Control of Western flower thrips through jasmonate-triggered plant immunity

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Chapter 4

Phenotypic variation in constitutive and jasmonic acid-mediated induced defenses against Western flower thrips in chrysanthemum

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Western flower thrips (WFT), *Frankliniella occidentalis*, is a severe insect pest of Chrysanthemum [*Chrysanthemum* × *morifolium* Ramat. (Asteraceae)]. Here we have explored whether variations in constitutive and inducible levels of trichome density and activity of the defensive enzyme polyphenol oxidase (PPO) correlate with WFT resistance in chrysanthemum. First, our results showed that both non-glandular and glandular leaf trichome densities significantly varied among 95 different chrysanthemum cultivars. Additional analyses in a subset of 12 of those cultivars, displaying contrasting levels of trichome densities, showed significant variations in PPO activities as well. Yet, constitutive levels of trichome density and PPO activity did not correlate with chrysanthemum resistance to WFT. Exogenous application of the phytohormone jasmonic acid (JA) to six selected cultivars further showed that activation of JA defenses increased chrysanthemum resistance to WFT, but this effect was cultivar-dependent. JA-mediated induction of WFT resistance was not explained by variations in non-glandular leaf trichomes nor PPO activity. Taken together, our results show that trichome density and PPO might not play a relevant role on chrysanthemum defenses against WFT, however, this does not exclude the existence of other glandular trichome-associated chemical defenses that were not addressed in our study.

Keywords: chrysanthemum; constitutive defense; *Frankliniella occidentalis*; induced defenses; jasmonic acid; polyphenol oxidase; trichome density

1 Introduction

Chrysanthemum [*Chrysanthemum* × *morifolium* Ramat. (Asteraceae)], which was first bred in China and Japan ca. 3000 years ago, is one of the economically most important ornamental crops worldwide (Fletcher, 1992). Its production, however, is negatively affected by a high susceptibility to multitude of arthropod pests. A major arthropod pest of chrysanthemum is the Western flower thrips (WFT), *Frankliniella occidentalis* [Pergande]. WFT is also one of the most serious greenhouse pests in agricultural and horticultural crops worldwide (Mouden *et al.*, 2017). This tiny insect has piercing-sucking mouth parts, and it can cause two types of damage: direct damage through feeding on leaves, flowers and fruits, thus reducing plant growth and affecting product appearance and market quality (de Jager, CM *et al.*, 1995; de Jager, KM *et al.*, 1995), and indirect damage through the transmission of devastating virus diseases (Maris *et al.*, 2003). Currently, the use of insecticides has been the most common strategy for WFT control. Determining chrysanthemum defense mechanisms against WFT would facilitate breeding for resistant cultivars and, therefore, reduce the application of pesticides (de Jager, CM *et al.*, 1995).

To defend themselves against arthropod herbivores, plants have evolved sophisticated constitutive and inducible defenses. Constitutive defenses are defined as physical structures or chemical components present in the plant prior to herbivory, and controlled by genetics or environmental factors (Rosner & Hannrup, 2004; Franceschi *et al.*, 2005). Induced defenses are initiated upon herbivore attack, and regulated by the type of herbivore-associated damage, environment, as well as the plant genetics and ontogeny (Karban & Myers, 1989; Franceschi *et al.*, 2005; Köhler *et al.*, 2015). In the framework of Integrated Pest Management, breeding for enhanced constitutive and inducible plant defenses against herbivorous pests is considered a relevant strategy to achieve pest control (Bottrell, 1979; Mirnezhad *et al.*, 2010; War *et al.*, 2012). Both constitutive and inducible defenses have been observed to vary within and among plant species. In some cases plants with high constitutive chemical or physical defenses display weaker inducible defenses (Agrawal *et al.*, 2002; Wittstock & Gershenzon, 2002). Yet, some authors have reported a positive correlation between the two types of resistance mechanisms as well (Zangerl & Berenbaum, 1990; Siemens & Mitchell-Olds, 1998), while others have reported no correlation at all (Brody & Karban, 1992; Thaler & Karban, 1997; English-Loeb *et al.*, 1998; Underwood *et al.*, 2000). Exploration of these variations and identification of resistant chrysanthemum genotypes that combine both defense strategies would be of fundamental importance for plant breeding and pest control.

Host plant resistance to arthropod herbivores is based on morphological and/or chemical traits that can confer antixenotic and/or antibiotic properties. Among the plant defense-associated morphological structures, leaf trichomes have been associated to plant resistance against arthropod herbivores in different plant species (Levin, 1973; Dalin *et al.*, 2008). Trichomes are hairy epidermal structures mainly found in leaves and stems, that can be classified as non-glandular and glandular (Glas *et al.*, 2012). Non-glandular trichomes function as physical hurdles, hindering the ability of insects to access the leaf surface and thus to feed and/or oviposit (Duffey, 1986). Glandular trichomes provide a physical barrier in plants, but they can also chemically repel or poison arthropod herbivores (Wagner, 1991). In chrysanthemum, the presence of non-glandular and glandular leaf trichomes has been described by several authors (Vermeer & Peterson, 1979; Deng *et al.*, 2010; He *et al.*, 2011; Sun *et al.*, 2013). Furthermore, density of non-glandular trichomes and the size of glandular trichomes have been positively correlated with enhanced chrysanthemum resistance to aphids

in three chrysanthemum cultivars (He *et al.*, 2011). However, the study of He *et al*. (2011) used a rather low number of cultivars, and a greater number of genotypes would be needed to determine the role of chrysanthemum trichomes on pest resistance. Furthermore, whether the density of both glandular and non-glandular leaf trichomes is important for chrysanthemum resistance to other insect pests, such as WFT, is also unknown.

We have previously reported that leaf content in chlorogenic acid was positively correlated with chrysanthemum resistance to WFT (Leiss *et al.*, 2009b). Disruption of plant tissues by herbivory triggers the oxidation of chlorogenic acid by plant polyphenol oxidases (PPOs) and peroxidases. This can result in the production of highly reactive quinones that inhibit the digestion of plant proteins by herbivores (Stout *et al.*, 1994; War *et al.*, 2012). Notably, higher activities of PPO have been associated to increased resistance to diverse arthropod herbivores in tomato (Mahanil *et al.*, 2008; Bhonwong *et al.*, 2009). In chrysanthemum, He *et al* (2011) reported higher constitutive levels of PPO activity in an aphid-resistant cultivar, suggesting a possible role of this defensive enzyme in chrysanthemum resistance to herbivory.

Although both trichome density and PPO activity are constitutively expressed in plants, their expression can be modulated by abiotic and biotic factors (Biesiada & Tomczak, 2012; Hauser, 2014; Escobar-Bravo *et al.*, 2017; Escobar-Bravo *et al.*, 2018), as well as by defense elicitors. For instance, application of the phytohormone jasmonic acid (JA) has been reported to induce trichome densities in tomato (Boughton *et al.*, 2005; Escobar-Bravo *et al.*, 2017) and Arabidopsis (Traw & Bergelson, 2003) among other plant species. Similarly, JA can also induce PPO in diverse plant species (Thaler *et al.*, 1996; Constabel & Ryan, 1998; Chen *et al.*, 2018).

In the present study we investigated whether constitutive and inducible levels of non-glandular and glandular trichome densities, as well as PPO activity correlate with chrysanthemum resistance to WFT. For this, we first determined trichome density in 95 chrysanthemum cultivars. Thereafter, we selected 12 of these cultivars to further test whether constitutive levels of trichome densities and PPO correlate with WFT resistance. Finally, we also determined whether JA-mediated induction of plant defenses against WFT varies among chrysanthemum cultivars, and whether this might be explained by the differential expression of trichomes and PPO-associated defenses.

2 Materials and methods

2.1 Plants and insects

A total of 95 chrysanthemum cultivars provided by three Dutch chrysanthemum breeders [Dekker Chrysanten (Hensbroek), Deliflor Chrysanten (Maasdijk) and Dümmen Orange (De Lier)] were used in our study (**Table 1**). Cuttings were individually planted in plastic trays (4 cm × 4 cm × 6 cm) filled with potting soil. At 14 d after planting, plants were transplanted to plastic pots (9 cm × 9 cm × 10 cm) containing the same potting soil. Plants were randomly placed in a climate room provided with 20°C, 70% RH, 113.6 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR) and L16:D8 photoperiod.

Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) were obtained from a colony reared on chrysanthemum flowers (cultivar 'Euro Sunny') in a climate room at 23°C, 60% RH and L12:D12 photoperiod.

Table 1 Breeding IDs of the 95 chrysanthemum cultivars used in this study. Cultivars can be identified according to breeding ID at the different breeding companies: Dekker Chrysanten (cultivars 1–28), Deliflor Chrysanten (cultivars 29–63) and Dümmen Orange (cultivars 64–95).

Cultivar	Breeding ID	Cultivar	Breeding ID	Cultivar	Breeding ID	Cultivar	Breeding ID
1	DC-1	25	DC-25	49	48837	73	56072
2	DC-2	26	DC-26	50	48639	74	56168
3	DC-3	27	DC-27	51	9403	75	56701
4	DC-4	28	DC-28	52	41475	76	56703
5	DC-5	29	26741	53	45644	77	56713
6	DC-6	30	31563	54	45785	78	56817
7	DC-7	31	8713	55	48286	79	57352
8	DC-8	32	7688	56	40931	80	57709
9	DC-9	33	30600	57	43339	81	57773
10	DC-10	34	21697	58	90753	82	57993
11	DC-11	35	13185	59	36318	83	58498
12	DC-12	36	8578	60	43110	84	59209
13	DC-13	37	8393	61	44339	85	64952
14	DC-14	38	4875	62	48942	86	65001
15	DC-15	39	48864	63	9361	87	37511
16	DC-16	40	47287	64	42215	88	37577
17	DC-17	41	57067	65	42377	89	37630
18	DC-18	42	55229	66	42629	90	42415
19	DC-19	43	55238	67	42909	91	25533
20	DC-20	44	46885	68	50223	92	22898
21	DC-21	45	55223	69	50858	93	48015
22	DC-22	46	55115	70	51643	94	20880
23	DC-23	47	45728	71	56068	95	49230
24	DC-24	48	90633	72	56069		

2.2 Experimental design

To investigate phenotypic variations in constitutive and inducible chrysanthemum defenses associated to WFT resistance, we performed three different experiments. First, we determined constitutive levels of non-glandular and glandular trichome densities on leaves of 95 chrysanthemum cultivars at 35 d after planting (Experiment 1). Second, we selected 12 out of the 95 chrysanthemum cultivars to further determine whether constitutive levels of trichome density and polyphenol oxidase (PPO) activity correlated with WFT resistance (Experiment 2). For this, chrysanthemum plants were sampled for determination of non-glandular and glandular trichome density and PPO activity, or used for non-choice whole plant thrips bioassays, at 35 d after planting. Third, we tested whether application of the phytohormone jasmonic acid (JA) enhanced WFT resistance in 6 selected cultivars, and whether this was explained by the induction of trichome- and PPO-associated defenses (Experiment 3). For this experiment, each JA-treated chrysanthemum plant was sprayed with approximately 5 ml of 3 mM JA (Cayman, Ann Arbor, Michigan, USA) in 2.4% aqueous ethanol solution as described by Redman *et al* (2001). Each control plant was sprayed with a similar volume of a mock solution consisting of 2.4% aqueous ethanol. Seven days after the hormone treatment, mock- and JA-treated plants were sampled for determination of non-glandular and glandular trichome density, PPO activity, or used for non-choice whole plant thrips bioassays. We selected this sampling time because previous studies in tomato and *Arabidopsis* have determined that a significant increment of trichome density can be observed at 7 days after the hormone application (Boughton *et al.*, 2005; Yoshida *et al.*, 2009). In addition, higher PPO activities can be detected up to 7 days of JA induction in tomato (Thaler *et al.*, 2001).

2.3 Non-choice whole plant thrips bioassay

Non-choice whole plant bioassays were performed as described by Leiss *et al* (2009a). For this, individual plants were placed into WFT-proof cages consisting of perspex plastic cylinders (50 cm height and 20 cm diameter) closed at the top with a displaceable ring of nylon gauze (120 µm mesh size). Ten adult WFT (8 females and 2 males) were added to each plant. Plants were maintained in a climate room provided with 113.6 µmol photons m^{-2} s^{-1} of PAR, 16L:8D of photoperiod, 25°C and 70% RH. WFT feeding damage, hereafter referred to as 'silver damage', was visually scored for each leaf of the plant, and expressed as the damaged area in mm^2, at 7 days after infestation. Whole plant silver damage was calculated by adding up the damage of each individual leaf.

2.4 Analysis of trichome density and morphology

In all the experiments, densities of glandular and non-glandular trichomes were determined on the adaxial leaf surface of the third leaf from the apex. Two pictures were taken in the middle part of the leaf at both sides of the main vein, each covering an area of 12 mm^2, using a stereomicroscope (MZ16, Leica Microsystems, Wetzlar, Germany). Trichome number was counted in both pictures using the software 64-bit Fiji ImageJ (http://fiji.sc/Fiji), and the average of the two measurements was expressed as number of trichomes per cm^2.

2.5 Scanning electron microscopy (SEM)

SEM analysis was conducted on the third leaf from the apex. Leaves were fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (PBS), pH 7.2, at room temperature. Samples were then dehydrated in acetone series of 50, 70, 90, 96, and 100% (v/v) and then dried in a Bal-Tec CPD 030 Critical Point Dryer with liquid CO_2 (Leica Microsystems). The samples were coated with gold in a Polaron SEM coating unit E5100. SEM images were taken with a JEOL 6400 scanning electron microscope at the Microscopy Unit of the Institute of Leiden (The Netherlands).

2.6 Determination of PPO activity

PPO activity was determined in the third leaf from bottom following the methodology described in Stout *et al*. (1998). Briefly, 0.150 g of leaf tissue without midrib flash frozen in liquid nitrogen, ground with a tissue lyser (Qiagen, Hilden, Germany) and homogenized in a 2 ml tube with 1.25 ml ice-cupper 0.1 M pH 7.0 phosphate buffer containing 7% polyvinylpolypyrolidine and 0.4 ml of 10% Triton X-100. The homogenate was vortexed for 2 min and centrifuged for 10 min at 11,000 × g and 4°C. Five microliters of the supernatant were added to 1 ml of 2.92 mM chlorogenic acid solution in pH 8.0 phosphate buffer. The optical density (OD) at 470 nm was recorded in a spectrophotometer (UV-1800, Shimadzu) every ten seconds for one minute. PPO activity was defined as the increment of OD values per min per gram of fresh weight.

2.7 Statistical analysis

Normality and homogeneity of data residuals were first checked using Kolmogorov-Smirnov and Levene's tests, respectively. We used one-way ANOVA to test significant differences in non-glandular trichome densities (Experiment 1) and PPO activity (Experiment 2) among cultivars. Data were square root transformed for non-glandular trichome density to meet the requirements of the ANOVA. Differences in glandular trichome density (Experiment 1) and silver damage symptoms (Experiment 2) among cultivars were analyzed by Kruskal-Wallis test. As a measure for the phenotypic variation across cultivars, the coefficient of variation (CV) was calculated. CV is a standardized measure of the dispersion of a sample. CV is expressed as the ratio of the standard deviation (δ) to the mean (μ), i.e. $CV = \delta/\mu$ (Sokal &

Rohlf, 1995). A high CV value means high phenotypic variation for the studied trait. The relationships between non-glandular and glandular trichome densities (Experiment 1), silver damage and PPO activity or trichome density (Experiment 2), and between constitutive and induced resistance indexes (Experiment 3) were determined by Pearson or Spearman correlation tests. In the Experiment 3, the effects of the hormone treatment, plant genotype and their interaction on silver damage, PPO activity and glandular trichome density were analyzed by Generalized Linear Models (GLM) using linear distribution and identity link functions. Differences among groups were tested by Fisher´s least significant difference (LSD) post-hoc test. The constitutive resistance index (CRI) was calculated for each cultivar in Experiment 3 by dividing the silver damage symptoms by the silver damage symptoms of a "reference cultivar". The "reference cultivar" was selected based on its lowest silver damage symptoms. Accordingly, the cultivar with the lowest silver damage has the highest constitutive resistance index, set as 1. The induced resistance index (IRI) was calculated for a given cultivar as the percent reduction in silver damage symptoms in JA-treated plants respect to controls: [(average of silver damage detected on JA-treated plants – average silver damage detected on mock-treated plants) / average of silver damage in mock-treated plants] (Brody & Karban, 1992). Statistical analyses were performed by using the SPSS software package (version 25; SPSS Inc., Chicago, IL, USA). All detailed statistics are included in Table S1.

3 Results

3.1 Non-glandular and glandular trichome densities vary among chrysanthemum cultivars

The morphological analysis of the chrysanthemum leaves revealed two types of leaf trichomes, non-glandular and glandular trichomes, the latter having a bean-shape structure and coinciding with the description reported by He *et al*. (2011) (**Fig. 1**).

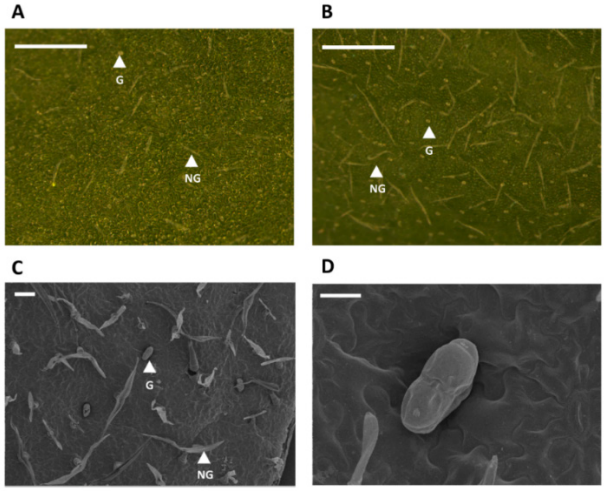

Fig. 1 Representative micrographs of non-glandular and glandular trichomes on chrysanthemum leaves. Light micrographs of trichomes in the adaxial leaf surface of (**A**) a chrysanthemum cultivar displaying low trichome density and (**B**) a cultivar displaying high trichome density. Scanning electron microscopic images of the adaxial leaf surface of chrysanthemum leaves (**C** and **D**). (**D**) Glandular trichome. White arrows indicate the position of non-glandular (NG) and glandular (G) trichomes. The white bars represent 100 μm in (A), (B) and (C), and 30 μm in (D).

Density of non-glandular trichomes differed strongly among cultivars (ANOVA, $P < 0.001$), ranging from 33 trichomes/cm² (cultivar 83) to 350 trichomes/cm² (cultivar 24) (**Fig. 2A**). The CV of non-glandular trichomes density was 37%. Similarly, glandular trichome densities varied strongly among cultivars (Kruskal-Wallis, $P < 0.001$), with a coefficient of variation of 113% (**Fig. 2B**). Out of the ninety-five cultivars analyzed, twenty of them had no glandular trichomes, and the highest density of glandular trichomes was 211 trichomes/cm². Densities of both non-glandular and glandular trichomes were positively correlated (**Fig. 3**; two-tailed Spearman, $P = 0.008$).

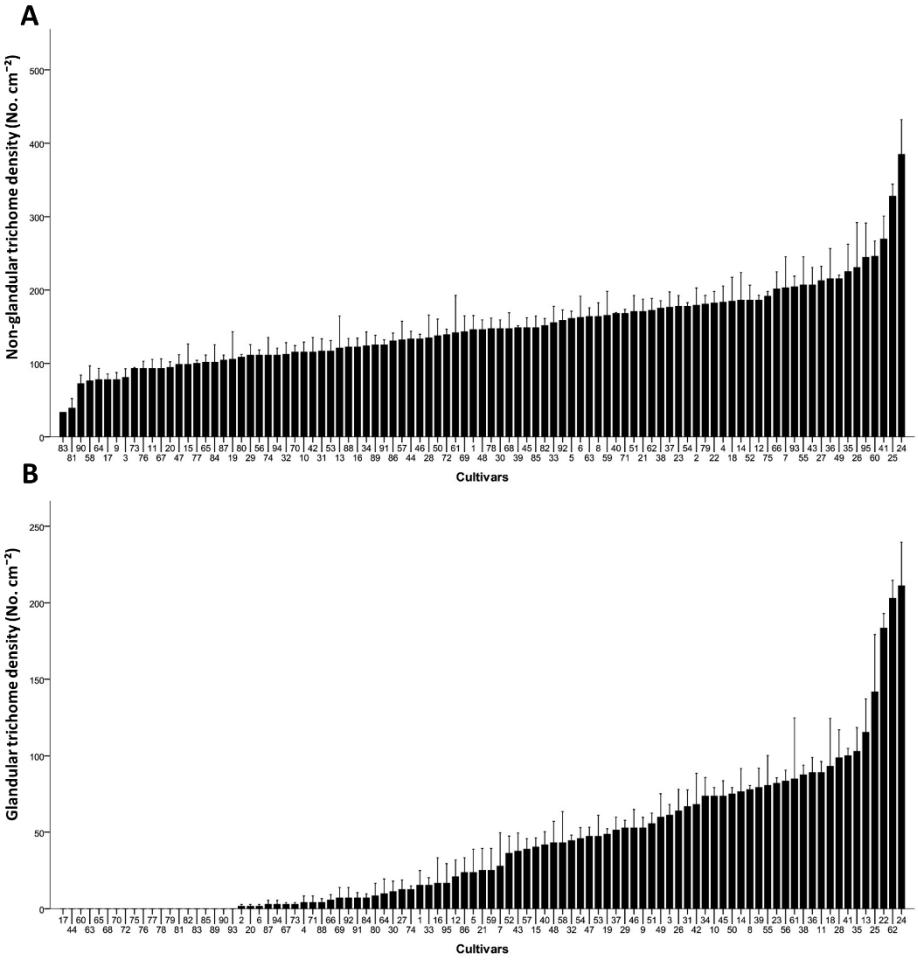

Fig. 2 Phenotypic variation in non-glandular and glandular trichome densities. Bars depict the mean (± SEM, $n = 3$) of leaf (**A**) non-glandular and (**B**) glandular trichome density analyzed in 95 chrysanthemum cultivars at 35 days after planting. Trichome density was determined on the adaxial side of the third leaf from the apex.

3.2 Trichome density and PPO levels do not correlate with chrysanthemum resistance to WFT

To determine whether trichome density was correlated with chrysanthemum resistance to WFT, we selected 12 chrysanthemum cultivars differing in trichome densities and tested their levels of WFT resistance using non-choice whole plant bioassays. Silver damage symptoms

significantly differed among the chrysanthemum cultivars (Fig. S1A; Kruskal-Wallis test, $P < 0.001$). No significant correlations between silver damage and non-glandular (**Fig. 4A**; two-tailed Pearson, $P = 0.564$) or glandular trichome density (**Fig. 4B**; two-tailed Pearson, $P = 0.715$) were observed.

To further test whether variation in WFT susceptibility correlated with differences in leaf chemical defenses, we also determined constitutive levels of PPO activity in the selected twelve cultivars (**Fig. 4C**). PPO activity significantly differed among the chrysanthemum cultivars (Fig. S1B; ANOVA, $P = 0.002$). However, the CV of PPO (i.e. 15%) was smaller than that of silver damage, i.e. 38%. PPO activity did not correlate with the silver damage symptoms (**Fig. 4C;** two-tailed Pearson, $P = 0.355$).

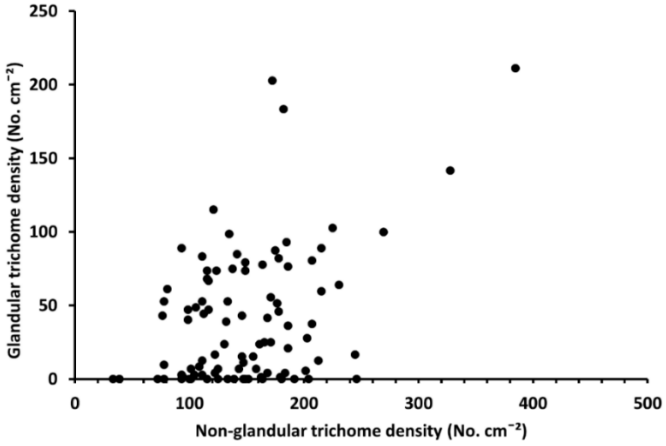

Fig. 3 Scatter plot depicting the relationship between non-glandular and glandular trichome densities. Non-glandular and glandular trichome density were measured in 95 chrysanthemum cultivars at 35 days after planting. Each dot corresponds to the mean of three plant replicates per cultivar. Trichome density was determined on the adaxial side of the third leaf from the apex.

3.3 Induction of chrysanthemum resistance to WFT differed among chrysanthemum cultivars, and it is not explained by variations in PPO and trichome levels

Constitutive levels of non-glandular and glandular trichomes, as well as PPO activity, were not correlated with chrysanthemum resistance to WFT. Thus, we further explored whether a higher inducibility of these defenses might explain the differences in WFT susceptibility among chrysanthemum cultivars. For this, we selected six cultivars differing in WFT resistance levels, PPO and trichome densities, and determined the induction of these defenses after application of JA. Silver damage symptoms significantly differed among the cultivars (**Fig. 5A**; GLM, $P < 0.001$ for genotype). Application of JA significantly reduced silver damage symptoms (GLM, $P < 0.001$ for hormone treatment) and this effect was depended on the chrysanthemum cultivar (GLM, $P < 0.001$ for the interaction).

JA significantly induced PPO activity in all the chrysanthemum cultivars (**Fig. 5B**; GLM, $P < 0.001$ for hormone treatment), and this induction was also dependent on the cultivar (GLM, $P < 0.001$ for interaction). Non-glandular trichome density varied among cultivars (GLM, $P < 0.001$ for genotype) (**Fig. 5C**), and it was significantly induced by JA (GLM, $P = 0.025$ for hormone treatment) depending on the cultivar (GLM, $P = 0.027$ for interaction). Glandular trichome density significantly varied among cultivars as well (**Fig.**

5D, GLM, $P < 0.001$). However, JA did not increase glandular trichome densities in any chrysanthemum cultivars (GLM, $P = 0.922$ for hormone treatment; $P = 0.541$ for interaction).

Induced production of non-glandular trichome (**Fig. 6A**; two-tailed Pearson, $P = 0.190$) or PPO activity (**Fig. 6B**; two-tailed Pearson, $P = 0.824$) did not correlate with the silver damage symptoms in JA-treated plants. Finally, CRI did not correlate with IRI for these 6 cultivars (**Fig. 6C**; two-tailed Pearson, $P = 0.944$).

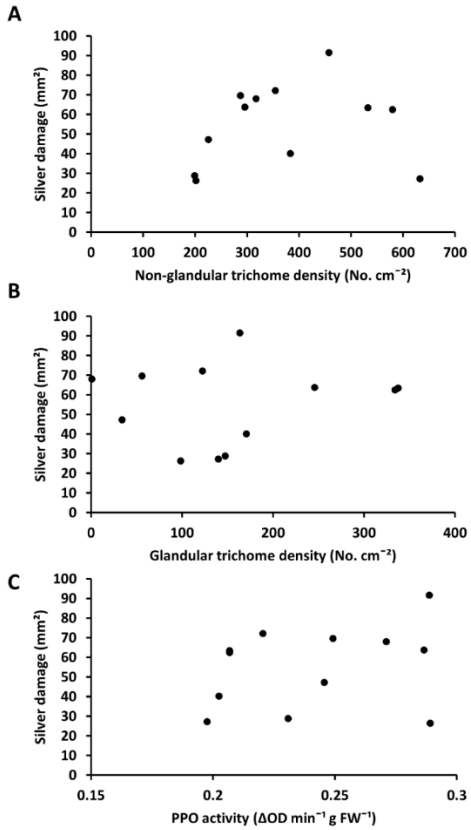

Fig. 4 Relationship between Western flower thrips resistance and putative defense-related traits in chrysanthemum. Scatter plots depicting the relationship between (**A**) silver damage and polyphenol oxidase (PPO) activity levels, (**B**) silver damage and non-glandular trichome density, and (**C**) silver damage and glandular trichome density. Plants were sampled for PPO activity and trichome density, or subjected to non-choice whole plant thrips bioassays, at 35 days after planting. Silver damage symptoms were evaluated at 7 days after Western flower thrips infestation. The plots display data obtained from 12 chrysanthemum cultivars. Each dot corresponds to the mean of five plant replicates per cultivar for PPO and trichome density, and of ten plant replicates per cultivar for silver damage symptoms.

4 Discussion

In the present study, we have demonstrated that constitutive and inducible resistance to WFT strongly differ among chrysanthemum cultivars. However, this variation could not be explained by the presence or induction of non-glandular and glandular trichomes, nor by the activity of the defensive protein PPO.

First, we showed that constitutive levels of non-glandular and glandular trichome densities varied considerably among chrysanthemum cultivars. A few studies have described the presence of trichomes in chrysanthemum leaves and the variation of trichome densities among cultivars (Stavrinides & Skirvin, 2003; He *et al.*, 2011; Sun *et al.*, 2013). However, those studies included only two or three chrysanthemum genotypes. Here we present a comprehensive analysis on trichome density using a large and significant representation of chrysanthemum cultivars. Further analyses on twelve selected chrysanthemum cultivars, differing in trichome densities, showed that these genotypes also displayed differences in their chemical defenses, i.e., PPO activity. However, our results revealed that variation in constitutive levels of non-glandular and glandular trichome densities, as well as PPO activities, were not correlated with WFT resistance in chrysanthemum.

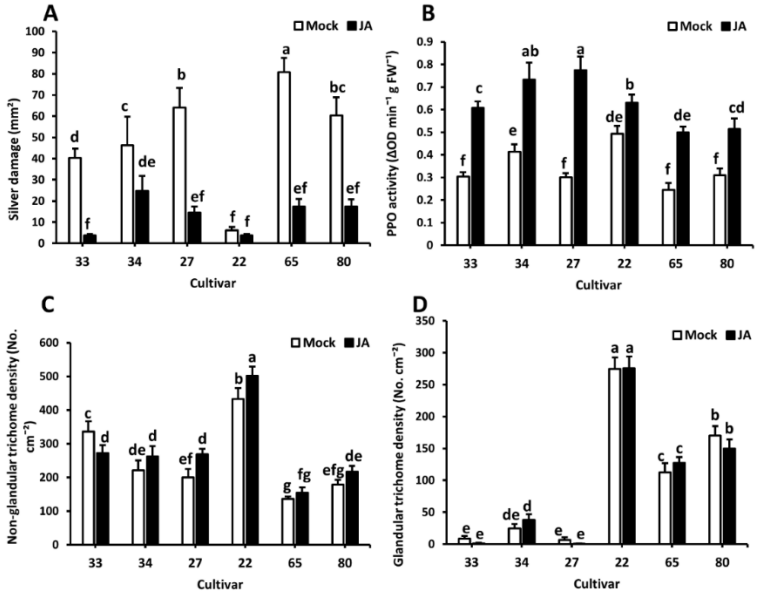

Fig. 5 Phenotypic variation in jasmonic acid-mediated induction of Western flower thrips resistance among chrysanthemum cultivars. Six different chrysanthemum cultivars were treated with mock or jasmonic acid (JA) solutions at 28 d after planting. Plants were sampled for determination of PPO activity and trichome density or subjected to non-choice whole plant bioassays at 7 days after the hormone treatments. (**A**) Silver damage symptoms (mean ± SEM, $n = 7$) evaluated in mock- and jasmonic-acid treated chrysanthemum plants at 7 days after Western flower thrips infestation. (**B**) PPO activity, (**C**) non-glandular trichome density and (**D**) glandular trichome density (mean ± SEM, $n = 5$-7) determined in mock- and JA-treated chrysanthemum plants. Different letters indicate significant differences among groups compared by Fisher's least significant differences (LSD) test at $P \leq 0.05$.

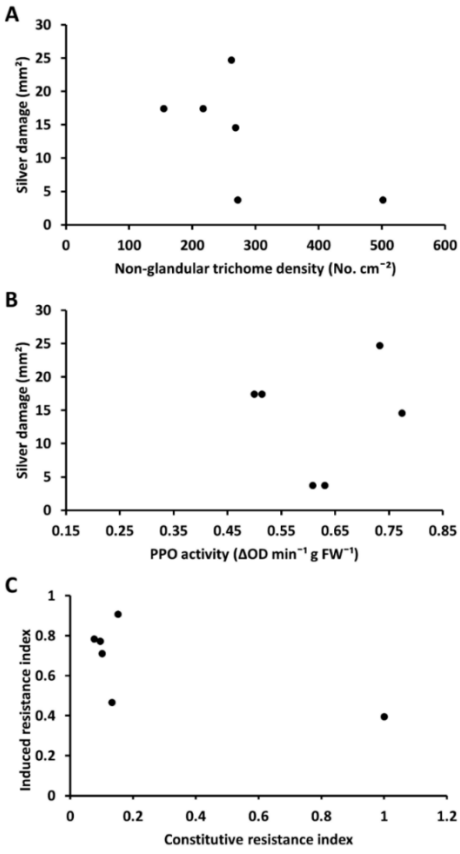

Fig. 6 Relationship between JA-associated induced defenses and chrysanthemum resistance to Western flower thrips. Scatter plots depicting: (**A**) the relationship between silver damage and non-glandular trichome density, and **(B)** silver damage and polyphenol oxidase (PPO) activity in jasmonic acid (JA)-treated plants corresponding to six different chrysanthemum cultivars. Each dot corresponds to the mean of five plant replicates per cultivar for PPO activity, and of seven plant replicates per cultivar for silver damage symptoms. Plants were treated with a JA solution at 28 days after planting and sampled for PPO activity, trichome density or subjected to non-choice whole plant thrips bioassays at 7 days after the hormone treatments. Silver damage symptoms was evaluated at 7 days after Western flower thrips infestation. And (**B**) relationship between constitutive resistance index (CRI) and induced resistance index (IRI).

Positive correlations between non-glandular or glandular trichome density and plant resistance to arthropod herbivores have been documented for many plant species (Mauricio, 1998; Handley *et al.*, 2005). Furthermore, in chrysanthemum, He *et al* (2011) observed a higher density of non-glandular trichomes in an aphid-resistant cultivar. Yet, trichome-mediated effects on herbivore performance might depend on the herbivore species. For instance, Tian *et al.* (2012) reported that high density of non-glandular trichomes in cultivated tomato (*Solanum lycopersicum*) leaves negatively influenced the feeding behavior and growth of the Colorado potato beetle (*Leptinotarsa decemlineata*), while it stimulated the growth of the moth *Helicoverpa zea*. The same authors described that the presence of tomato glandular trichomes hampered *H. zea* growth, but it did not have any effect on the Colorado potato beetle. In chrysanthemum, the chemical composition of leaf glandular trichomes has not been previously reported, nor it was characterized in our study. Therefore, we do not rule

out the possibility that differences in the production of trichome-derived allelochemicals, rather than the density, might still play a role in chrysanthemum resistance to WFT. Indeed, differences in the profiles and/or abundance of glandular trichomes-derived allelochemicals determine the levels of plant resistance in many plant species. For instance, production of methyl ketones by type-VI glandular trichomes in the wild tomato species *S. hirsutum* fam. *glabratum*, which are absent in the glandular trichomes of cultivated tomatoes, confers resistance to multitude of arthropod pests (Antonious *et al.*, 2005). Moreover, within tomato species, variations in the amount of glandular trichome-derived compounds are also reported (Ghosh *et al.*, 2014; Kim *et al.*, 2014). Interestingly, He *et al*. (2011) reported that the gland size of the glandular trichomes present in the leaves of an aphid-resistance chrysanthemum genotype were larger than in the susceptible genotypes. This might be associated to a higher production and storage of trichome-derived chemicals. In addition, Levin (1973) described that the trichome morphology, i.e., height, might be associated to cotton (*Gossypium hirsutum*) resistance to herbivores. Additional studies are thus needed to determine 1) whether glandular trichomes in chrysanthemum are biochemically active, 2) the compounds they produce, and 3) whether these putative compounds might confer anti-herbivory properties and explain differences in WFT susceptibility among cultivars.

PPO activity has been observed to correlate with the resistance of several plant species against herbivorous insects, e.g., in alfalfa (*Medicago sativa*) (Wei *et al.*, 2007) and in eggplant (*Solanum melongena*) (Bhattacharya *et al.*, 2009). The lack of correlation of PPO with chrysanthemum resistance to WFT might be explained by the absence of other chemical defenses. In a previous study, we reported that chlorogenic acid levels positively correlate with chrysanthemum resistance to WFT (Leiss *et al.*, 2009b). Variations in the levels of foliar chlorogenic acid, one of the main enzymatic substrates of PPO, might determine the effectivity of PPO-associated defenses. It would be interesting to perform additional chemical analysis to test the potential correlation between PPO levels and chlorogenic acid levels in future studies.

Finally, our results further showed that exogenous application of the phytohormone JA reduced WFT damage in chrysanthemum. Activation of JA signaling has been reported to confer resistance to WFT in diverse plant species (Li *et al.*, 2002; Abe *et al.*, 2009; Escobar-Bravo *et al.*, 2017; Chen *et al.*, 2018). Yet, our study constitutes the first report on the effects of JA on chrysanthemum defenses against WFT. Moreover, we showed that the effect of JA on chrysanthemum resistance to WFT was genotype-dependent (**Fig. 4A**). Phenotypic variation in the inducibility of plant defenses within plant species has been previously reported (Brody & Karban, 1992; English-Loeb *et al.*, 1998; Agrawal, 1999; Underwood *et al.*, 2000; Sauge *et al.*, 2006). This can be explained by differences in the chemical profiles and/or the presence of potentially inducible defense traits among genotypes within a plant species. In this sense, exogenous application of JA or its volatile form methyl jasmonate (MeJA) has been found to increase non-glandular and glandular trichome densities on newly formed leaves in tomato (Boughton *et al.*, 2005) and Arabidopsis (Traw & Bergelson, 2003), which in some cases resulted in an enhanced resistance to arthropod herbivores (Escobar-Bravo *et al.*, 2017). Additionally, JA is also reported to induce the expression of the defensive protein PPO, an effective defense against some herbivore species (Constabel *et al.*, 1995; Thaler *et al.*, 1996; Cipollini *et al.*, 2004; Chen *et al.*, 2018). We hypothesized that induction of these defenses might increase chrysanthemum resistance to WFT. However, our results demonstrated that, despite the positive effect of JA on non-glandular trichome density and PPO activity, the induction of these defenses could not explain the increased resistance to WFT. Furthermore, JA did not affect glandular trichome density in chrysanthemum. There might be several explanations for this: first, JA is not

associated with or it needs to cooperate with other phytohormones in glandular trichome formation in chrysanthemum (Hare & Walling, 2006; Xue *et al.*, 2018). Alternatively, chrysanthemum might need longer times (> 7 days) to generate new trichomes, as the period needed to observe changes in trichome densities in plants after herbivory or JA induction ranges from days to weeks (Dalin *et al.*, 2008). Taken together, what contributes to the differentially JA-mediated induction of chrysanthemum resistance to WFT is still unknown. Additional research to determine variations in leaf chemical responses to JA treatment among chrysanthemum cultivars might help to answer this question.

Theory predicts that constitutive defenses are negatively correlated with induced defenses in plants (Herms & Mattson, 1992). However, induced defenses did not correlate with constitutive defenses in our study (**Fig. 6C**). Moreover, some chrysanthemum genotypes displaying the lowest silver damage symptoms under control conditions (**Fig. 5A**; genotype 33) also experienced a stronger reduction in WFT-associated damage after JA application (tenfold reduction) than the most susceptible ones (e.g., genotype 80, three-fold reduction). Our findings agree with those described in in cotton (*G. hirsutum*), in which plant constitutive resistance to spider mites (*Tetranychus turkestan*) did not correlate with herbivory-induced resistance (Brody & Karban, 1992). Similar results were also obtained in soybean (*Glycine max*), where constitutive resistance to Mexican bean beetles (*Epilachna varivestis*) did not significantly correlated with herbivore-induced resistance (Underwood *et al.*, 2000). In another example, JA-mediated induced resistance to diamondback [*Plutella xylostella* (L.)] in crucifers did not correlate with their constitutive resistance to the same herbivore (Zhang *et al.*, 2009). The lack of correlation between induced and constitutive resistance in chrysanthemum suggests the possibility to breed for cultivars with high levels of both types of resistance.

In conclusion, our results showed that constitutive and induced chrysanthemum resistance to WFT varied substantially among chrysanthemum cultivars. However, neither constitutive and/or inducible levels of PPO activity and leaf trichome density can be considered as useful markers for the identification of WFT resistant genotypes. Yet, we identified chrysanthemum genotypes that displayed high basal and JA-mediated induced levels of WFT resistance, revealing the possibility to breed for both resistances.

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Supplementary materials

Table S1 Detailed statistical analysis performed for data displayed in each figure.
Fig. S1 Phenotypic variation in Western flower thrips resistance and polyphenol oxidase activity among chrysanthemum cultivars.

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Supplementary materials

Table S1 Detailed statistical analysis performed for data displayed in each figure

Figure	Panel	Statistical test	Factor and statistic value	Degree of freedom	Significance
Fig. 2	A	ANOVA	Genotype; $F = 6.59$	$df1 = 94$, $df2 = 190$	$P < 0.001$
	B	Kruskal-Wallis	Genotype; $\chi^2 = 250.1$	$df = 94$	$P < 0.001$
Fig. 3	-	Spearman correlation	$r = 0.269$; $N = 95$	-	$P = 0.008$
Fig. 4	A	Pearson correlation	$r = 0.186$, $N = 12$	-	$P = 0.564$
	B	Pearson correlation	$r = 0.118$; $N = 12$	-	$P = 0.715$
	C	Pearson correlation	$r = 0.293$; $N = 12$	-	$P = 0.355$
Fig. 5	A	GLM	Genotype; *Wald* $\chi^2 = 70.979$	$df = 5$	$P < 0.001$
			JA or Mock; *Wald* $\chi^2 = 111.912$	$df = 1$	$P < 0.001$
			Interaction; *Wald* $\chi^2 = 33.160$	$df = 5$	$P < 0.001$
	B	GLM	Genotype; *Wald* $\chi^2 = 58.106$	$df = 5$	$P < 0.001$
			JA or Mock; *Wald* $\chi^2 = 195.343$	$df = 1$	$P < 0.001$
			Interaction; *Wald* $\chi^2 = 25.740$	$df = 5$	$P < 0.001$
	C	GLM	Genotype; *Wald* $\chi^2 = 261.895$	$df = 5$	$P < 0.001$
			JA or Mock; *Wald* $\chi^2 = 5.034$	$df = 1$	$P = 0.025$
			Interaction; *Wald* $\chi^2 = 12.661$	$df = 5$	$P = 0.027$
	D	GLM	Genotype; *Wald* $\chi^2 = 1036.121$	$df = 5$	$P < 0.001$
			JA or Mock; *Wald* $\chi^2 = 0.010$	$df = 1$	$P = 0.922$
			Interaction; *Wald* $\chi^2 = 4.057$	$df = 5$	$P = 541$
Fig. 6	A	Pearson correlation	$r = -0.619$; $N = 6$	-	$P = 0.190$
	B	Pearson correlation	$r = -0.118$; $N = 6$	-	$P = 0.824$
	C	Pearson correlation	$r = -0.037$; $N = 6$	-	$P = 0.944$
Fig. S1	A	Kruskal-Wallis	Genotype; $\chi^2 = 48.8$	$df = 11$	$P < 0.001$
	B	ANOVA	Genotype; $F = 3.32$	$df1 = 11$, $df2 = 48$	$P = 0.002$

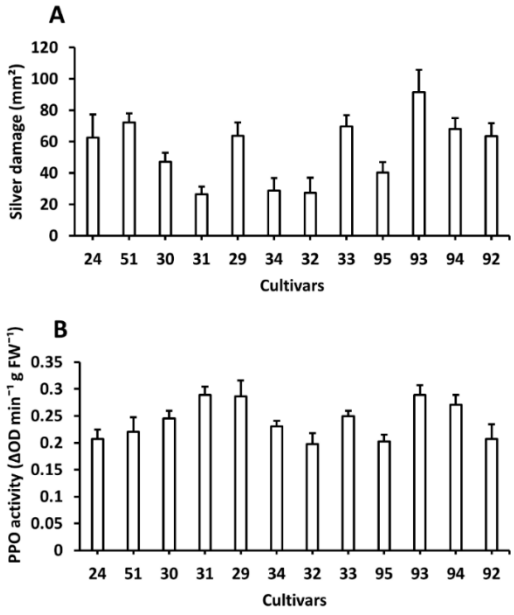

Fig. S1 Phenotypic variation in Western flower thrips resistance and polyphenol oxidase activity among chrysanthemum cultivars. (**A**) Silver damage symptoms (mean ± SEM, n = 10) and (**B**) polyphenol oxidase (PPO) activity (mean ± SEM, n = 5) were determined in 12 different chrysanthemum cultivars. Plants were sampled for PPO activity measurement or used for non-choice whole plant bioassays at 35 days after planting. Western flower thrips (WFT) leaf damage ('silver damage') was determined at 7 days after WFT infestation.