Green defense against thrips: Exploring natural products for early management of western flower thrips

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CHAPTER SEVEN

SUMMARY AND DISCUSSION

Summary and General Discussion

In this final chapter I summarize the main findings and discuss the overall contribution of the thesis by presenting a critical opinion about the present work and future research needed to follow up strategies for early thrips management. In doing so, the discussion is considered in light of our main research question: 'Can we employ plant derived compounds as a strategy to boost the plant's defense system?'

Whether modern agriculture without conventional pesticides will be possible or not is a matter of debate. This debate is only meaningful within the context of environmental awareness on one hand, and the availability of sustainable substitutes on the other.

The general introduction, in **chapter one**, discusses the need for changes in the techniques and compounds that, until recently, have been the mainstay for dealing with pest insects. Additionally, it offers a brief overview of the concept sustainable use of pesticides, from a regulatory context with some insight on changes triggered by the implementation of the regulatory framework. The need for increased agricultural production, together with the necessity to reduce insecticide use, has stimulated scientists to study the ability of plants to survive and adapt to insect attacks over aeons of time and, how such innate defense systems can be adopted or manipulated in agricultural settings. Consequently, the importance of phytochemicals and their use as candidates for pest management has increased during recent years. Against this background, subsequent sections in the introduction focus on the methodology by describing the key research aim of this project and the experimental research system. As a contribution to the changing legislation and evolving societal attitudes concerning environmental issues, this project aims to explore the use of plant-derived compounds for early management strategies in tomato (*Solanum lycopersicum* L.) and *Chrysanthemum morifolium* (Ramat) to combat one of the most economically important insect species – western flower thrips (WFT; *Frankliniella occidentalis*). To manipulate and augment chemical basal defenses, known putative defense secondary metabolites were exogenously applied (**chapter four**) whereas, in **chapter five** and **six** external application of plant hormones were explored as a means to trigger innate defense responses against WFT.

Chapter two reviews the concept and the main control tactics of Integrated Pest Management (IPM) in light of WFT. The acronym 'IPM' has gained global recognition and is endorsed as the future paradigm for crop protection, resonating with public and political perceptions. The holistic concept of IPM emphasizes on systems approaches by integrating preventative and therapeutic intervention measures which are briefly introduced. Among the topics considered here, this chapter particularly focusses on two key approaches as areas of major progress for sustainable pest management, being (1) host plant resistance, the selection or development (via traditional breeding or genetic modification) and the use of crop plants that possess defensive traits against herbivores, and (2) biological control, the use of living organisms as natural enemies of crop pests. Knowledge gaps are identified and innovative approaches are emphasized, highlighting the advances in -omics technologies, from genomes to metabolites, which have been a major driver for the adaptation of systems-based approaches. Such integrative approaches, particularly when put in the context of high throughput screening (HTS), enable a comprehensive view of defense mechanisms and provide a powerful tool to drive innovation in crop protection.

Amongst such innovative approaches, the search for environmentally acceptable substitutes has amplified (Adeyemi, 2010; Isman et al., 2006; Kortbeek et al., 2019; Lorsbach et al., 2019). Biopesticides appear in the horizon as an attractive solution for sustainable plant protection (Villaverde et al., 2013). Accordingly, **chapter three** discusses main strategies for selecting bioactive plant-derived secondary metabolites as sustainable alternative candidates for crop protection agents. This chapter provides a simplified guide, from plants to practice, and comprises three essential elements. The first element describes a robust, reliable and quantitative eco-metabolic approach to screen for bioactive metabolites followed by (2) a rigorous validation process to study and verify the insecticidal activity. Examples are provided to illustrate the respective advantages of using naturally based crop protection agents as regards to pesticides, in meeting sustainability objectives. Ten selected metabolites were surveyed which, beyond their natural function as defensive chemical weapons against insect attack, appear to provide numerous desirable health benefits. Central to the control of pest insects is the question of how these green substituents can be formulated and promulgated. Many of the insecticidal metabolites are crystalline solids with limited water solubility which thus, potentially hamper commercial formulation. As such, the third element describes a strategy for improving the solubility of sparingly soluble compounds. Natural Deep Eutectic Solvents (NADES), a new class of innovative environmentally benign solvents composed of a wide range of primary metabolites, were evaluated as a concept for enhancing solubility and stability of scarcely water soluble secondary metabolites. This chapter is further supported with experimental results demonstrating the solubilizing capacity of six insecticidal metabolites in a variety of NADES. The highest solubilities were achieved using lactic acid: 1,2-propanediol in a molar ratio of 2:1 and 1,2-propanediol: choline chloride: water (molar ratio 1:1:1).

Hence, this investigation was further taken up with the final aim to apply this concept as a pre-sowing seed treatment (e.g., soaking) for enhancement of tomato defenses against WFT (**chapter four**). The effect of eight different NADES on tomato seed germination and plant growth performance was studied prior to exploring their potential as vehicles for insecticidal plant secondary metabolites. Exogenous applications of three representative model compounds (beta-alanine, sinapic acid and chlorogenic acid) were evaluated for their ability to protect tomato plants from their most vulnerable stage onwards. NADES has proven to be an interesting solvent to significantly improve solubility of plant metabolites. However, in exploring its use as a carrier solvent for defensive secondary metabolites, both phenolic compounds as well as beta-alanine did not improve thrips resistance in terms of silver damage symptoms. Furthermore, an inherent drawback of exogenously applied NADES is its' phytotoxic effect on seed germination and viability, particularly when the duration of tomato seed soaking was increased. The work described in this chapter was therefore, a main driver to explore inducible responses as an alternative component of the resistance arsenals.

Modulation of endogenous insecticidal metabolite levels by exogenous application

The production of the impressive arsenal of endogenous insecticidal molecules has been exploited by man throughout history and, still serve either directly or as lead compounds for semi-synthesis of modern agrochemicals (Lorsbach et al., 2019). The search for lead compounds from natural sources however, may be complicated by the co-occurrence of numerous plant metabolites as well as the precense of plant matrices (Inui et al., 2012). Exogenous application with pure phytochemicals may thus, not always be able to fully reveal their insecticidal potency, especially when the *in planta* insecticidal activity is due to synergy or interactions between different constituents present in the plant. Such interactions may occur through different mechanisms including: bioavailability, activation of pro-metabolites or deactivation of active compounds into inactive metabolites, inhibition of binding to target proteins or interference with cellular transport processes (Efferth and Koch, 2011; Jeschke, 2016). Among these mechanistic reasons, the importance of bioavailability has been highlighted throughout the experimental chapters.

Seed treatments are routinely applied worldwide to protect seeds and early developmental stages from attack by insect pests and pathogens (Brandl, 2001) however, they only provide a transient window of systemic protection which often declines as the bioactive compound is degraded or diluted as growth progresses (Quérou et al., 1998). This might very well explain the lack of improvement in trips resistance upon tomato seed soaking with plant secondary metabolites (**chapter four**). Additionally, various other factors, including soil moisture, water solubility, substrate retention, treatment dose and formulation as well as seed coat permeability, all influence the translocation and the final efficacy of an active ingredient applied as seed treatment (Quérou et al., 1998). For many decades, crop plants have typically been bred and cultivated for constitutive defenses whereas, inducible responses are strongly underutilized management tactics (Stout et al., 2002). Alternatively, seed treatments with plant-derived elicitors enable young plants to mount defenses early in the growth cycle by triggering innate defense responses (Song et al., 2017). Being mediated through the plant's complex metabolic pathways where many feedback and trade-off mechanisms operate, induced defenses may represent a more durable form of resistance (Stout et al.,2002; Bruce et al., 2017).

Accordingly, **chapter five** seeks to induce defenses against thrips at the seed stage using the ubiquitous plant hormone jasmonic acid (JA). Elicitor-mediated induction of plant defenses is a widely accepted eco-friendly strategic approach for modern plant protection as it aims to substantially minimize the use of toxic pesticides (Vallad and Goodman, 2004; Steenbergen et al., 2018). Seeds are receptive to direct application of exogenous phytohormones without being constrained by the inherent costs of defense and could therefore, offer broad-spectrum protection at the early stages of plant development (Rajjou et al. 2006; Worrall et al., 2012; Haas et al., 2018). However, previous work has demonstrated that these beneficial effects of JA seed treatments are not consistent among tomato cultivars (Smart et al., 2013). This is in line with the work described in chapter five, where I examined the extent of variation of resistance inducibility in eight commercial tomato cultivars. Among the evaluated cultivars, their response to exogenous JA seed application differed inherently.

Silver damage symptoms were only reduced in JA-seed treated plants of the tomato cultivar Carousel. Enhancement of resistance, however, was not associated with activation of defense-related traits such as polyphenol oxidase activity (PPO), trichomes nor with induction of volatiles. Assessment of these traits early in the growth phase could shed more light on the mediated defense responses. Most promising however, was the fact that sulfuric acid scarification significantly augmented responsiveness to seed applied JA in the non-responsive cultivar Moneymaker. This observation lends added support to the contention that seed coat permeability is undoubtedly an important factor for facilitation of JA absorption (i.e. bioavailability) and thus, increased physiological activity. Additionally, this outcome creates opportunities for tomato breeding programs to select for genetic traits that affect seed permeability as well as for private seed companies to pre-treat non-responsive cultivars.

Commercial utility of elicitors

Nonetheless, the observed variability in host responsiveness between cultivars constitutes a major obstacle, because one of the foremost conditions for successful commercialization of elicitor-mediated induced resistance is consistent efficacy. Intriguingly, in scientific literature, Moneymaker along with tomato cultivars Aisla Craig, Micro-Tom and Castlemart are frequently used to represent tomato as a plant model system. Despite being regarded as a model plant of the Solanaceae family, the fundamental question remains whether or not it is correct to refer to tomato as model species in general whilst, there are significant differences among individual cultivars. The variability in responsiveness in different cultivars clearly demonstrated the necessity to include multiple cultivars as biological test system to establish consistent statements. Moreover, it shows that neglect of this issue at the screening stage for new chemical elicitors unnecessarily contributes to an excess of false-negative findings. To avoid potential lead candidates being incorrectly classified as inactive, it is important to distinguish true-negative results from false-negative elicitors. Alternatively, transgenic plants and cells containing defense gene promotor-reporter gene fusions may provide convenient, sensitive and specific assay systems for screening potential enhancers of natural resistance to herbivores (Doerner et al., 1990; Corrado and Karali, 2009; Halder and Kombrink, 2018). Elicitors can be introduced by treating young seedling or cell suspension cultures. Subsequently, for example, the response can be monitored in a high throughput fashion using a microplate reader imaging system for visualizing reporter degradation (Larrieu et al., 2015).

Despite the inherent potential of elicitors for sustainable agricultural use, an imperative question is why very few have achieved commercial success? Adoption of such approaches have a strong regulatory dimension which may act as a barrier to the development of reliable and efficacious commercially viable products. Moreover, the development of a new active compound is expensive and time consuming. The vast majority of commercial products claiming to induce disease resistance often involve SA-mediated signalling (Gorelick and Bernstein, 2014). On the contrary, not much headway has been made in the commercialisation of products controlling herbivory. Large-scale application of JA can be prohibitively costly but, perhaps of bigger importance is the well-known growth repressing action of JA, particularly at higher doses (Redman et al., 2001). Screening for or designing structurally related analogues that retain the resistance-inducing capacity of JA (Haider et al., 2000) but, are less active in growth repression may be more promising in this respect. It is also crucial to recognise that the agricultural utility of JA-induced defenses do not produce an immediate knockdown effect and rarely confers complete resistance (Stout et al., 2002). Nonetheless, one of the main advantages of induced resistance responses is that it can be compatible with, or even enhance biocontrol of plant pests by promoting plant attractiveness to natural enemies and therefore, could be considered as a parallel or sequential approach (Smart et al., 2013; Bruce et al.,2017; Pappas et al., 2017). Finally, elicitor induced resistance is differentially effective against different pests and thus, hormonal crosstalk following exogenous JA treatment may leave some plants more vulnerable to pathogens associated with the SA-regulated pathway.

Challenges in irreproducibility

In the context of mechanistic crosstalks, the complex interaction between the root promoting auxin hormone indole-butyric acid (IBA) and JA was explored in **chapter six**. Recent studies demonstrate that auxins also affect a multitude of plant defense responses through complex interactions among multiple hormone pathways, including JA (Kazan and Manners, 2009; Robert-Seilaniantz et al., 2011; da Costa et al., 2013). In search of effective dipping treatments to protect cuttings during their vegetative stage, we serendipitously observed that water dipping of IBA-coated cuttings repeatedly reduced herbivory, both by thrips as well as by leaf miner. Furthermore, separation of IBA and talcum powder implies a possible confounding effect of the carrier chemical. Assessment of polyphenol oxidase activity (PPO), 14 days after dipping treatment, suggests that neither direct induction nor priming of plant defenses are involved. Future experiments aiming at understanding the early signaling events, including hormonal signaling networks, from a more holistic perspective may help to explain the physiological basis involved in conferring protection against herbivores. Overall, however, results were highly variable and thus, do not allow us to deduce conclusive statements concerning the involvement of auxin-mediated defense responses. A possible explanation of the anomalies appears to hinge on the large phenotypical variation in cuttings generated from stock plants and warrants the necessity to improve intra- and inter- variability and reproducibility, which both form a cornerstone in science for critically assessing the correctnes of scientific claims and conclusions drawn (Plesser, 2018). It is therefore, strongly advised that the putative defensive role of auxins should be exploited and integrated in coherent models of response and function in model plant species prior to translation to commercially important horticultural crops. Our study provides an interesting starting point to investigate alternative roles of auxins in commercial propagation and may add, in addition to their growth regulating function, a promising defense strategy to the horticultural toolbox of chrysanthemum propagators. Future work with auxin formulations is needed to determine whether such an approach is of commercial value over a broader range of herbivores.

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