Article

Incidence of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and defense mechanisms in *Gerbera hybrida*

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Abstract

The existence of natural variation at different levels of whitefly infestation (*Trialeurodes vaporariorum*) in gerbera genotypes (*Gerbera hybrida*) allows the existence of different levels of resistance or susceptibility to the insect. In this investigation the natural variation of gerbera was evaluated at the incidence of *T. vaporariorum*. In a randomized complete block design, ten gerbera genotypes were analyzed in different phenological stages without incidence (SI) and with incidence (CI) of whitefly. Nymphs were counted, in a cm^2 at the center of the underside of the leaves and total adults per leaf. The relative content of chlorophyll (USPAD), the content of phenolic compounds (Cf), the enzymatic activity of peroxidases (AePOX) and the antioxidant capacity DPPH (Caox) were measured. Gerbera genotypes showed significant differences in the incidence of whiteflies ($p \leq 0.01$), as well as in Cf, AePOX and Caox, both in SI and CI ($p \leq 0.01$), which suggests differences in insect preference a its host and the ability of genotypes to respond to whiteflies. In CI with respect to SI, they increased the content of Cf (1.4 times), AePOX (4 times) and Caox (12 times). USPAD decreased in the genotype with the highest incidence of the insect (-4%). The significant correlations between the number of insects (nymphs) with Cf (-0.71^{**}) and AePOX (-0.65^{**}) and Caox with Cf (-0.73^{**}) and AePOX (-0.76^{**}), showed that the feeding of the insect interferes with the metabolism of its hosts and triggers integral responses in the defense mechanism of plants, with differences in resistance that can be used in genetic improvement.

Keywords: enzymatic activity of peroxidases, genetic variability, phenols.

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Introduction

Gerbera (*Gerbera hybrida*) is one of the most commercially important ornamentals in the State of Mexico (Andrade and Castro, 2018). The main producers in the floricultural region of the south of the state are the municipalities of Villa Guerrero, Tenancingo, Coatepec Harinas and Zumpahuacán, which generate a production of 1 108 384 t per crop cycle (SIAP, 2016). One of the pests that affects the gerbera crop is the whitefly *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (Parrella *et al*., 2014; Reddy, 2016).

For its control, insecticides are commonly applied whose improper use has led to the development of hemiptera resistance (Pérez-Sandoval *et al*., 2011; Bass *et al*., 2015). The use of genotypes resistant to this pest, detectable in genetic variability, can be an easily adoptable, economical, environmentally safe control method and can be an alternative to reduce the use of insecticides (Sulistyo and Inayati, 2016; Hoshino *et al*., 2017).

Conventional genetic improvement has received great benefits from the genetic variation of gerbera and has resulted in the development of genotypes with aesthetic and performance characteristics (Senapati *et al*., 2013; Pradhan *et al*., 2017; Mangroliya *et al*., 2018). According to the perception of producers and marketers of the aforementioned floricultural region, approximately 200 varieties of gerbera are cultivated that, in addition to being morphologically different, also show differences in susceptibility to pests and can be used as a genetic resource. In this sense, variations between gerbera and chrysanthemum genotypes have been reported in the synthesis of secondary metabolites and antioxidant enzymes as defense mechanisms in response to pest attack (Krisps *et al*., 2001; He *et al*., 2011).

For example, Sierra *et al*. (2014) mention that the presence of certain secondary metabolites in plants causes insects, such as whiteflies, to have preference for certain plants and repellency for others due to variations in compounds such as coumarins, terpenes and steroids. In plant species, the stress caused by insects induces antioxidant enzymes such as peroxidases, which participate in the defense mechanism with the synthesis of cell wall polymers such as lignin and suberine, natural physical barriers (Dicko *et al*., 2005; Zhao *et al*., 2016).

In gerbera, evidence of variation between genotypes could be exploited in the search for materials resistant to whiteflies, which would imply a decrease in production costs, particularly in the use of insecticides for the control of this pest. That is why the objective of this research was to evaluate the variation of 10 genotypes of *G. hybrida* in the incidence of *T. vaporariurum*, content of phenolic compounds, enzymatic activity of peroxidases, antioxidant capacity and relative chlorophyll content.

Materials and methods

Experiment location

The research was conducted from September 2016 to December 2017, at the facilities of the University Center Tenancingo of the Autonomous University of the State of Mexico, which are located at km 1.5 of the Tenancingo-Villa Guerrero road, Tenancingo, State of Mexico a 18º 97’ 03’’ north latitude and 99º 61’ 17’’ west longitude and at an altitude of 2 200 m.

Vegetative material

Ten genotypes of *G. hybrida* obtained by *in vitro* culture were used. Seven of them developed at the University Center UAEM Tenancingo and the company Comprehensive Services for Horticulture and identified as: Sofia (Sof), Estrella (Est), Andrea (And), Magda (Mag), Lisieka (Lis), Carmin (Car) and Morelia (Mor), the remaining three are commercial varieties of names Dino (Din), Opera (Ope) and Completa (Com).

Establishment of the experiment

Seedlings acclimated for six to eight weeks with averages of 10 cm in height and 6 leaves were established under greenhouse in 20 L plastic pots with previously disinfected substrate, composed of a mixture of peat and perlite expanded in a 2:1 ratio (v/v), commonly used for macetry in the region. The plants were established in an experimental design of randomized complete blocks with 10 repetitions. The experimental unit was a pot with a plant.

Fertilization of the crop

It was according to Surin (2011) with modifications in micronutrients. The pH was adjusted in a range of 5.5 to 6 and electrical conductivity of 2 to 2.7 mS m^{-1}.

Incidence of the whitefly *Trialeurodes vaporariorum*

The gerbera infestation was by natural invasion of the insect at the beginning of the emission of the floral chapter, during a period of 30 days in which no insecticide applications were made. Nymphs were quantified, without considering urging, in a square centimeter to the center of the underside of mature leaves and the total of adults per complete mature leaf. Measurements were made 30 and 60 days after the infestation (Morales and Cermeli, 2001).

Evaluation of biochemical variables

They were determined in mature leaves with a higher relative chlorophyll content, recorded in 58 ±3.5 SPAD units, (SPAD-502, Minolta Camera Co., Osaka Japan). They were measured in conditions of no incidence (SI), in the vegetative stage of the plants, prior to the infestation of the insect and with incidence of the insect (CI) at the beginning of the chapter emission and in flowering. The following variables were measured by spectrophotometry (Genesys 10S UV-Vis Thermo Scientific).

Phenolic compounds (Cf). They were determined by the folin-ciocalteu (FC) method described by Waterman and Mole (1994). Samples of 100 mg of leaf were macerated in 5 mL of methanol (50%) and incubated at 100 °C for five min, centrifuged at 5 400 rpm for 5 minutes to separate and preserve the supernatant at 4 °C. At 0.15 mL of supernatant the sample was added the same amount of FC reagent and 0.5 mL of 20% Na_2CO_3, they were added to 4.5 mL with distilled water and allowed to stand in the dark for 30 min. The calibration curve was made with gallic acid (10 mg in 10 mL of 50% methanol) as a standard at concentration intervals of 0.005 mL. The results were expressed in mg of gallic acid equivalents per gram of the analyzed sample.

Enzymatic activity of peroxidases (AePOX). It was determined according to the method described by Anderson *et al*. (1995). Samples of ± 50 mg of mature leaf were macerated in 200 µL of 50 mM extraction buffer of pH 7.2 potassium phosphate, 1 mM of ethylene diamine tetraacetic acid (Edta) and 1% of polyvinylpyrrolidone (PVP). The extract was centrifuged at 6 000 rpm for 5 min and the 0.020 mL volume supernatant was used to quantify AePOX in a reaction mixture buffer with 50 mM sodium phosphate pH 7, 3.33 mM guaiacol, 4 mM H_2O_2, at 25 ° C, in a final volume of 3 mL. As a blank, reaction buffer without supernatant was used.

The enzymatic activity was determined by the oxidation of the guaiac substrate in the presence of H_2O_2 at 470 nm for three min in 30 s intervals (extinction coefficient of guaiacol ε= 26.6 Mm^{-1} cm^{-1}, in the nmol equation min^{-1} mg^{-1}). Enzymatic activity Pox = (Abs) (ε) (final test volume/sample volume) (mg of protein).

DPPH antioxidant capacity (Caox). It was determined by the 1,1-diphenyl-2-picryl hydracil (DPPH) method according to Abe *et al*. (1998). The sample extraction methodology was the same as for Cf. A solution of DPPH at 250 µM in 80% methanol (98.58 mg in 1000 mL of methanol) was used. To 2 750 mL of the DPPH solution, 0.25 mL of the gerbera extract supernatant was added and allowed to stand for one hour. Readings were made at 517 nm absorbance. The spectrophotometer blank was 80% methanol. The calibration curve was performed with ascorbic acid (10 mg in 10 mL of 80% methanol), with concentration ranges of 0.005 mL. Each sample was analyzed in triplicate. The results were expressed in mg of equivalents of ascorbic acid per gram of the analyzed sample.

Statistical analysis

The data obtained were analyzed with the statistical package Info Stat (Di Rienzo *et al*., 2016) and submitted to analysis of variance (α= 0.05), where there were significant differences, a comparison of means with the Duncan test was applied (α= 0.05). Multiple correlation analysis was also done.

Results and discussion

Trialeurodes vaporariorum incidence

In the occurrence of adults plus whitefly nymphs, highly significant differences ($p\leq 0.01$) were found among the ten gerbera genotypes evaluated. The variation of genotypes with extreme values was 1 to 43 between Com and Lis, respectively (Figure 1). The comparison of means grouped the genotypes into four categories, defined in this study, according to their incidence in: low (A), Com, Din, Ope, Sof, And and Car, moderate low (AB), Est and Mor , moderate high (B), Mag and high (C), Lis.

The presence of *T. vaporariorum* in gerbera has been documented by several authors (Berndt and Meyhöfer, 2008; Parrella *et al*., 2014; Rojas *et al*., 2018). The variation in the response between genotypes could respond to differences in morphological characteristics of plants, which can act as physical barriers to insects (Taggar and Gill, 2012; Belete, 2018) and factors related to plant metabolism and mechanisms Antioxidants such as the participation of secondary metabolites (Zhang *et a*l., 2017) and the activity of certain enzymes (Taggar *et al.*, 2014; Zhao *et al*., 2016).

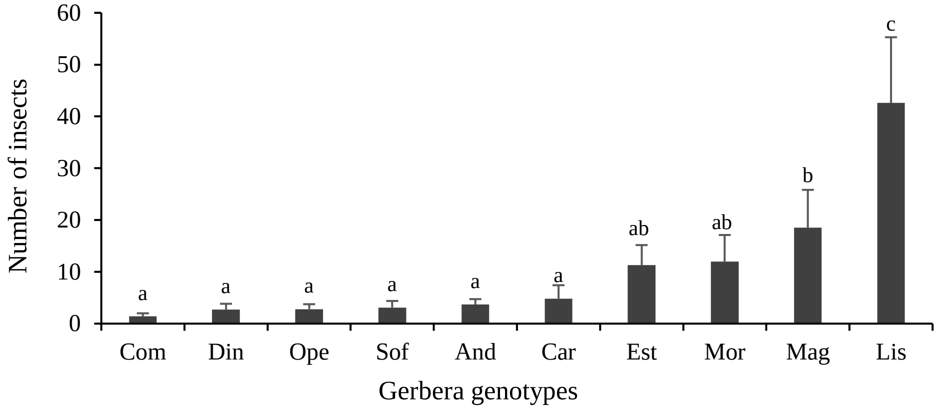

Figure 1. Incidence of *trialeurodes vaporariorum* (adults plus nymphs) in *Gerbera hybrida* in the phenological stages of the beginning of floral chapter emission and flowering. Error bars= standard error. Values with the same letter are statistically equal according to the Duncan test ($p \leq 0.05$).

In this regard, studies in gerbera, highlight the variation between genotypes in the synthesis of secondary metabolites in response to the attack of pests (Krisps *et al*., 2001), which constitute an important element for their use in the development of resistant varieties (Broekgaarden *et al*., 2011; Mitchell *et al*., 2016; Hoshino *et al*., 2017).

In studies conducted by Lucatti *et al*. (2010) with genotypes of *Solanum lycopersicum* L., suggested that the variation between their populations to the incidence of whitefly allows the selection of genotypes with some resistance to the insect for genetic improvement and obtaining resistant varieties. Those genotypes that showed lower incidence of *T. vaporariorum* could be used in the genetic improvement of gerbera to develop resistant or tolerant varieties to this pest, which would imply a decrease in the use of insecticides destined for its control.

Cf Content

Highly significant differences ($p \leq 0.01$) were found in the Cf content in both SI and CI between the ten genotypes evaluated (Figure 2). In SI, the difference between extreme values was almost double between Ope and Mor, which suggests that those genotypes with higher content could have an advantage in insect repellency over those that presented lower values of this metabolite, by synthesizing them as permanent defense strategy. However, defense chemicals such as phenolic compounds are considered expensive for plants due to resources consumed in their biosynthesis (induced) or the ecological consequences of their accumulation as constitutive expression (Lattanzio, 2013).

In CI, an average increase of 40% in the Cf content with respect to SI was observed, and in particular, there was a variation in response between genotypes from 5 (Ope) to 108% (Mor). Genotypes such as Ope and Car, even though their increase from SI to CI was low, remained among the genotypes with the highest Cf values, showing high values from SI.

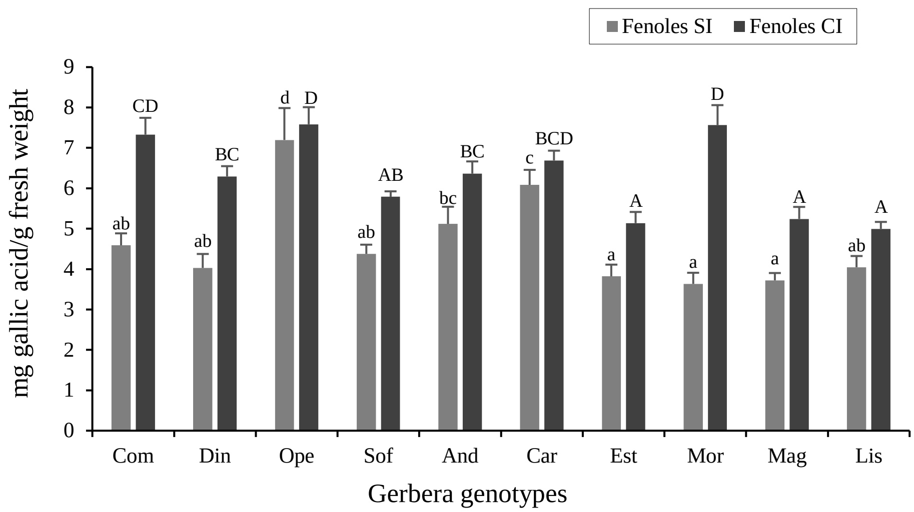

Figure 2. Content of phenolic compounds (cf) in mature leaves of ten genotypes of *Gerbera hybrida* without incidence (SI) in vegetative stage and with incidence (CI) of *T. vaporariorum* at the beginning of emission of floral chapter and flowering. Error bars correspond to the standard error. Lowercase letters= comparison of SI means; uppercase letters= comparison of CI means. Values with the same letter are statistically equal according to the Duncan test ($p \leq 0.05$).

The fact that those genotypes that had a significant increase from SI to CI such as Mor and Com, suggests that they induce the synthesis of this metabolite as a defense mechanism towards *T. vaporariorum* and according to the incidence of the Com insect could present some resistance to The whitefly has a lower incidence of the insect and a high concentration of Cf in CI. Lis, Est and Mag genotypes had the lowest concentrations of Cf in both SI and CI, suggesting that they may be more susceptible to the insect not having other defense mechanisms other than these compounds.

Different studies report the participation of phenolic compounds such as chlorogenic acid, caffeic acid, cinnamic acid (Sandhyarani and Usha, 2013), ferulic acid, catechin (Zhang *et al*., 2017), coumarins (Rehman *et al*., 2012), tannins and lignin (War *et al*., 2012: Vaca-Sánchez *et al*., 2016), to counteract insect stress as a defense mechanism. Tannins, for example, exhibit some toxicity to pests and can significantly reduce the growth, development and survival of insects (War *et al*., 2012), others such as lignin strengthen the cell wall of plants, and act as a barrier physics towards insects (Lattanzio, 2013; Sandhyarani and Usha, 2013).

Sierra *et al*. (2014) mentioned that the presence of secondary metabolites including phenolic compounds in plants, causes insects, such as whiteflies to have preference for certain plants and repellency for others due to variations in the concentration of secondary metabolites. Likewise, the increase in the content of phenols in plants is considered a common response to insect damage (Sandhyarani and Usha, 2013).

In this investigation, the results indicate a significant increase in the Cf content with the presence of the insect, which indicates that this metabolite has an important participation in the defense mechanisms of the plants possibly to counteract the stress of the plant generated by *T.*

vaporariorum Similar results were reported by Zhang *et al*. (2017) who indicate an increase in the content of phenolic compounds in genotypes of *Nicotiana tabacum* after the incidence of *T. vaporariorum* and *Bemisia tabaci*. In soybeans, aphid-resistant genotypes showed a greater increase in phenolic compounds than those susceptible (Jiang *et al*., 2009). The variation in Cf synthesis between gerbera genotypes in both SI (Ope and Car), and CI (Com), suggests differences in the type of response of plants to the insect, according to a metabolic priority by the plant (Strauss *et al*., 2002).

Enzymatic activity of peroxidases (AePOX)

There were highly significant differences ($p \leq 0.01$) between the genotypes studied for AePOX in both SI and CI measurements (Figure 3). In SI the difference between extreme values of enzyme activity was 20 times between Din and Mor. From SI to CI, a 4 times average increase in AePOX was observed. The variation between extreme CI values was 1 to 3 times between Car and Lis, respectively. Those genotypes with higher AePOX in SI (Com and Din), showed a lower increase in enzyme activity compared to other genotypes in CI, however, their previous values (in SI) allowed them to be on par with those genotypes with high AePOX in IC (Figure 1). In contrast, the genotypes that had a significant proportional increase from SI to CI were Mor and Lis, although their values remained low compared to other genotypes.

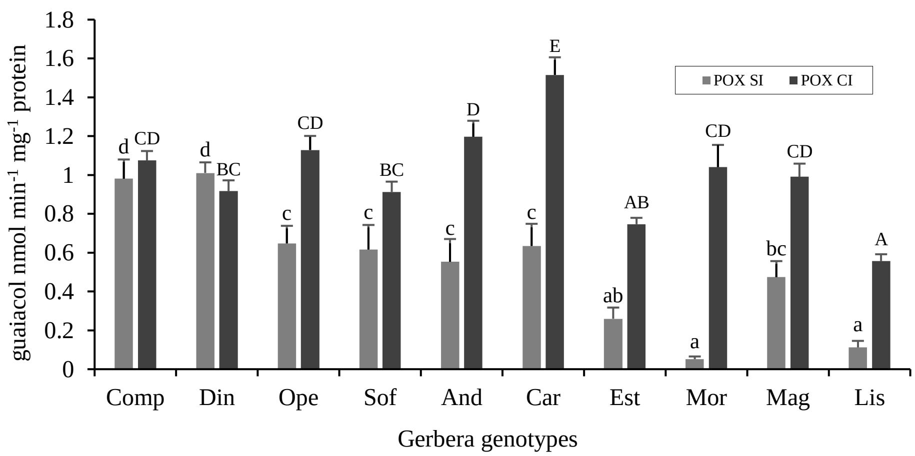

Figure 3. Enzymatic activity of peroxidases (AePOX) in mature *Gerbera hybrida* leaves; no incidence (SI), in vegetative stage; and with incidence (CI) of *T. vaporariorum* at the beginning of emission of floral and flowering chapter. Error bars correspond to the standard error. Lowercase letters= comparison of SI means; uppercase letters= comparison of CI means. Values with the same letter are statistically equal according to the Duncan test ($p \leq 0.05$).

In the plant-insect interaction, the participation of antioxidant enzymes such as POX and polyphenol oxidases (PPO) has also been reported (Jiang *et al*., 2009; Sandhyarani and Usha, 2013), which catalyze the oxidation of a wide variety of phenols for the synthesis of lignin and suberine that accumulate in the cell wall or for the formation of quinones, toxic to insects (Lattanzio *et al*., 2006; Lopez-Nicolas and García Carmona, 2010; Shigeto and Tsutsumi, 2016).

In this study, the differences in AePOX between gerbera genotypes evaluated, both in SI and in CI, indicate natural variation between genotypes in the synthesis of this enzyme possibly regulated at the level of transcription or translation (Cosio and Dunand, 2009; Shigeto and Tsutsumi, 2016). In genotypes of other ornamentals of the same family as *Crysanthemum grandiflorum*, variation in the expression of antioxidant enzymes (super oxide dismutase and phenyl ammonium lyase) has also been reported, including POXs, both without incidence and with incidence of aphids and suggest the participation of these enzymes in insect resistance (He *et al*., 2011).

Those genotypes that more induced the enzyme from SI to CI, such as Lis, Mor and Car, guided the exercise of their energy resources differently (Strauss *et al*., 2002). Similar results to this research on the increase in the enzymatic activity of POX with the incidence of whitefly were obtained in black lentil genotypes (*Vigna mungo* L.) affected by *Bemisia tabaci*, where those more resistant to insect stress showed a greater increase in AePOX than those susceptible (Taggar *et al*., 2014). Zhao *et al*. (2016) reported an increase in the enzymatic activity of POX and catalase in tobacco plants after the presence of *B. tabaci*, in comparison to the control plants. On the other hand, Mor's ability to promote AePOX with the high incidence of *T. vaporariorum* suggests the participation of the enzyme as a defense mechanism.

DPPH antioxidant capacity (Caox)

Highly significant differences ($p\leq 0.01$) were found among the ten gerbera genotypes evaluated in both SI and CI (Figure 4). In SI, although there was variation between almost 2:1 genotype, their values were less than 0.l mg of ascorbic acid per gram of fresh weight in all genotypes evaluated. In contrast, in CI the proportional increase in each genotype varied from six to 20 times with respect to SI. Lis that stood out with intermediate values of antioxidant capacity, showed low values of Cf and AePOX content, which may explain the higher incidence value of *T. vaporariorum*, otherwise Com that presented low values of antioxidant capacity and high values of Cf and AePOX, in addition to the lower incidence values of the insect.

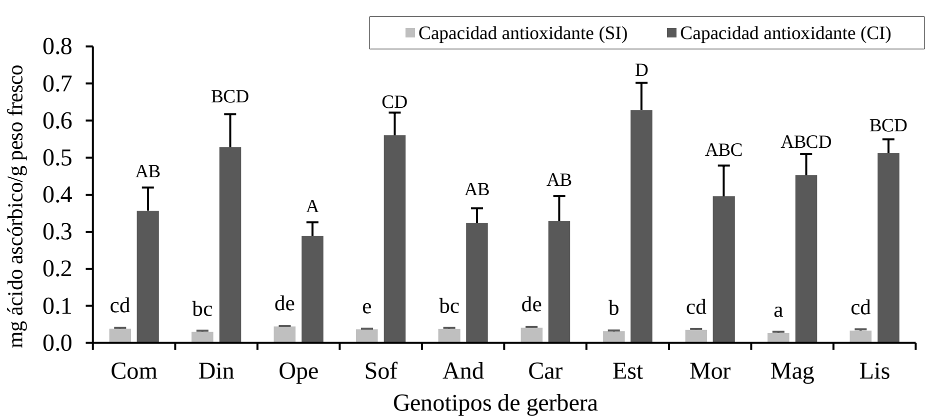

Figure 4. Antioxidant capacity DPPH (Caox) of *Gerbera hybrida* without incidence (SI), in vegetative stage and with incidence (CI) of *T. vaporariorum* at the beginning of the emission of floral chapter and flowering. Lowercase letters= comparison of SI means, capital letters= comparison of CI means. Error bars correspond to the standard error. Values with the same letter are statistically equal according to the Duncan test ($p\leq 0.05$).

The higher antioxidant capacity values suggest a higher production of reactive oxygen species (EROS) as a result of different types of stress in the plant such as the incidence of insects or infection by pathogens, mechanical damage, wounds by herbivores or abiotic factors (Mittler, 2002; Benezer-Benezer *et al*., 2008). In fact, only egg oviposition promotes an increase in EROS, such as the hypersensitive response induced by pathogens, which lead to the desiccation of the egg and its subsequent detachment of plants (Hilker and Fatouros, 2016).

In other species such as tobacco (*Nicotiana tabacum* L.), whitefly infestation increased H_2O_2 levels by 77.3% more than in control plants (Zhao *et al*., 2016). H_2O_2, in addition to being a signaling molecule under stress conditions and in the physiological processes of plants (Sharma *et al*., 2012), is used as a substrate by peroxidases for both lignification and suberization of the cell wall, such as for the oxidation of phenolic compounds during insect injuries, which indirectly converts it into an antioxidant enzyme (He *et al*., 2011).

Relative content of chlorophyll (USPAD)

Highly significant statistical differences ($p \leq 0.01$) were presented in both SI and CI evaluations (Figure 5), but with an average discrete variation of 15% between extreme values between genotypes of both measurements. Likewise, there was an average increase of SI to CI of 8%; however, in two genotypes their values decreased, and they corresponded to those that showed the highest insect incidence. These results suggest that the variation in the relative chlorophyll content between genotypes is due to different factors such as the variability of the species, the phenological stage of the plant in which it was evaluated, and the same incidence of pests as the whitefly.

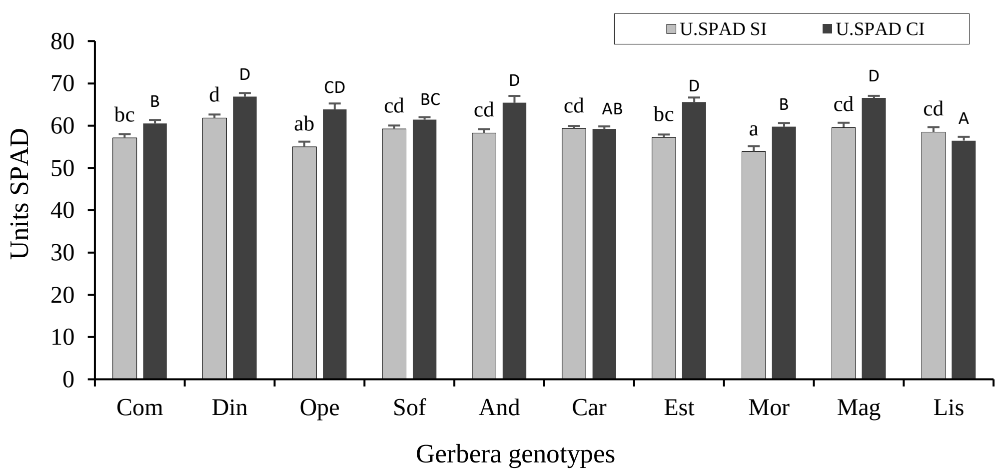

Figure 5. Relative content of chlorophyll (USPAD) in *Gerbera hybrida* of photosynthetically active leaves without incidence (SI), in vegetative stage and with incidence (CI) of *T. vaporariorum* at the beginning of the emission of floral chapter and flowering. Lowercase letters= comparison of SI means, capital letters= comparison of CI means. Error bars correspond to the standard error. Values with the same letter are statistically equal according to the Duncan test ($p \leq$ 0.05).

Goławska *et al*. (2010) cites that the relative content of chlorophyll is one of the most important parameters in the relationship between plants and insects. A significant reduction in the relative chlorophyll content due to insect infestation has been reported in different plant species (Goławska *et al*., 2010; Huang *et al*., 2014). Kerchev *et al*. (2012) suggested that insect herbivory far from stimulating photosynthesis reduces carbon fixation and this response occurs by reprogramming the gene expression of the primary metabolism.

Correlation analysis

The negative and highly significant correlations of the incidence of insects, particularly nymphs, with phenols (r= -0.71) and activity of the POX enzyme (r= -0.65) (Table 1), suggest an influence of these biochemical variables on protection of plants, as already mentioned by different authors (Sandhyarani and Usha 2013; Taggar *et al*., 2014). Also, the phenolic content was positively correlated and highly significant with AePOX (r= 0.62).

Table 1. Pearson correlation matrix of six variables measured in *Gerbera hybrida*.

	Adults (A)	Nymphs (n)	A + n	Cf	AePOX	USPAD	Caox
Adults (A)	1						
Nymphs (n)	0.84^{**}	1					
A + n	0.99^{**}	0.89^{**}	1				
Cf	-0.55	-0.71^{**}	-0.6	1			
AePOX	-0.66^{**}	-0.52	-0.65^{**}	0.62^{**}	1		
USPAD	-0.51	-0.13	-0.45	-0.14	0.04	1	
Caox	0.32	0.27	0.32	-0.73^{**}	-0.76^{**}	0.15	1

A + n= adults plus nymphs; Cf= Phenolic compounds; POX= enzymatic activity of peroxidases; U SPAD= SPAD units; Caox= antioxidant capacity; **= highly significant values.

In this association between variables it is important to highlight that peroxidases, in addition to catalyzing the lignification of the cell wall and detoxifying peroxides produced due to oxidative stress, produce phenoxy and other oxidative radicals that in association with phenols that act as deterrents for feeding and they produce toxins that reduce the digestibility of plant tissue to insects (Dicko *et al*., 2005; Shigeto and Tsutsumi, 2016).

The antioxidant capacity was negatively and highly significant correlated with Cf (r= -0.73) and AePOX (r= -0.76), which suggests that the phenols and the activity of the enzyme in its action to minimize damage by the insect, neutralize free radicals indirectly represented by antioxidant capacity. The relative chlorophyll content was not significantly correlated with any variable; however, the trend of negative correlation with number of insects was similar to that reported by Huang *et al*. (2014).

Conclusions

Gerbera genotypes showed variation in the incidence of *T. vaporariorum*, as well as in the content of phenolic compounds and in the AePOX, which indicates genetic variation of this species, and preferences of the insect towards certain hosts. Those genotypes with lower incidence of the insect can be used to develop resistant or tolerant varieties as alternatives in floricultural production. The ability of those genotypes both without incidence and with incidence, in the synthesis of enzymatic antioxidant compounds (AePOX) and non-enzymatic (phenolic compounds), to counteract the oxidative stress caused by the insect, probably gives them some bioprotection as a defense mechanism against *T. vaporariorum* infestation.

Acknowledgments

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