in the US. Combining natural resources such as kaolin clay with plant-based repellents such as limonene offers a non-chemical, alternative solution to control these pests. While showing promising efficacy, kaolin applications must be monitored in conjunction with weather as heavy rainfall may wash away treatments. Limonene-scented kaolin can also augment traditional control when tank mixed and applied simultaneously on tomato. These findings offer growers an effective and much-needed control option for maintaining row crop vegetables as widely used insecticide programs have decreased in efficacy due to increased insecticide resistance.

## Author contributions

NJ, JF and XM designed the experiments; NJ, TP, JF, and MP carried out experiments. NJ, TP, and XM wrote the manuscript and conducted the statistical analyses. All authors read, edited and approved the final manuscript.

## Data availability statement

All data was ultimately collected through funding provided by the University of Florida and is, therefore, UF property. The data are available upon reasonable request.

## Declaration of competing interest

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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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cropro.2022.105905>.

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