Control of Western flower thrips through jasmonate-triggered plant immunity

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Chapter 6

Summary and discussion

Crop production is severely hampered by the attack of arthropod pests and the pathogens they transmit. Current pest control mainly depends on the use of pesticides, which entails a serious risk for the environment and the human health. An alternative strategy is to enhance host plant resistance to pests and pathogens using elicitors that increase the expression of defense-associated traits (Benhamou, 1996; Stout *et al.*, 2002). One of the most extensively studied defense elicitor is the phytohormone jasmonic acid (JA) (Campos *et al.*, 2014). JA controls both constitutive and inducible plant defenses (Li *et al.*, 2002; Li *et al.*, 2004). Artificial application of this phytohormone has been described to activate JA signaling and to induce a wide array of chemical and morphological responses in plants that, in many cases, increase their resistance to herbivorous arthropods (Thaler *et al.*, 1996; Abe *et al.*, 2009; Maes & Goossens, 2010). Nevertheless, both constitutive and inducible plant defenses against arthropod herbivores can vary within and among plant species. Furthermore, these defenses might differ in their nature and magnitude within the plant canopy, which can determine herbivore preference and performance (Lee *et al.*, 2017). In this thesis, I have investigated whether these variations in constitutive and inducible defenses within-plant and/or inter-genotypes correlate with differences in susceptibility to the Western flower thrips (WFT) *Frankliniella occidentalis* in cultivated tomato (*Solanum lycopersicum*) and commercial chrysanthemum (*Chrysanthemum* × *morifolium* Ramat). In addition to the effects of JA on plant defenses, I have explored whether the action of bacterial-derived defense elicitors might mimic the positive effects of JA on tomato and chrysanthemum defenses against this insect pest.

In Chapter 2 we investigated whether the induction of JA-associated defenses varied along the tomato plant canopy, and whether this explains the differential distribution of WFT-associated damage between developing and fully-developed leaves. Our results showed that JA treatment enhanced tomato resistance to WFT, but the magnitude of this induction was much stronger in developing leaves compared to already fully-developed ones at the time of application. Levels of the defensive-related protein polyphenol oxidase (PPO), type-VI trichome densities and the content in trichome-derived volatiles were all much highly induced in developing leaves than in fully developed ones after the hormone treatment. We hypothesized that the stronger induction of these anti-herbivore defenses in young developing leaves explains why these leaves were less preferred by WFT. Hence, type-VI trichomes and the production of their derived allelochemicals are important tomato defenses that can confer resistance to WFT as well (Escobar-Bravo *et al.*, 2018). From an ecological point of view, a stronger induction of these defenses in developing leaves can increase the protection of those plant tissues that contribute more to plant fitness (Constabel *et al.*, 2000). Indeed, young leaves are photosynthetically more active and, therefore, a rich source of nutrients for the plant but also the feeding target of herbivores. How plants can modulate the magnitude of JA-associated defense responses is not clear, but there are several hypotheses that might explain this phenomenon. For instance, developing leaves might act as sink tissues, where the carbohydrates are preferentially allocated and used for the production of chemical defenses (Arnold & Schultz, 2002; Arnold *et al.*, 2004). In addition, a higher light caption by apical developing leaves might increase their sensitivity to JA, and thus confer a higher capacity to display JA-associated defense responses (Constabel *et al.*, 2000; Ballaré, 2011). Interestingly, despite the reduced capacity of fully-developed leaves to increase trichome densities, the production of terpenes per trichome was higher than in developing leaves. This finding suggests tissue-specific responses of the trichome biosynthetic machinery to the phytohormone JA. Notably, differential expression of terpene synthases along the tomato canopy has been previously described (Besser *et al.*, 2009). Yet, it would be interesting to

determine how terpene-related biosynthetic genes respond to JA treatment in different tomato organs as well.

In Chapter 3, to explore the effect of other JA-mimic elicitors on tomato defenses against WFT, we investigated the action of *Pseudomonas syringae* pv tomato DC3000 (*Pst*) infection and the phytotoxin it produces, coronatine (COR). Furthermore, we investigated whether other *Pst*-derived defense elicitors might enhance tomato resistance to WFT. Our results showed that infiltration of *Pst* or COR reduced WFT-associated leaf damage, concomitant with the activation of JA-associated responses. Yet, COR also activated salicylic acid (SA) signaling in infiltrated leaves, while *Pst* did not. This suggests that tomato plants respond differently to *Pst* and COR to some degree, which was confirmed by the slightly different metabolome profiles of *Pst*- and COR-infiltrated leaves. Unexpectedly, activation of JA signaling in *Pst*- and COR-infiltrated plants did not induce the production of type VI leaf trichomes in newly formed leaves. This could be explained by the different COR and jasmonates effects on plant physiology (Uppalapati & Bender, 2005; Tsai *et al.*, 2011). Finally, our results showed that, besides COR, other defense elicitor/s present in *Pst*-derived culture medium can enhance tomato resistance to WFT as well. The nature of the defense elicitor/s present in the medium, however, is unknown and requires further research. Yet, our data showed that this induction was mediated by the activation of JA signaling. Whether *Pst*-derived culture medium affects another defense and growth-related signaling pathways was not tested, and it would require additional investigation. In line with this, it would be also interesting to test whether inoculation with *Pst*-derived culture medium might enhance plant resistance to other important pests and pathogens of tomato. Altogether, our findings highlight the potential use of defense elicitors derived from *Pst* DC3000 for tomato protection against WFT. Yet, the effect of *Pst*-derived elicitors on the production of flowers and fruits, and the fruit biomass of tomato plants needs further investigation.

Leaf trichomes and PPO activity have long been associated with plant resistance to arthropod herbivores in different plant species (Levin, 1973; Dalin *et al.*, 2008; Mahanil *et al.*, 2008; Bhonwong *et al.*, 2009). In Chapter 4 we investigated whether there are variations in constitutive and inducible levels of trichome density and PPO activity among different chrysanthemum cultivars, and whether this variation correlated with WFT resistance. Our results showed that both non-glandular and glandular trichome densities varied significantly among chrysanthemum cultivars. However, differences in trichome densities did not explain the levels of chrysanthemum susceptibility to WFT. Still, whether chrysanthemum glandular trichomes produce allelochemicals, and whether differences in plant susceptibility are associated to the production of these putative compounds was not further investigated and needs additional research. Constitutive levels of PPO activity did not correlate with chrysanthemum resistance to WFT either. We hypothesized that the lack of correlation between PPO activity and chrysanthemum resistance to WFT results from the insufficient expression levels of this enzyme or the deficiency in other chemical defenses. Previous work in our laboratory demonstrated that chlorogenic and feruloyl quinic acids levels positively correlated with chrysanthemum resistance to WFT (Leiss *et al.*, 2009). These phenolic compounds can be oxidized by PPO and peroxidases, which produces derived compounds that can alter the nutritional quality of plant tissues for herbivorous arthropods (Felton & Duffey, 1991). Additional studies to determine the possible correlation between PPO levels and phenolic acid leaf content, and chrysanthemum resistance to WFT are thus needed. Finally, using a subset of cultivars, we also showed that exogenous JA application significantly enhanced chrysanthemum resistance to WFT. Interestingly, this induction was cultivar-dependent, and it was not explained by increases in leaf trichomes nor PPO activity. Our results suggest the existence of other JA-induced defense mechanisms in chrysanthemum

responsible for this induced resistance. Furthermore, our data showed that WFT-resistant genotypes displayed both high constitutive and highly inducible defenses against WFT, which opens new venues for chrysanthemum breeding.

Having demonstrated that JA application can enhance chrysanthemum resistance to WFT (Chapter 4), we further investigated (Chapter 5) whether local and systemic defense responses to exogenous JA application vary along the plant canopy in chrysanthemum, and correlate with WFT resistance levels. First, our results showed that apical (leaf 9-10 from the bottom) chrysanthemum leaves were more susceptible to WFT than basal (leaf 4-5 from the bottom) ones. The metabolomic analyses revealed that basal leaves displayed higher content in phenolic compounds and lower concentrations of amino acids when compared to apical leaves. This can explain why basal leaves were less preferred by WFT, as they might be less nutritious for herbivorous arthropods (Behmer *et al.*, 2002). Furthermore, the higher content in phenolic compounds might have conferred increased deterrent properties against WFT (Leiss *et al.*, 2009; Demkura *et al.*, 2010; Leiss *et al.*, 2013). In addition, our data showed that variations in constitutive levels of PPO activity along the plant canopy could not explain the differences in WFT susceptibility. This is in line with previous results described in Chapter 4, where variations in PPO activities among different chrysanthemum cultivars did not correlate with WFT resistance levels. We also demonstrated that local application of JA can enhance WFT resistance in systemic chrysanthemum leaves, but that this effect depended on the site of the hormone application. While local application of JA on apical leaves reduced the silver damage symptoms per plant, local application of JA on basal leaves did not. Specifically, the leaves developed after the JA induction (leaves 13-18) experienced a stronger reduction in silver damage symptoms when the below and adjacent leaves 9 and 10 were locally induced. The metabolomic analysis, however, demonstrated that both basal and apical leaves responded to the JA treatment only locally. Thus, how local treatment of apical leaves enhanced WFT resistance in newly formed leaves is still unknown. Further metabolomic and hormonal analysis are needed to determine these systemic responses in chrysanthemum.

Tomato and chrysanthemum: Differences and similarities in constitutive and JA-associated defense responses

WFT is an important pest of tomato and chrysanthemum. Here, we have shown that the pattern of WFT-associated damage varies along the plant canopy in both plant species. As WFT is a generalist herbivore, we speculated that this pattern might be associated with the distribution of the chemical and physical defenses within the plant, and that WFT would feed more on less protected leaf tissues. Overall, our data supported this hypothesis. But the pattern of damage along the canopy was opposite in the two species, as the morphological and chemical defenses against WFT differed between tomato and chrysanthemum. We showed that a higher density of type-VI glandular trichomes in apical developing leaves coincided with a higher accumulation of trichome-associated volatiles per leaf and less silver damage symptoms in tomato. Notably, further analyses in our laboratory demonstrated that type-VI trichome-associated allelochemicals play a fundamental role in tomato defenses against WFT (Escobar-Bravo *et al.*, 2018). In chrysanthemum, however, densities of non-glandular and glandular trichomes was not associated to WFT resistance (Chapter 3). Furthermore, our data showed that in chrysanthemum, WFT caused less silver damage symptoms in basal leaves than in apical ones (Chapter 4). Interestingly, basal leaves presented higher levels of the phenolic compound chlorogenic acid, which has been positively associated with WFT resistance in chrysanthemum (Leiss *et al.*, 2009). When compared to tomato, however, previous experiments in our laboratory showed that a higher

production of chlorogenic acid in the leaves did not affect WFT resistance (Mirnezhad, 2011). It would be interesting to determine the within-plant distribution of other plant secondary and primary metabolites in tomato as well. The comparison with the chemical profiles of chrysanthemum might give some clues about common defense patterns against WFT in both plant species. Finally, whether basal chrysanthemum leaves might greatly contribute to plant fitness, and this is the reason they are better protected against WFT herbivory also needs further investigation.

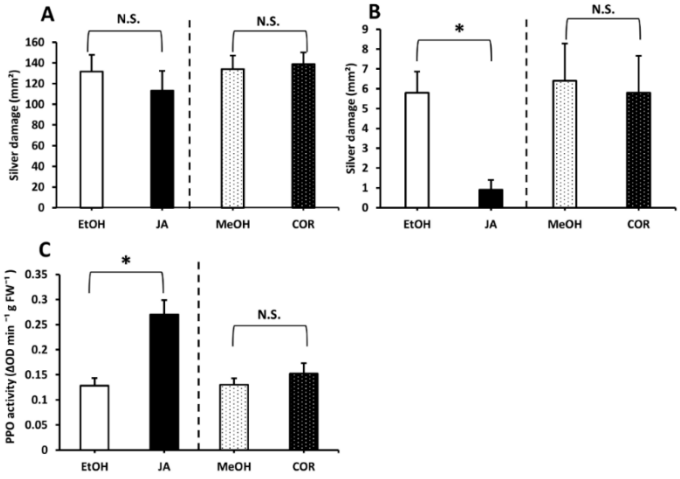

Fig. 1 Local chrysanthemum defense-associated responses to COR and JA infiltration. Chrysanthemum cuttings (cv. Morreno Pink) were grown in a climate room (20°C, 70% RH, 113.6 µmol m^{-2} s^{-1} of photosynthetically active radiation and L16:D8 photoperiod). At 19 d after planting, two leaves (leaf 3 and 4 from the bottom) were pressure-infiltrated with: 1) ethanol solution (EtOH, 0.6%, solvent for jasmonic acid dilution), 2) jasmonic acid (JA, 3 mM), 3) methanol solution (MeOH, 0.32%, solvent for coronatine dilution) or coronatine (COR, 10 µM). At 7 days after infiltration, plants were sampled for determination of polyphenol oxidase (PPO) activity or used for whole-plant non-choice thrips bioassays. Silver damage symptoms determined in (**A**) the whole plant and (**B**) the infiltrated leaves at 7 days after WFT infestation (mean ± SEM, $n = 10$). (**C**) Polyphenol oxidase activity (PPO) (mean ± SEM, $n = 5$) was determined in infiltrated leaves. Differences in PPO levels and silver damage symptoms between EtOH- and JA-treated plants, and MeOH and COR-treated plants, were determined by Student *t*-test. Asterisk indicates significant differences at $P \leq 0.05$. N.S. not significant. The methodology used for the PPO activity measurements and non-choice whole plant bioassays is the same as the described in Chapter 5. The methodology used for JA or COR infiltration is the same as the described in Chapter 3.

Exogenous application of the phytohormone JA enhanced both tomato and chrysanthemum resistance to WFT. Yet, while application of JA increased type-VI trichome densities in newly formed tomato leaves (Chapter 2), the application of this hormone did not affect the production of glandular trichomes in chrysanthemum (Chapter 4). In addition, we found that whereas local application of JA generally induces systemic chemical responses in tomato (Chapter 2), it failed to induce systemic responses in chrysanthemum leaves (Chapter 5). Moreover, JA-mediated enhancement of chrysanthemum resistance to WFT strongly depended on the site of the hormone application along the plant canopy (Chapter 5). In a further attempt to determine whether this was a specific response to JA, we have tested local responses to COR as well. Local application of COR did not affect chrysanthemum resistance to WFT (**Fig. 1A, B**), nor induced PPO activity in treated leaves (**Fig. 1C**). The molecular mechanisms that explain the differences in COR-mediated induced responses between

tomato and chrysanthemum are unknown. However, it might be explained by the capacity of the F-box protein coronatine insensitive1 (COI1) and JAZ complexes to recognize COR, as the binding of COR to COI-JAZs complexes is highly specific (Katsir *et al.*, 2008).

In conclusion, we showed that constitutive and inducible chemical and morphological defenses against WFT differ between tomato and chrysanthemum plants. Furthermore, we demonstrated that both plant species respond differently to bacteria-derived defense elicitors, such as the phytotoxin coronatine. This highlights the plant species-specificity of these interactions and the possible limitation for the use of pathogen-associated molecular patterns to enhance the plant immune system (Quintana-Rodriguez *et al.*, 2018). This study thus provides knowledge and novel strategies for WFT control. Yet, further comprehensive work is needed to evaluate the influence of these induction strategies on plant fitness.

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