An Acad Bras Cienc (2022) 94(2): e20191244 DOI 10.1590/0001-3765202220191244
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CROP SCIENCE

Melon genotypes with resistance to *Liriomyza sativae* Blanchard (Diptera: Agromyzidae)

JOSIELMA M. DE OLIVEIRA, JACKSON L. ARAÚJO, JOSÉ WAGNER S. MELO & NIVIA S. DIAS-PINI

Abstract: The vegetable leaf miner (*Liriomyza sativae*) is considered one of the main melon pests, causing serious problems for producers in all growing regions. A promising type of pest control has been use of resistant cultivars, in isolation or associated with other types of control. This study aimed to evaluate the resistance of melon genotypes to *L. sativae*. Twenty-one melon genotypes and one commercial "Goldex" hybrid (susceptibility pattern) were evaluated in two experiments. In the first experiment, we observed the non-preference of *L. sativae* for oviposition and feeding by quantifying the number of eggs and feeding punctures, both on the adaxial side and on the abaxial face of the leaves. In the second experiment, we observed the antibiosis effect through *L. sativae* larval and pupal viability. Genotype CNPH 06-1047-341 showed the lowest preference for oviposition (high resistance), with low egg values on both leaf sides (0.3 eggs/plant). In genotypes CNPH 06-1047-313, CNPH 06-1047-346, CNPH 11-1071-27, CNPH 11-1071-39, CNPH 11-1071-43, and CNPH 11-1071-53, we observed a higher preference for the adaxial side, whereas for the other genotypes and the commercial hybrid there was no discrimination between leaf sides. In relation to antibiosis, genotypes CNPH 06-1047-339, CNPH 06-1047-333, CNPH 06-1047-330, CNPH 06-1047-334, CNPH 06-1047-331, CNPH 06-1047-343, CNPH 10-1056-313, CNPH 06-1047-346, and CNPH 06-1047-341 presented lower larval and pupal viability. Genotype CNPH 06-1047-341 was the least preferred for oviposition and feeding and the most promising as a source of resistance to *L. sativae*.

Key words: *Cucumis melo*, antixenosis, antibiosis, vegetable leaf miner.

INTRODUCTION

Melon cultivation (*Cucumis melo* L.) has stood out in the world scenario as a crop of great social and economic importance (FAO 2017). Nevertheless, despite the great potential that this crop represents for the trade balance of fruit exports in Brazil, there are phytosanitary problems that demand urgent solutions to ensure that the melon production chain remains competitive in the globalized market (Araújo et al. 2007). In this context, the incidence of pests is one of the main risk factors that can prevent the production and commercialization of melon, given the inadequacy of the characteristics demanded by the consumer market (Araújo et al. 2007, Sales Junior et al. 2004), especially in relation to the total soluble solids content (ºbrix), consistency, and aesthetic appearance of the fruits (Sales Junior et al. 2004).

The leaf miner, *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) has stood out among the pests that cause significant impacts on the production of melon, given the direct and indirect damages that it causes throughout the crop cycle (Araújo et al. 2007, Celin et al. 2017). Female adults cause perforations along the leaf blade, especially at the apex of the plant, which are caused from feeding and oviposition

(Parrella et al. 1985). The greatest damage is observed by the feeding of the larvae in the leaf mesophyll, with formation of mines that can dry the leaves, consequently reducing the photosynthetic capacity of the plant (Costa et al. 2017) and resulting in fruits with low market quality (Araújo et al. 2007). At high levels of infestation, leaves can prematurely fall, affecting the growth or even the survival of the plant (Costa et al. 2017, Johnson et al. 1983). In the latter case, defoliation can lead to direct exposure of the fruits to sun rays, causing burns and depreciating their commercialization (Lima & Chagas 2014).

The most used type of control of *L. sativae* has been the chemical method (Capinera 2001, Ferguson 2004), with the use of systemic and/or contact pesticides (Agrofit 2018). The exclusive and continuous application of these products has been shown to be ineffective, besides making the activity expensive (Hidrayani et al. 2005), given the low turnover of active principles, which creates a favorable scenario for the development of pest resistance (Guimarães et al. 2009). In addition, it has adversely affected the population of natural enemies, further contributing to the worsening of the problem (Hossain & Poehling 2006).

An alternative and promising way to control pests is the use of resistant plants because they have inherited genetic characteristics that make them more resistant than others under equal conditions (Rossetto 1973). The use of melon cultivars resistant to *L. sativae*, in isolation or associated with other pest control methods, can lead to a number of benefits for both producer and consumer, as well as the environment, from the conservation of natural enemies and reduced use of chemical pesticides (Basij et al. 2011).

There are few published works on the evaluation of the resistance of melon genotypes to *L. sativae*; we also highlight the difficulty in identifying materials resistant to vegetable leaf miner (Nunes et al. 2013). Therefore, the identification of resistance sources in available germplasm banks is one of the first steps to obtain resistant cultivars. Thus, this study aimed to evaluate the resistance of melon genotypes to *L. sativae*.

MATERIALS AND METHODS

The experiments were conducted in the laboratory and in a greenhouse of the experimental unit of Embrapa Agroindústria Tropical, located in Fortaleza, State of Ceará, Brazil.

Rearing of *L. sativae* in laboratory

The rearing of *L. sativae* began with insects collected in the melon producing regions of the municipality of Mossoró, State of Rio Grande do Norte, Brazil, and was based on the methodology proposed by Braga Sobrinho et al. (2011). We used jack beans, *Canavalia ensiformis* (L.) (Fabaceae), as the host plant. This plant was used to avoid the preimaginal conditioning of insects. The vases containing the infested plants were housed in wooden cages (100 x 100 x 100 cm), covered with voile and kept in the laboratory under controlled environmental conditions (27 ± 2° C; 75 ± 10% of RH; 14h of photophase). Adults were fed with a 10% honey and water solution.

Melon genotypes

We evaluated twenty-one melon genotypes from the Melon Genetic Improvement Program of Embrapa (Brazilian Agricultural Research Corporation), and the commercial "Goldex" Hybrid was included in the experiment as a control (susceptibility pattern) (Table I).

Seeds of the melon genotypes were seeded in expanded polystyrene trays with 128 cells. At 21 days of sowing, the seedlings were transplanted

Table I. Selected melon genotypes for the *L. sativae* resistance test and corresponding codes used in the study.

Genotype	Code	Genotype	Code
CNPH 10-1056-313	313	CNPH 11-1071-26	AC26
CNPH 06-1047-330	330	CNPH 11-1071-27	AC27
CNPH 06-1047-331	331	CNPH 11-1071-35	AC35
CNPH 06-1047-333	333	CNPH 11-1071-37	AC37
CNPH 06-1047-334	334	CNPH 11-1071-39	AC39
CNPH 06-1047-339	339	CNPH 11-1071-42	AC42
CNPH 06-1047-341	341	CNPH 11-1071-43	AC43
CNPH 06-1047-343	343	CNPH 11-1071-53	AC53
CNPH 06-1047-346	346	CNPH 11-1071-55	AC55
CNPH 11-1071-23	AC23	CNPH 11-1071-56	AC56
CNPH 11-1071-25	AC25	GOLDEX	Commercial Hybrid

to polyethylene vases, 10.5 cm in diameter and 7.5 cm high, with a capacity of approximately 0.5 kg of substrate. Fine sand (sterilized) and commercial compost (HS FLORESTAL®) were used as substrate, in a 1:1 ratio. The plants remained in greenhouse, irrigated twice a day until reaching the vegetative stage of three final leaves (approximately 15 days).

Antixenosis bioassay

The non-preference of *L. sativae* for feeding and oviposition was evaluated in a confinement test with choice. Vases with melon plants (21 genotypes + commercial hybrid) were randomly distributed in a wooden cage (115 x 380 x 90 cm), covered with voile, under controlled environmental conditions (27 ± 2°C; 75 ± 10% of RH; 14h of photophase). Then, the proportion of eight newly emerged adult insects (four couples) per plant was released into the cage for infestation. A completely randomized design with three replicates was used. The experimental unit was a vase with one plant (with three final leaves), each one corresponding to one replicate. The plants were confined with the insects for twenty-four hours. After 24 hours, the number of eggs and feeding punctures were quantified, both on the adaxial side and on the abaxial side of the leaves. A 4 cm^2 circular area was delimited in the central region of the leaves to quantify feeding punctures. The visualization of the structures was performed with the aid of a stereo microscope (50x), Stemi 508 Zeiss.

Antibiosis bioassay

The antibiosis effect of the genotypes on immature *L. sativae* (larva and pupa) was evaluated in a confinement test with choice. Vases with melon plants (21 genotypes + commercial hybrid) were randomly distributed in a wooden cage (115 x 380 x 90 cm), covered with voile. The plants remained in the laboratory under controlled environmental conditions (the same as in the previous test). The design was completely randomized, with six replicates. Then, the proportion of eight newly emerged adult insects (four couples) per plant was released into the cage for infestation. The plants were confined with the insects for twenty-four hours. After the infestation period, they were removed from the cage and transferred to the greenhouse, where they remained until the larvae hatched and the miners emerged. After the emergence of the miners, the plants went back to the laboratory for the counting of larvae. Subsequently, the plants were distributed on the

bench, and each individual leaf was placed in a disposable cup (150 ml), properly identified, to collect the pupae and also as a way of protecting the larvae from biotic agents of mortality. The emergence of pupae was observed daily, which were quantified and stored in properly identified glass tubes, sealed with film paper until adults emerged and were quantified. With the values of larvae, pupae, and adults, we determined the *L. sativae* larval and pupal viability in the genotypes evaluated.

Larval viability was calculated by equation: $LV = \frac{NP}{NL}100$, where LV corresponds to larval viability, NP corresponds to number of pupae, and NL corresponds to number of larvae. Pupal viability was calculated by equation: $PV = \frac{NA}{NP}100$, where PV corresponds to pupal viability, NA corresponds to the number of adults emerged, and NP corresponds to the number of pupae.

Data analysis

Data on non-preference for oviposition and feeding and antibiosis among genotypes were transformed into $\sqrt{(X + 0.5)}$ in order to present a normal distribution. Then, we performed an analysis of variance and compared the means using Dunnett's test ($\alpha = 0.05$). The values of non-preference for feeding and oviposition on a leaf side were transformed to $\sqrt{X}$ and the means were compared by student's t-test ($\alpha = 0.05$). We used the statistical program SAS® (2004).

RESULTS

Antixenosis. In the evaluation of non-preference of *L sativae* for oviposition, significant differences ($F_{21.44} = 4.12$; $P < 0.0001$) were observed between genotypes and the commercial hybrid (susceptibility pattern) (Figure 1). Genotype CNPH 06-1047-341 showed the lowest preference

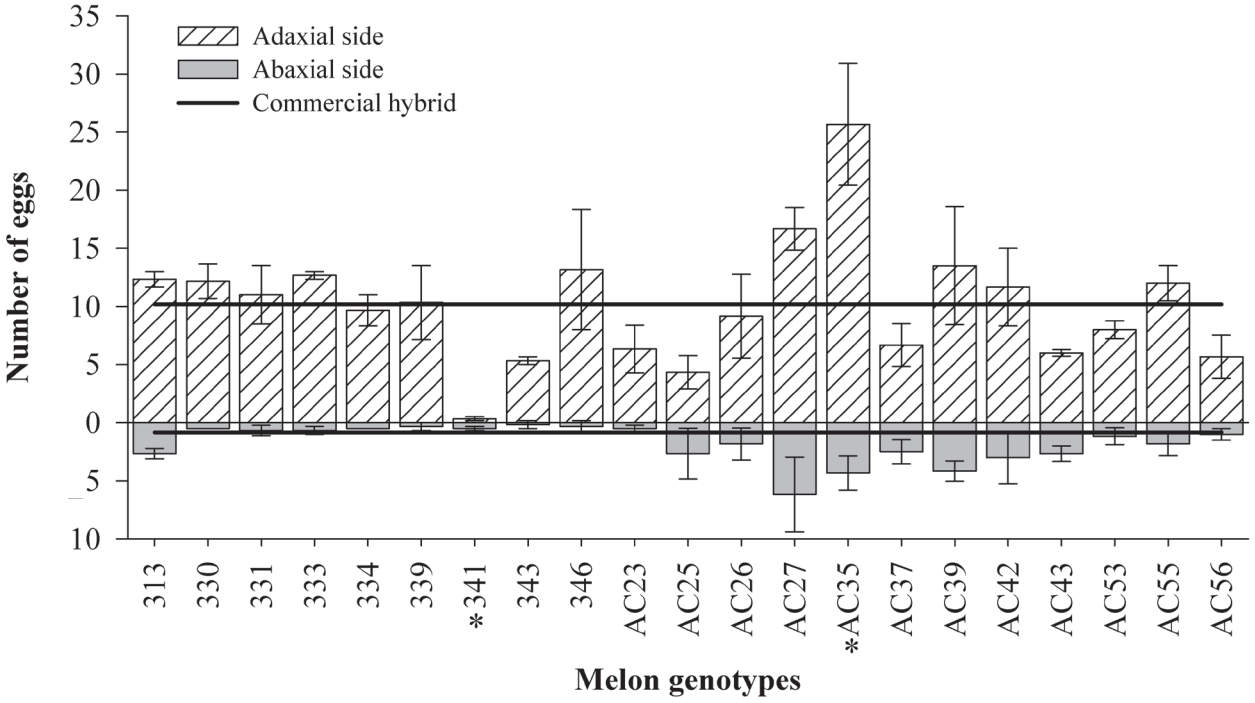

Figure 1. No preference of *L. sativae* for oviposition in melon genotypes. Genotypes followed by an asterisk indicates a significant difference in relation to the commercial hybrid by Dunnett's test (α = 0.05). Line in bold (mean number of eggs in the leaves of the susceptible standard genotype), Bars (mean number of eggs in leaves of genotypes with standard error).

(high resistance), with low egg values on both leaf sides (0.3 eggs/plant). In contrast, genotype CNPH 11-1071-35 had the highest preference (high susceptibility), and we found more eggs than the mean obtained in the commercial hybrid and in the other genotypes. Genotypes CNPH 06-1047-343, CNPH 11-1071-23, CNPH 11-1071-25, CNPH 11-1071-37, CNPH 11-1071-43, and CNPH 11-1071-56 also had a lower preference for oviposition (moderate resistance), but it was not enough to differ statistically from the commercial hybrids (susceptibility pattern).

In relation to the non-preference for one of the leaf sides for oviposition, we observed that there were significant differences (t = 2.83, DF = 4, *P* <0.047) in some genotypes between the number of eggs on the adaxial side and on the abaxial side (Figure 1). In genotypes CNPH 10-1056-313, CNPH 06-1047-346, CNPH 11-1071-27, CNPH 11-1071-39, CNPH 11-1071-43, and CNPH 11-1071-53, *L. sativae* females had a higher preference for the adaxial side, whereas there was no discrimination between leaf sides for the other genotypes and the commercial hybrid.

In the evaluation of feeding punctures, significant differences were also observed between genotypes and the commercial hybrid ($F_{21, 44}$ = 6.29, *P* <0.0001) (Figure 2). Genotypes CNPH 06-1047-330, CNPH 06-1047-334, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 11-1071-37 had less preference for feeding on both leaf sides, which differs from the commercial hybrid (susceptibility pattern). Genotypes CNPH 06-1047-339, CNPH 11-1071-23, CNPH 11-1071-25, CNPH 11-1071-26, CNPH 11-1071-39, and CNPH 11-1071-56 had less feeding punctures; however, their values did not differ from the commercial hybrid.

In relation to the non-preference for one leaf side for feeding, significant differences (t = -2.88; DF = 4; *P* <0.044) were observed in genotypes CNPH 06-1047-331, CNPH 06-1047-346,

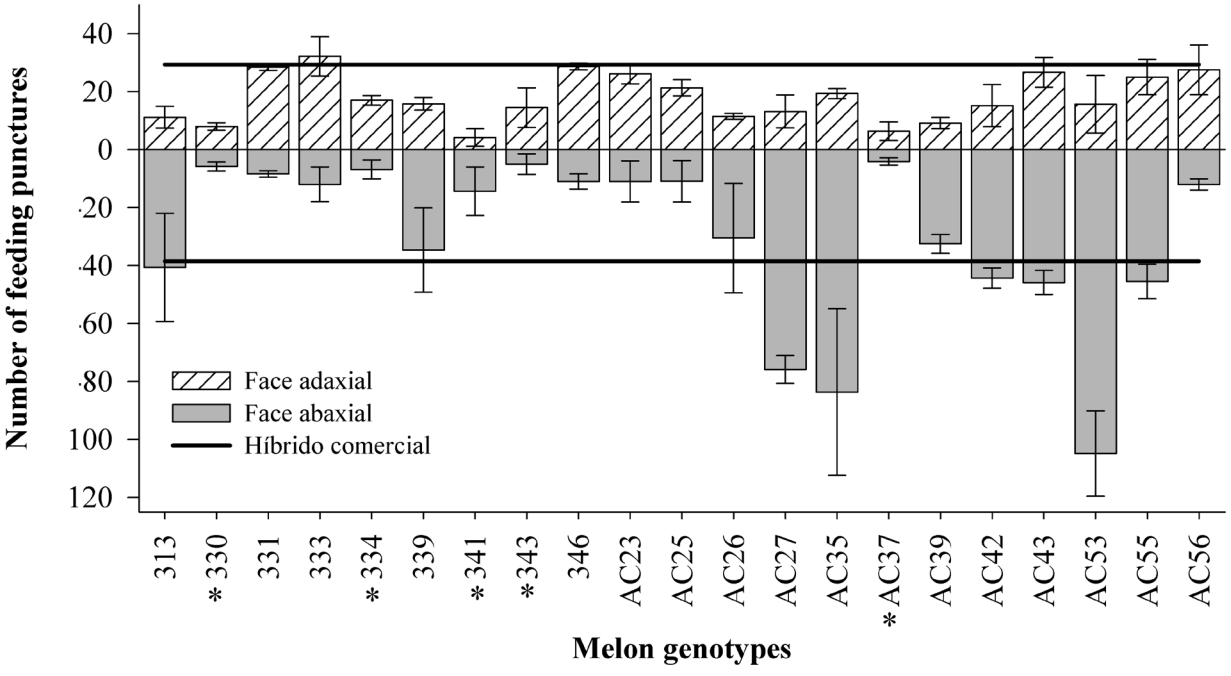

Figure 2. No preference of *L. sativae* for feeding in melon genotypes. Genotypes followed by an asterisk indicates a significant difference in relation to the commercial hybrid by Dunnett's test (α = 0.05). Line in bold (mean feeding punctures in the leaves of the susceptible standard genotype), Bars (mean feeding punctures in the abaxial and adaxial sides of the leaves of genotypes with standard error).

CNPH 11-1071-26, CNPH 11-1071-39, CNPH 11-1071-42, CNPH 11-1071-43, and CNPH 11-1071-53, in which *L. sativae* females had a greater preference for the abaxial side, which was the opposite of oviposition, with a greater number on the adaxial side. In genotype CNPH 11-1071-27, higher feeding was observed in the adaxial side, which also had the highest oviposition. As for the other genotypes, no significant difference was observed between the adaxial and abaxial sides.

Antibiosis

The percentage of *L. sativae* larval survival ranged from 81.5% (CNPH 11-1071-42) to 0.33% (CNPH 06-1047-341). Genotypes CNPH 10-1056-313, CNPH 06-1047-330, CNPH 06-1047-331, CNPH 06-1047-333, CNPH 06-1047-334, CNPH 06-1047-339, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 06-1047-346 had low larval viability, differing statistically from the commercial hybrid ($F_{21, 110}$ = 27.09; P <0.0001), which had mean larval viability of 60.8% (Figure 3). Some of these genotypes had larval viability below 5%. We observed that larval development did not occur in most eggs counted for these genotypes, with the interruption in the pest cycle.

In the evaluation of pupal viability, we observed that genotypes CNPH 10-1056-313, CNPH 06-1047-330, and CNPH 06-1047-331 showed high pupal mortality and consequently no emergence of the insect in the adult phase. Therefore, the analysis was performed with the genotypes that presented at least one adult individual, with significant differences only between genotypes CNPH 06-1047-334, CNPH 06-1047-341, and the commercial hybrid ($F_{18, 95}$ = 6.35, P <0.0001) (Figure 4).

DISCUSSION

The results of this study demonstrate that: (i) genotype CNPH 06-1047-341 was the least preferred for oviposition, with high resistance,

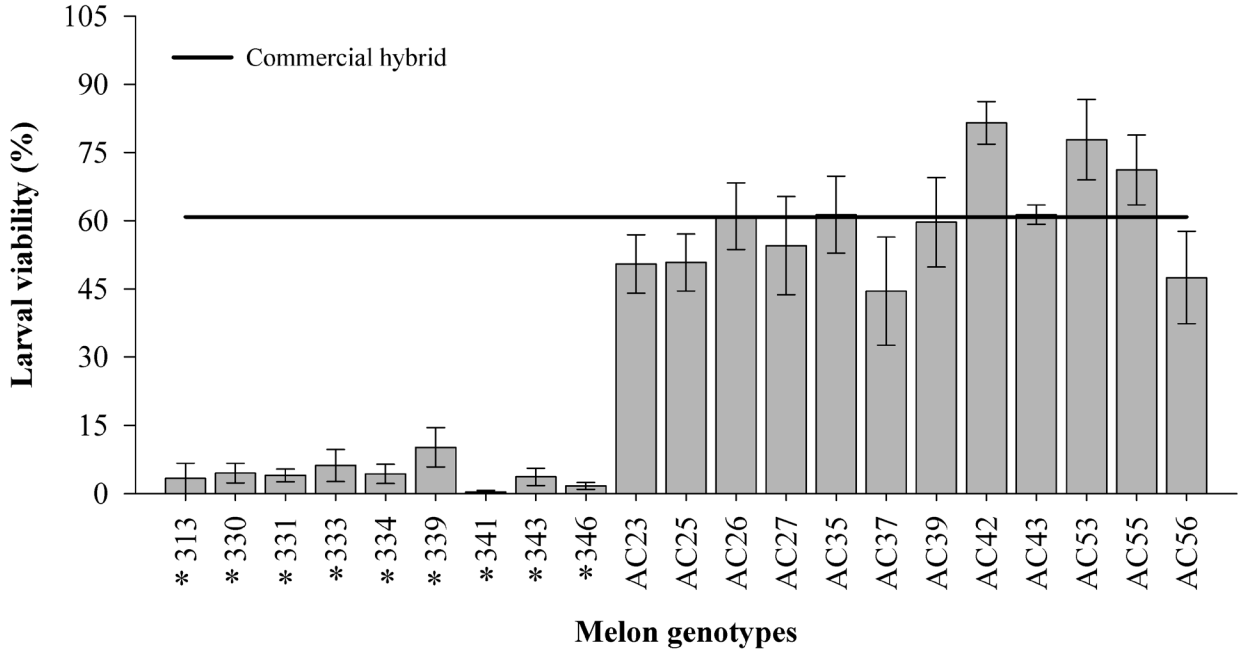

Figure 3. *L. sativae* larval viability in melon genotypes. Genotypes followed by asterisks indicates a significant difference in relation to the commercial hybrid by Dunnett's test (α = 0.05). Line in bold (mean larval viability of the susceptible standard genotype), Bars (mean larval viability of genotypes with standard error).

while genotype CNPH 11-1071-35 was the most preferred, with high susceptibility; (ii) genotypes CNPH 06-1047-330, CNPH 06-1047-334, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 11-1071-37 had the lowest preference for feeding; (iii) there is difference in the preference for one of the leaf sides, varying according to genotype; (iv) genotypes CNPH 10-1056-313, CNPH 06-1047-330, CNPH 06-1047-331, CNPH 06-1047-333, CNPH 06-1047-334, CNPH 06-1047-339, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 06-1047-346 showed antibiosis, with reduced larval and pupal viability.

The mechanism of resistance by non-preference is determined by the behavioral reaction of the insect in relation to the host plant, when it is most used as food, oviposition site, or shelter when compared to others under the same conditions (Lara 1991). There are reports that plants are capable of promoting varied responses to insect behavior through stimuli or appeal related to color, odor, texture, and other characteristics that may affect the attractiveness of these insects (Coelho et al. 2009, Taiz et al. 2017). Thus, it is possible that the non-preference for oviposition observed in genotype CNPH 06-1047-341 is related to one of these structural or morphological characteristics, in particular, epidermal formations such as hair. Studies with this crop (Coelho et al. 2009, Nunes et al. 2013) and also with other crops, such as, Cassava (Strucker et al. 2017), and soy (Lima & Lara 2004), have demonstrated that the presence of trichomes, as well as their density and size, can act directly on the behavior of mites and insect pests, interfering with oviposition (Coelho et al. 2009, Nunes et al. 2013, Strucker et al. 2017), feeding (Silva et al. 2008), and locomotion (Cardoso 2008, Matos et al. 2009). In relation to the high susceptibility of genotype CNPH 11-1071-35, it is possible that there is a combination of factors other than the characteristics related to morphology, such as: greater attractiveness from the emission of volatiles produced by

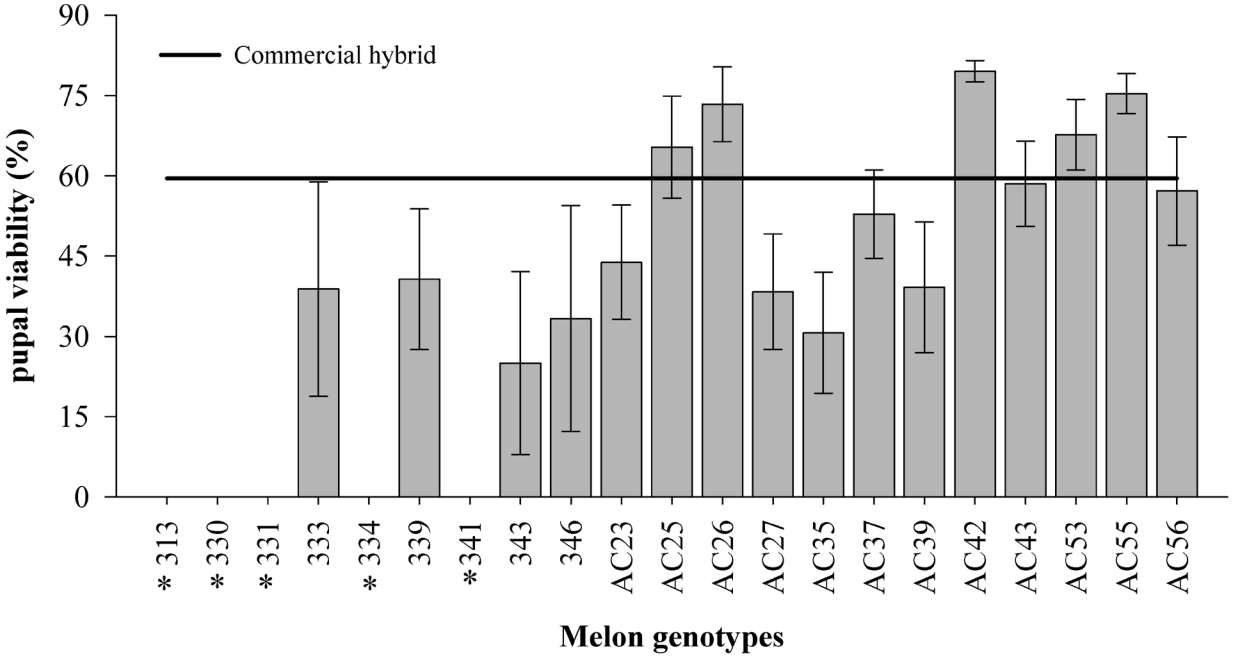

Figure 4. ***L. sativae*** **pupal viability in melon genotypes. Genotypes followed by asterisks indicates a significant difference in relation to the commercial hybrid by Dunnett's test (α = 0.05). Line in bold (mean pupal viability of the susceptible standard genotype), Bars (mean pupal viability of genotypes with standard error).**

plants (Ceruti 2007). It is known that insects recognize and locate their host plants by detecting the characteristic volatile mixtures emitted by them (Ceruti 2007, Riffel & Costa 2015), and, depending on the context in which it is inserted, a certain group of compounds can be attractive or repellent for a particular insect species (Schoonhoven et al. 2005). However, the biological function of these compounds in the insect-plant interaction, either for *L. sativae* attractiveness and/or repellency, was not determined in this evaluation, which can be the subject of future works.

The reduced number of feeding punctures observed in genotypes CNPH 06-1047-330, CNPH 06-1047-334, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 11-1071-37 may indicate that these genotypes have antixenosis resistance. Probably, the lower use of these genotypes is related to the perception of some repellent substance or because of phagodeterrence. The discrimination of a potentially host plant requires a stimulus that will be perceived by a highly developed sensory system; if this stimulus is positive, the insect will move to the plant and the stimulant substance will be considered attractive, otherwise it will be repellent. Once in contact with the plant, the insect performs a test bite and, for this end, the *L. sativae* female uses its ovipositor apparatus to perforate the leaf. After the damage, the liquid is spilled, which will be absorbed by both the female and the male. If the insects continue feeding, the substances will be phagostimulants, however if they are induced to stop feeding, the substances will be phagodeterrent (Seffrin et al. 2008). It is worth mentioning that this type of resistance based on antixenosis is always desirable, either for feeding or oviposition, since there is an expectation regarding the minimization of production losses from a lower use of the plant by the pest, which will result in higher production and better quality fruits (Basij et al. 2011).

Regarding non-preference for one leaf side for feeding and oviposition, several factors may be involved, among them physical barriers and visual or biochemical stimuli that act in sequence, resulting in the acceptance or rejection of a host (Panda & Khush 1995). The variation among genotypes shows that one of the sides seemed more appropriate for larval feeding, survival, and development. Thus, these results related to the discrimination of the insect for one leaf side suggests that the evaluation should be performed with data from both sides, which will certainly result in a better interpretation of the behavioral analysis of the pest.

In antibiosis, the insect feeds normally on the plant, but it may have adverse effects on its biology, negatively acting on parameters such as number of instars, weight, growth, reproduction, survival, among others (Lara 1991). Thus, antibiosis is related to the presence of chemical substances produced by plants (toxic metabolites, enzymatic and reproductive inhibitors) or associated with the absence/deficiency of essential nutrients of isolated or joint action (Painter 1951, Lara 1991). Phytochemical studies have shown that *C. melo* presents a rich source of volatile compounds (Beaulieu & Grimm 2001, Albert & Pitrat 2006), triterpenoids (Ibrahim et al. 2016, 2018), sterols (Akihisa et al. 1987), and flavonoids (Muller et al. 2013). Among the triterpenoids, cucurbitacins have been the most studied, mainly because they present a wide pharmacological potential (Shen et al. 2009, Zhou et al. 2017). These cucurbitacins are bitter substances found especially in plants of the Cucurbitaceae family, which can both provide protection against herbivory of generalist species and serve as food attraction for specialist species (Metcalf et al. 1980,

Mendell et al. 1971). Therefore, the larval and pupal mortality observed in genotypes CNPH 10-1056-313, CNPH 06-1047-330, CNPH 06-1047-331, CNPH 06-1047-333, CNPH 06-1047-334, CNPH 06-1047-339, CNPH 06-1047-341, CNPH 06-1047-343, and CNPH 06-1047-346 is probably related to the action of these secondary metabolites, whose constitution may have affected the metabolism of these insects, causing their death (Gullan & Cranston 2012). Similar results have been obtained in melon strains by Celin et al. (2017), who have observed low *L. sativae* larval viability soon after the larvae began feeding on the leaf mesophyll. Nunes et al. (2013) have observed promising results for plant resistance in melon genotypes, as they obtained a melon genotype that possibly affected the development of pupae and, consequently, the emergence of Liriomyza spp. adults, presenting a possible antibiosis effect.

There is variability among the melon genotypes evaluated in relation to resistance to *L. sativae*. There is an antixenosis and antibiosis effect among the tested genotypes. Genotype 341 is the least preferred for oviposition and feeding and the most promising as a source of resistance to *L. sativae*.

Acknowledgments

Authors acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico [J.W.S. Melo - PQ - 306372/2017-7], and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for providing the scholarships and Embrapa Agroindústria Tropical for financial support.

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How to cite

OLIVEIRA JM, ARAÚJO JL, MELO JWS & DIAS-PINI NS. 2022. Melon genotypes with resistance to *Liriomyza sativae* Blanchard (Diptera: Agromyzidae). An Acad Bras Cienc 94: e20191244. DOI 10.1590/0001-3765202220191244.

Manuscript received on October 14, 2019;
accepted for publication on May 6, 2020

JOSIELMA M. DE OLIVEIRA[1]
https://orcid.org/0000-0001-7864-7662

JACKSON L. ARAÚJO[1]
https://orcid.org/0000-0001-7746-2279

JOSÉ WAGNER S. MELO[1]
https://orcid.org/0000-0003-1056-8129

NIVIA S. DIAS-PINI[2]
https://orcid.org/0000-0002-3664-812X

[1]Universidade Federal do Ceará, Departamento de Fitotecnia – Fitossanidade, Campus do Pici, Av. Mister Hull, 2977, Pici, 60356-000 Fortaleza, CE, Brazil

[2]Centro Nacional de Pesquisa de Agroindústria Tropical, Embrapa Agroindústria Tropical, Rua Doutora Sara Mesquita, 2270, Pici, 60511-110 Fortaleza, CE, Brazil

Correspondence to: **Nivia da Silva Dias-Pini**
E-mail: nivia.dias@embrapa.br

Author contributions

NSD-P and JWSM conceived and designed research. JMO and JLA conducted experiments. JMO, JLA and JWSM analyzed data. JMO, JLA and NSD-P wrote the manuscript. All authors read and approved the manuscript.