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Strong dissuasive effect of a synthetic capsaicinoid against whitefly infestation in avocado trees

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Abstract

Whitefly infestation is a major threat to horticultural crops. The control of these sap-sucking insects could be performed by application of insecticides, which may be toxic to untarget species such as honeybees and other beneficial insects. Safety of consumers is a priority and urges the development of alternatives to control pathogens and pests, thus identification of novel natural and/or synthetic products to control insect pests not harmful for humans or pollinators is an urgent need. The persistent seasonal, yearly occurrence of whitefly infestations, offered the opportunity to test promising biostimulant, non toxic molecules as dissuasive products. In this report, we show that whitefly infestation in avocado trees in the field causes the spread of chlorotic halos, blade distortion and wilt of leaves. Through serial aspersions of *N*-vanillyl-octanamide (ABX-I), a synthetic capsaicinoid to the avocado trees, a clear repellent effect was observed against adult whiteflies that approached the trees at sunny hours but did not feed on leaves. The number of whiteflies resting on leaves decreased in the following days after capsaicinoid application. These data reveal the possibility to control whitefly pest via application of a capsaicinoid-related compound.

Keywords: whitefly, *N*-vanillyl-octanamide, capsaicinoids, avocado, insecticides.

Introduction

The production of avocado (*Persea americana* Mill.) is important for the economy of several countries, being México the largest producer of the world. The fruit has a growing demand owing its rich content of nutrients, antioxidants and vitamins, including magnesium, potassium, folic acid, omega 3, lutein and fibre (Lu *et al.,* 2009; Dreher and Davenport, 2013; Di Stefano *et al.,* 2017; Flores *et al.,* 2019). The Hass variety is the preferred by the consumer and the most widely cultivated. However, it has a number of destructive pests that halt growth of the tree, particularly during the first years after planting and reduce fruit quality and yield in adult trees (Walling, 2008; Guarnaccia *et al.,* 2016).

Whiteflies are insects that cause drastic losses to crops, having more than 1500 species already described, which associate with vascular plants, including angiosperms and gymnosperms. The insects feed on leaves for prolonged periods, extract nutrients from the phloem and may spread the growth of viruses and fungal pathogens (Martin, 1987; Martin *et al.,* 2000; Jones, 2003). Additionally, through the excretion of honeydew by whiteflies, the growth of sooty mold on leaves is induced and photosynthesis can be inhibited (Sani *et al.*, 2020). Hosts of high economical value susceptible to whitefly damage are citrus, avocado, squash, potato, cucumber, grape and tomato amongst nearly other 250 horticultural varieties and fruit trees (Walling, 2008; Malumphy *et al.,* 2009).

The infestation success of whiteflies is related to an imbalance of jasmonic acid-inducible defences that protect plants against insects, and such response is compromised not only in infested plants, but also in their neighbours enabling rapid spread of the pest (Zarate *et al.,* 2007; Zhang *et al.,* 2013; 2019). The monoterpenes β-myrcene and ρ-cymene as well as the sesquiterpene β-caryophyllene are released by tomato plants after initial infestation by the whitefly *Bemisia tabaci* (Gennadius) MAEN1, and application of β-myrcene or β-caryophyllene decreased JA levels, indicating that

these volatiles mediate the sensitivity of plants to these devastating insects (Zhang *et al.,* 2019). Other stressing factors such as drought, nutrient scarcity, hail falls and cold temperatures may further aggravate the stress imposed by whiteflies. In this sense, ABA signaling and glucosinolate accumulation play critical roles in plant resistance to the cabbage whitefly *Aleyrodes proletella* (Broekgaarden *et al.,* 2018).

Application of insecticides is a widespread strategy to control insect infestation in the field, but it has to be done in a regular manner and may lead to undesirable mortality of pollinators and other beneficial insects for ecosystem balance (Potts *et al.*, 2010; Sun *et al.*, 2018; Smith *et al.*, 2019). Different insecticides are used to control whiteflies, including neonicotinoids, pymetrozene, methamidophos, malathion, pyrethroids, endosulfan, pyriproxyfen and buprofezin. However, all of them have effects on non-target organisms and also contaminate the environment. In addition, whitefly biotypes resistant to these insecticides have emerged (Atansly *et al.*, 2016; Sani *et al.*, 2020). This has led to the development of alternatives that include biological control using entomopathogenic fungi that infect and kill whiteflies (Sani *et al.*, 2020). It is also necessary to find compounds with protective effects on crops against pests without adverse effects to beneficial insects or to the environment. In this scenario, the identification of natural, plant-derived metabolites offered promising compounds to control insect pests, with less or null deletereous effects to untarget species (Cantrell *et al.*, 2012; Yan *et al.*, 2018; Hernández-Carlos and Gamboa-Angulo, 2019).

Capsaicin is the bioactive molecule responsible of the pungent taste of hot chili peppers (Thiele *et al.*, 2008; Lu *et al.*, 2017). Capsaicin is not only a human edible compound with multiple potential benefits to health (Hayman and Kam, 2008), it is thought to be an environmentally friendly molecule and an interesting alternative to pesticides (Vázquez-Fuentes *et al.*, 2021). Indeed, it is increasingly being used as an insect repellent because its fast-acting and strong irritant effects. Capsaicin itself, or synthetic-derived capsaicinoids, may be applied to plant foliage to repel insect pests (Claros-Cuadrado *et al.*, 2019; Li *et al.*, 2019), but experimental proof of its bioactivity against whitefly infestation under field conditions remains to be gathered.

In this report, we show that whitefly infestation in avocado trees in the field correlates with the spread of chlorotic halos, and blade distortion of leaves. Application of *N*-vanillyl-octanamide, also termed ABX-I, a synthetic capsaicinoid, strongly reduced the number of winged adults resting on leaves at early morning and repels feeding when the whiteflies are most active during the sunny day hours. Our data provide the foundation for the use of capsaicinoids to protect plants from whitefly pests.

Materials and methods

Preparation and application of *N*-vanillyl-octanamide (ABX-I)

N-vanillyl-octanamide (ABX-I) was kindly provided by Applied Biotech SA de CV, México. The purity of the compound is >95%, confirmed by HPLC and RMN as described in Castillo and coworkers (2007). 300 mM ABX-I stock was prepared dissolving a given amount of the compound in a water:polysorbate 20 (7:3 v/v proportion), the final volume was adjusted to 20 liters with water. Applications were performed spraying the trees with the ABX-I solution using a sprayer backpack ensuring that the overall leaf and stem surfaces were gently covered.

In 2019 two monthly applications in March and April were done to 40 trees at 10 h in the morning when the whiteflies flew around and feed on leaves of trees from a newly stablished avocado orchard, located at 19° 29’ 43.9” N, 101° 43’ 33.1” W. These applications allowed to empirically note the dissuasive effect on the insects. In year 2020, ABX-I was applied in March, April and May at early morning (7 h in the morning) to 40 trees, when the insects were still resting on leaves. In 2021, comparable applications to 2020 were performed, which aided to empirically confirm the dissuasive effect of ABX-I and the reduction of leaf chlorosis symptoms.

Determination of whitefly infestation

Visual examination was done in the following four days after ABX-I application and quantitative measurements of infestation was done the next day after application by counting the insects lying on at least 10 leaves from previously selected and marked tree branches. Data recorded are from 10 independent trees selected from a section covering 40 trees of the orchard under high infestation.

Statistical analysis

One-way ANOVA followed by Tukey´s post hoc or Student t-test were performed to determine significant differences ($P< 0.05$) using GraphPad prism 5 software.

Whitefly sampling and imaging

Whiteflies were collected at 7 h early morning from infested avocado trees growing in the field during the

period 25 March-16 April 2020. The insects resting on leaves were carefully transported to the laboratory inside transparent plastic containers with lids, and small holes were done to the lids to enable aeration. 10 individuals were fixed in a solution of ethanol 70% an then mounted on slides and photographed using a dissecting microscope or a microscope with Nomarsky optics (Leica DM500B), and representative images were taken.

Results

Whitefly infestation in avocado

During 2019, one year after planting the avocado trees, whitefly colonies were found resting on the trees at early morning and start to fly as sunlight and temperature increased. Two ABX-I montly applications in March and April were done to 40 trees at 10 h in the morning when the whiteflies were flying around the leaves of trees and feeding on leaves. These applications allowed us to empirically note the dissuasive effect of ABX-I to the insects upon inspection of the whitefly population resting on the same trees prior and one day after application of ABX-I (**Figure 1 a-f**).

At the beginning of April 2020, winged adults started to be observed again, which suggests that the spread of the pest is season-dependent. The insects were noticeably observed flying during the sunny hours, and approaching the leaves of trees to feed on them, and resting at night and early morning on leaf undersides (**Figure 2 a-c**).

Some winged adults were collected and observed under Nomarsky optics microscopy. Lateral, ventral and dorsal views of the insects evidenced roughly 2 mm yellowish bodies with four whitish wings, in which the greater, anterior wings were decorated with black bands and/or spots (**Figure 3 a-d**). Examination of the head revealed a large stylet (**Figure 3a**), a needle-like structure used to suck sap from the vascular tissues of leaves.

Comparison of the obtained images with previous descriptions of whiteflies did not allow us to define the taxonomy of the insects, which require further detailed microscopic examination and/or molecular typification.

The spread of leaf chlorosis in avocado trees correlates with the feeding by whiteflies

The leaves in which the flies were detected, developed interveinal chlorosis and blade distortion from 8-10 days later (**Figure 4a, b**). Some of the damaged leaves falled, causing defoliation gaps in the tree. These data collectively suggest that the insect population and time of infestation coincided with leaf damage, although certainly, we cannot at this stage stablish a causal relation.

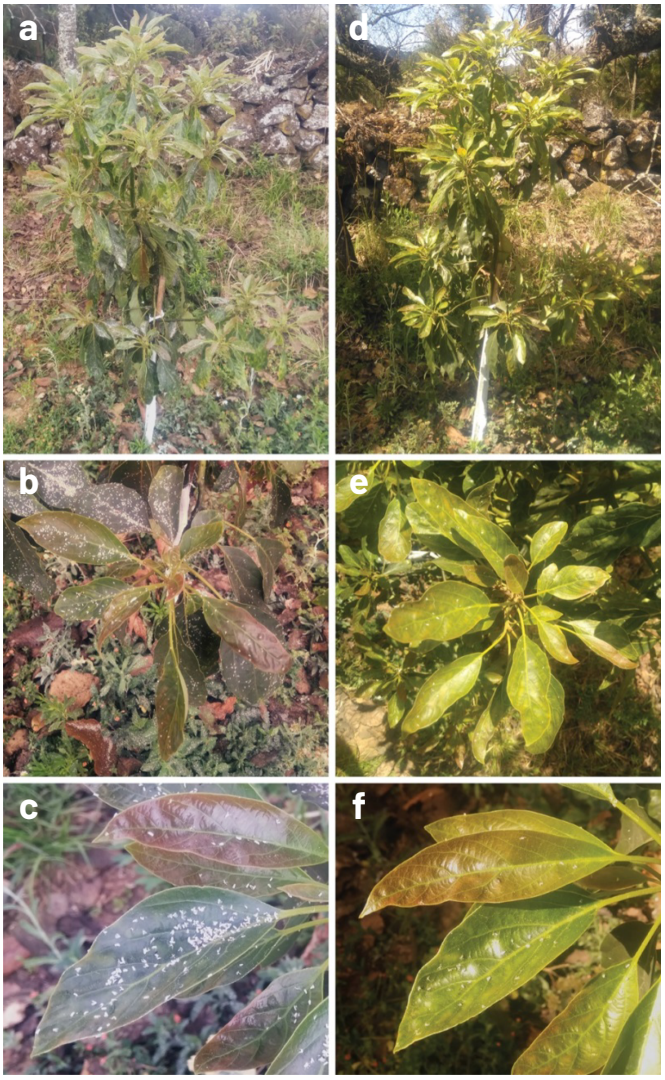

Figure 1. Whitefly infestation of avocado trees in 2019 spring. Photographs of a representative tree in a highly infested zone of the orchard showing the whitefly populations resting on its leaves before (**a**, **b**, **c**) and after one day of ABX-I application (**d**, **e**, **f**). The photographs were taken at 7 h, early morning. For reference, the height of the tree in **a** and **d** was 1.14 m.

ABX-I capsaicinoid compound decreases whitefly infestation

Capsaicinoids are natural bioactive compounds that may repel insects or kill them (Claros-Cuadrado *et al.,* 2019; Li *et al.,* 2019). *N*-vanillyl-octanamide can be produced by synthetic means and was named ABX-I (Castillo *et al.,* 2007). It differs from capsaicin by an octanamide group instead of 8-methyl-nonenamide. To further examine the bioactivity of ABX-I already observed during the

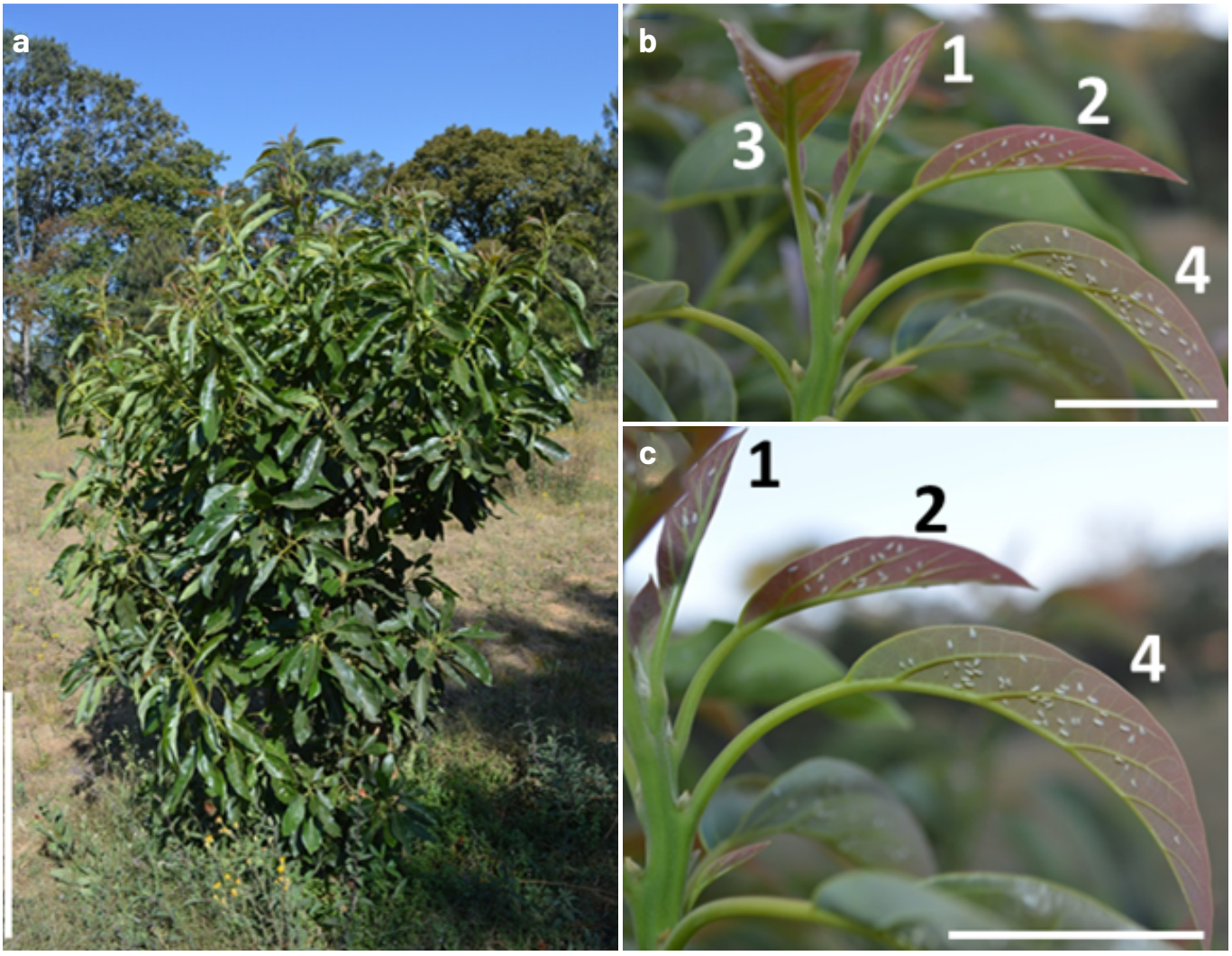

Figure 2. Whitefly infestation of avocado trees in 2020 spring. **a)** Phenotype of a 2.15 m avocado tree growing in the field at the beginning of spring season. **b)** A newly developed tree branch showing the leaves at progressive developmental stages marked with numbers. **c)** Close up of **(b)** to highlight the whiteflies resting on the abaxial side of leaves. All images were taken between 7-8 h in the morning, at a time where the insects were resting behind leaf blades. Scale bars **a**: 0.5 m; **b**, **c**: 10 cm.

previous year (2019), and to gather quantitative data of the antagonistic effects of ABX-I on the pest, in 2020 40 trees in a highly infested section of the orchard were thoroughly sprayed with a solution of 300 µM ABX-I, ensuring that leaves and stems were gently covered. A representative tree was chosen, showing the whitefly infestation prior and after ABX-I application. After each application, it was observed a clear repellent effect that lasted for several hours against the whiteflies, the insects approached the trees but did not feed on leaves. The next day, the same ABX-I-treated trees and leaves were examined and photographed early in the morning, showing the absence of the whiteflies (**Figure 5a, b**).

To determine the magnitude of the infestation prior to ABX-I application, the number of adult whiteflies was quantified in ten developing leaves within a single branch with their position on the branch marked with rising numbers as in **Figure 2b** and **2c**, from earlier stages indicating small leaves to well developed laminas, measuring 10-15 cm in length (stages 6-10). The number of insects increased from roughly 15 in stage 1 to roughly 130 in stage 10 leaves (**Figure 6a**). The average number of whiteflies per avocado branch was of 70 individuals (**Figure 6b**). Noteworthy, after ABX-I applications, the number of whiteflies decreased in leaves at all developmental stages and their average number per branch was of 10. An analysis of persistence of the insects on the branch indicated that the capsaicinoid effectively controlled the whitefly pest at the following 3 days after application, with only 12% of insect persistance (**Figure 6c**).

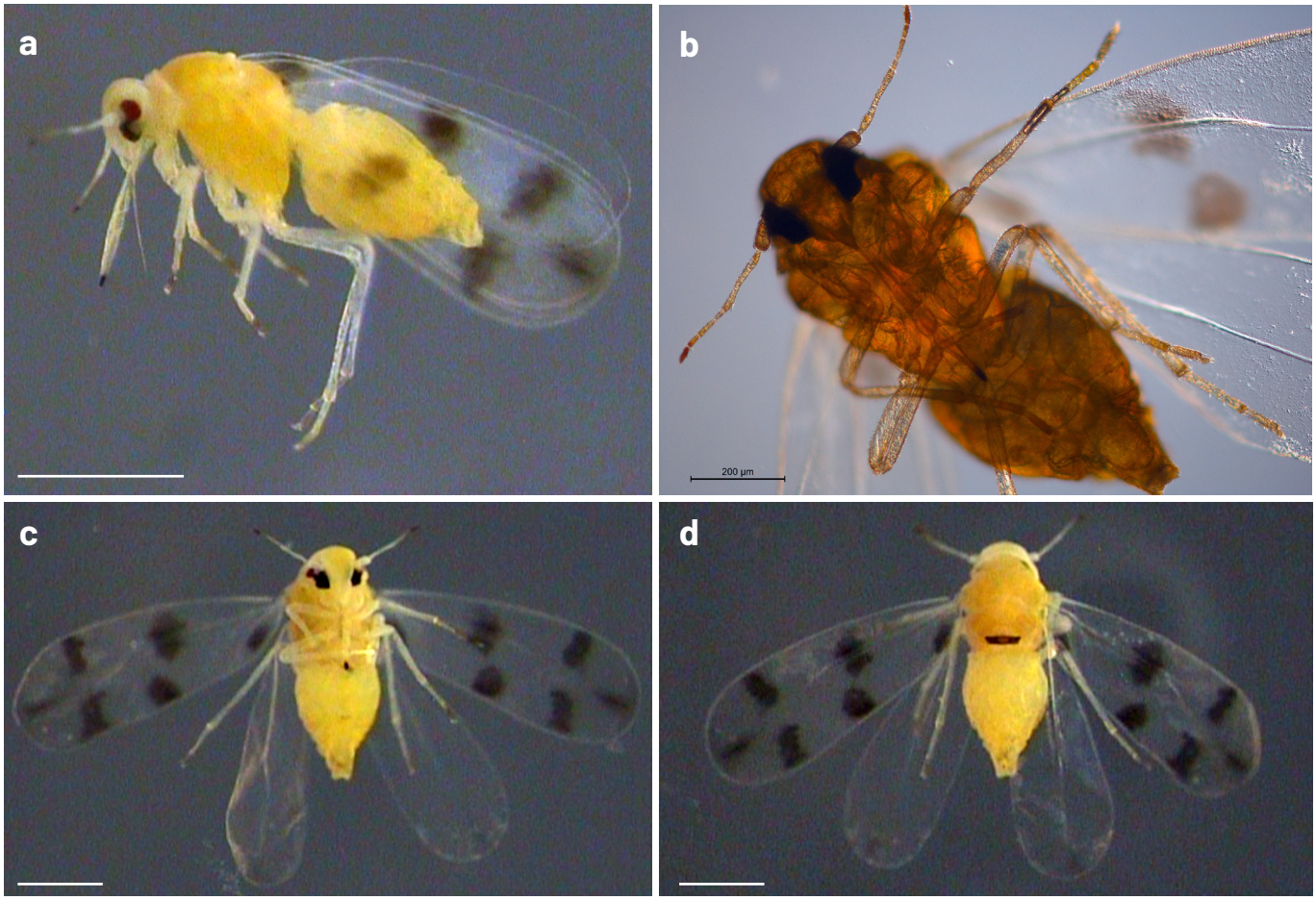

Figure 3. Adult phenotypes of whiteflies captured on leaves of avocado trees. **a**) Lateral, **b-c**) Ventral, **d**) Dorsal views of winged adults showing four wings, the anterior wings were decorated with black bands or spots. Scale bars: 1 mm.

Figure 4. Interveinal chlorosis in leaves as a major symptom of whitefly damage. **a**) Several branches of a tree showing the interveinal chlorosis on leaves. **b**) A single leaf showing the patchy appearance. Scale bars: 10 cm.

Discussion

In this report, we documented the seasonal whitefly visits in an avocado orchard in the field of Michoacán state, México. Weekly visual inspection to the orchard in three subsequent years after planting (2019-2021) evidenced the appearance of winged adults by the end of March to beginning of April and the population decreased with the first rains by June. This indicates that the pest may be seasonal and occurred by the spring.

Different whitefly genders from *Aleyrodidae* family

Figure 5. Representative images of whiteflies resting on avocado leaves before and after application of ABX-I. **a**) Photograph of adult whiteflies resting behind leaves at early morning prior to ABX-I application. **b**) Photograph of the same leaves after three serial applications of ABX-I. Note the decrease in the number of flies resting on leaves following capsaicinoid treatment. Scale bars in **a**= 7.5 cm, in **b**= 5 cm.

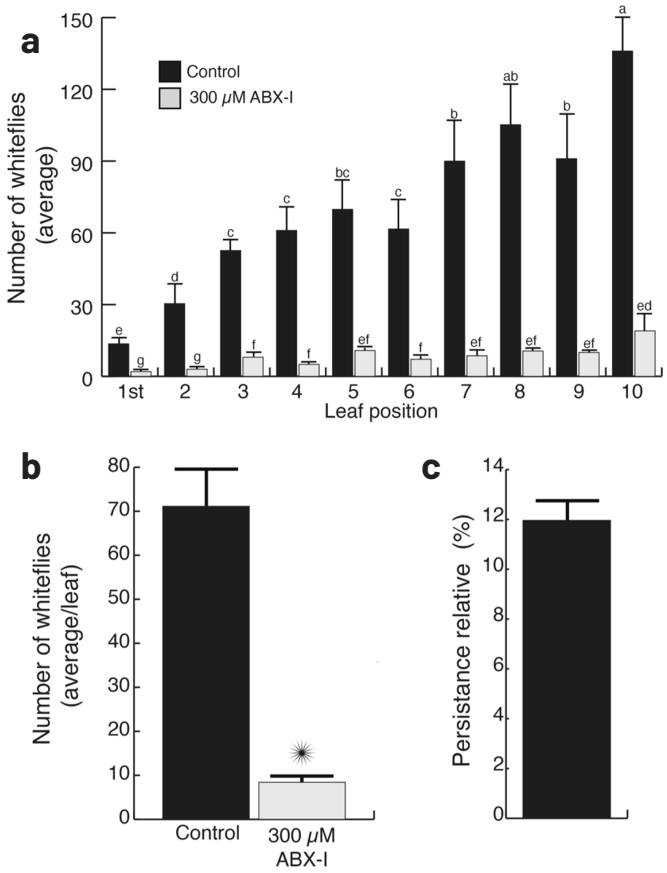

Figure 6. Quantification of the number of whiteflies on avocado leaves before and after application of *N*-vanillyl-octanamide (ABX-I). **a**) Average number of winged whiteflies resting on avocado leaves at early morning (7 h) prior to ABX-I application (black bars) or after application of ABX-I (light brown bars). **b**) Whitefly average from 10 leaves, **c**) Percentage of persistance of whiteflies after ABX-I application relative to number of whiteflies before ABX-I aplication. Bars in graphs show the means ± standard error, n=10. Different letters in **a**) indicate statistical differences as obtained by ANOVA Tukey´s post hoc analysis (*P< 0.05).* Asterisc in **b**) indicate statistical differences determined through a Student t-test (*P< 0.01*).

have been suggested as potential threats to avocado in diferent countries including México (Varón-Devia *et al.,* 2016). However, there is no evidence regarding the species infesting avocado orchards in Michoacán state, nor consequences of such infestation or specific control strategies. Current research identified some whitefly species from avocado orchards in the state of Morelos, México, including *Tetraleurodes perseae*, *Triaelurodes vaporariorum* and *Paraleyrodes minei* (García-Palacios *et al.*, 2016; García-Palacios *et al.*, 2020). *Tetraleurodes perseae* was found in highlands orchards, where the population increases as temperature raises, between February and April (García-Palacios *et al.*, 2016). Thus, the whiteflies spreads observed in our study are consistent with these previous reports and may be due to the rise of temperature at spring.

The insects started to be active when the first rays of the sun appeared and activity was highest at sunny hours, between 10-12 h in the morning, then aproached the trees to feed on leaves, which correlated with appearance of chlorosis, and leaf malformations at late days. The symptoms could be related to the feeding behaviour of the insects, which extract phloem sap using their stylet and compromise the nutritional status of leaf tissues. According to this assumption, leaf symptoms in most trees could be reversed by application of organic amendments or fertilizers during the rainy season of 2019-2021, which suggests the reversibility of the damage.

Another interesting observation was that the winged adults rested on the undersides of leaves at night and early morning, and thus the chemical control would be better if the product is applied at early morning rather that at sunny hours. Due to their flying behavior, pesticide applications at sunny hours would be ineffective for

whiteflies in avocado, since the insects can scape and move to distant trees or to wild plants. Moreover, at this time most pollinators such as honeybees were observed to feed on flowers of wild plants or flowers from avocado trees, and the application of insecticides or other bioactive molecules is thus not recommended. The application of ABX-I at early morning is highly recommended, when the whiteflies still rest behind avocado leaves and no pollinators were still active on the orchard.

For seasonal pests, plant protection may occur by the use of repellent substances that avoid insect feeding on leaves, and in consequence, halt popullation increases. Temporary suppression of whiteflies may be achieved either by application of insecticides or via alternative products. Capsaicin is the main bioactive component of hot chili peppers and is thought to play an adaptive and ecological role for the producing plants (Thiele *et al.,* 2008; Lu *et al.,* 2017). Capsaicin and few related molecules have been applied with success to control insect pests without toxic effects for pollinators or humans (Claros-Cuadrado *et al.,* 2019; Li *et al.,* 2019).

ABX-I inhibited growth of phytopathogens *Colletotrichum gloeosporioides*, *Botrytis cinere*a, *Colletotrichum acutatum*, *Fusarium* sp., and *Rhizoctonia solani* AG2 in vitro and spray application to tomato or avocado plants did not compromise growth and development, which uncovers its potential for the control of fungal diseases (Vázquez-Fuentes *et al.*, 2021). Here, we provide evidence that the compound dissuades whiteflies and also confers protection to avocado trees in the field. Whiteflies are known to feed on many different, wild and cultivated hosts and therefore, the protective function of ABX-I could be extended to other vegetables and crop species.

When controlling the whitefly pest, it should be kept in mind the extraordinary complexity of the insect-plant communication that determines host sensitivity. Monoterpene and sesquiterpene volatiles are emitted by plants that are attacked by whiteflies, which turn off the jasmonic acid-dependent defenses in their neighbours (Zarate *et al.,* 2007; Zhang *et al.,* 2013; 2019). It is at present unknown if the application of capsaicinoids actually disrupt such chemical communication, but it is an interesting possibility.

Conclusion

This report showed the potential of a synthetic, biostimulant capsaicinoid, ABX-I for the control of seasonal occurrence of whitefly pest in avocado trees. The leaf damage and chlorosis is reversible upon nutritional management and the number of whiteflies feeding on leaves could be decreased via a highly repellent effect upon application of the capsaicinoid, which opens the door for the use of capsaicin-related compounds to protect horticultural and fruit trees from the attacking insects.

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