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Trichome mimics: sprayable plant-based adhesives for crop protection against thrips

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Chapter 1

General Introduction

The problem of pesticide dependence in agriculture

By the year 2050, humanity is expected to exceed a population threshold of 9.7 billion (United Nations 2024). To avoid food shortages and economic difficulties, agricultural yields therefore need to increase substantially. However, at the same time we must limit our agricultural land use and decrease our ecological footprint to maintain a healthy environment and a good quality of life (Hunter et al. 2017). Currently, pests, diseases, and weeds, cause up to 40% losses in global crop production (FAO 2022). As our climate changes rapidly, these numbers and losses due to other factors will likely only further increase if we do not act (Chakraborty and Newton 2011; Hristov et al. 2020). To prevent such crop losses and to maintain overall plant health in our agricultural systems, various crop protection methods are already used. These methods include cultural practices (e.g., crop rotation, intercropping, and resistance breeding), chemical-based approaches (e.g., synthetic or botanical pesticides and fertilizers), and biological control methods (the suppression of pests and diseases using other organisms) (Viaene et al. 2006; Bashyala et al. 2022). Among these methods, chemical (or synthetic) pesticides play an important role. These pesticides encompass a range of substances, including herbicides, insecticides, and fungicides, each applied to manage specific threats. In 2022 alone, we used 3.7 million tons of pesticides, and this number is increasing annually (FAO 2024). The use of synthetic pesticides has greatly improved our agricultural productivity and stability, especially upon their introduction after World War II. However, the indiscriminate and large-scale use of these chemicals has come with severe negative consequences for nature, the environment and human health. For example, pesticides decrease microbial diversity and soil health by building up in the soil and can seep into ground water or spread with the wind to poison plants, animals, and humans alike. In addition, pesticide use has led to a loss of overall biodiversity (Mahmood et al. 2016). Continuous use of pesticides is making these problems worse every day. Meanwhile, pests are also evolving to become increasingly resistant to such pesticides. When this happens, more and more synthetic chemicals are required to achieve the same levels of agricultural pest control as before, or sometimes these chemicals simply stop working (Georghiou and Lagunes-Tejeda, 1991). Currently, over 500 crop pests are known to have evolved pesticide resistance, including major global crop pests such as western flower thrips (*Frankliniella occidentalis*, Pergande) (the model pest used in this thesis) which is becoming increasingly more problematic because of the build-up of resistance (Gao et al. 2012; Reitz et al. 2020). Given these severe negative aspects, the use of chemical pesticides for pest control is simply not sustainable, the sooner we stop using them, the better.

Integrated pest management and biopesticides as a potential solution

Fortunately, through increasingly stringent pesticide regulations and the development of more sustainable pest control approaches, crop protection has been transitioning from heavy reliance on conventional pesticides to a more holistic framework known as integrated pest management (IPM). This approach incorporates diverse control strategies that prioritize pest prevention, monitoring, and the use of non-chemical intervention methods to keep pest populations beneath economic threshold levels. The use of chemical pesticides is kept to a minimum and is considered to be a last resort measure (Angon et al. 2023). Some of the most common preventive and monitoring measures used in IPM to reduce the likelihood of pest infestations include intercropping, crop rotation, soil health management, organic fertilizers, the use of traps, lures, repellents and physical barriers, the promotion of general biodiversity and beneficial insect populations, and the use of pest resistant crop varieties (Barzman et al. 2015). Common intervention strategies include judicious pesticide use via targeted application and rotation of pesticides with different modes of action, the use of naturally sourced biopesticides that act against pests or stimulate plant innate defenses, and the use of biological control agents, such as predatory arthropods, parasitoids, pathogenic fungi, bacteria, and viruses (Chandler et al. 2011; Mouden et al. 2017). Especially the use of biopesticides — those pesticides derived from natural organisms or compounds — has recently gained momentum as a safer, more sustainable alternative crop protection method to synthetic chemicals (Khater 2012). Well-known examples of biopesticides include extracts from neem trees, pyrethrum (a botanical made from the flowerheads of *Chrysanthemum cinerariifolium* and *Chrysanthemum coccineum*), and solutions containing plant-based essential oils, all of which exhibit excellent insecticidal, repellent, oviposition inhibiting, and / or arthropod growth-disrupting properties, as well as being able to act as inducers of plant innate defenses (Regnault-Roger 1997; Dayan et al. 2009). Next to their effectiveness against pests, crop protection products featuring natural compounds are commonly found to be more easily biodegradable and to have less severe effects on non-target organisms such as pollinators and predatory insects than their synthetic counterparts. In general, the use of biopesticides for pest control is therefore considered as safer for the environment, although exceptions to this rule also exist and we still lack knowledge in many areas surrounding the effects of natural materials on plants, animals, and the rest of our environment. (Khater 2012; Dougoud et al. 2019).

Natural plant defenses as an inspiration for pest control

Plant defenses have long served as a source of inspiration for the development of innovative and sustainable pest control strategies. Over millions of years of evolution, plants have developed a wide array of physical and chemical mechanisms to defend themselves against herbivores and pathogens. Common examples of constitutive (continuous) and induced (only when stimulated) plant defenses are thorns, (glandular) trichomes, waxy exudates, and the production of toxic and repellent metabolites such as alkaloids, terpenoids, and phenolics (Fürstenberg-Hägg et al. 2013). Perhaps one of the most remarkable types of plant defenses are glandular trichomes. These specialized hair-like structures, located on the surface of stems, leaves, and flowers of many vascular plant species (LoPresti et al. 2015), provide an interesting mixture of both mechanical and chemical defense (Levin 1973). Next to physically hindering the movement of arthropods, the glandular cells at the tip of these hairs often contain or secrete diverse mixtures of compounds, including sticky resins, oils, or toxins that can immobilize or deter arthropods, thereby acting as a first line of defense against herbivory

(Wagner 1991). Some carnivorous plants such as *Drosera* spp. have taken this to the extreme, using their glandular trichomes to trap insects for their own nutrition (Fig. 1.1). (For more information about glandular trichomes and the ecology of sticky plants, see chapter 2.) While glandular trichomes themselves are not typically harnessed directly in crop protection, they have inspired the development of novel biopesticides that mimic their mode of action and may even provide additional benefits via stimulation of plant innate metabolomic defenses.

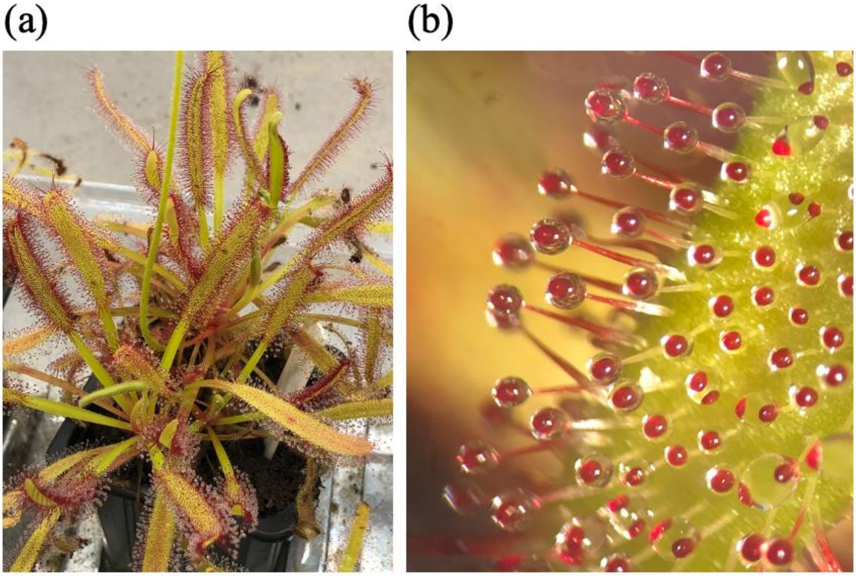

Fig. 1.1 The carnivorous plant *Drosera capensis* (a). Close up of the glandular trichomes of *D. capensis* which are covered in adhesive fluids that act as an effective trap for flies and other insects (b), - pictures by T.V. Bierman.

Plant-based sticky materials for crop protection

One promising, recently developed crop protection method, and the focus of this thesis, are sprayable solutions containing natural chemicals and adhesive droplets made from plant-based oils (Fig. 1.2). Inspired by the adhesive exudates produced by the glandular trichomes of sticky plants such as butterwort, tomato and tarweeds (Alcalá et al. 2010; Bar and Shtein 2019; Krimmel and Pearse 2016), these sprayable adhesives are aimed to provide plants with a physiochemical barrier against small arthropod pests such as thrips. In the NWA-funded project " Natural Plant Defense, Mimicking Natural Trichomes (www.naturalplantdefense.com), the effectiveness of multiple prototypes for thrips control have been tested, including solutions of natural chemicals that also contain adhesive droplets made from oxidized rice germ oil (RGO), similar formulations made from an oxidized mixture of sunflower oil, linseed oil and olive oil (MIX), and a more fluid adhesive spray consisting of a solution of natural chemicals containing an oxidized mixture of rice germ oil and olive oil (RGO-OLO).

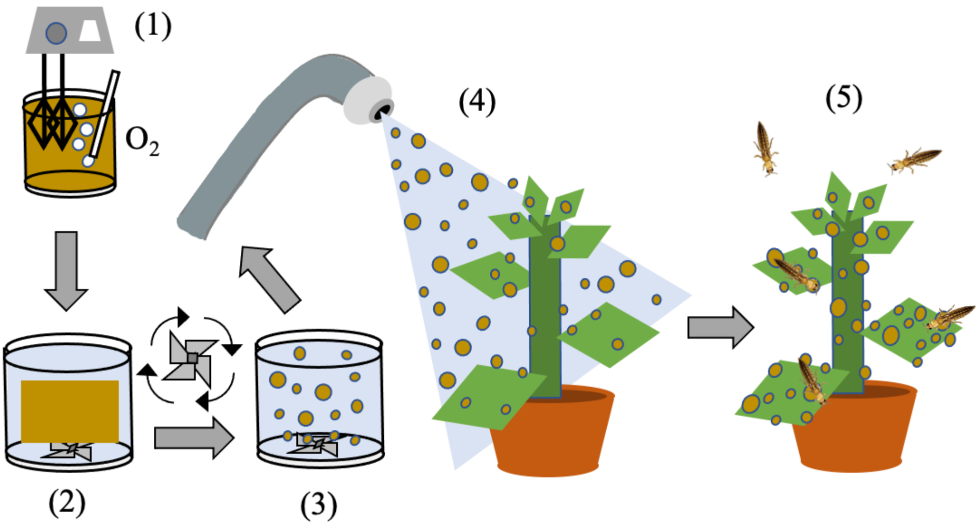

Fig. 1.2 Sprayable solutions with adhesive oil droplets for crop protection: plant-based oils are first stirred and oxidized under high temperature until the consistency and tackiness of the oil is vastly increased (1). The resulting semi-solid chunk of oil is then placed in a carrier solution of water and other natural chemicals (2) and ground into tiny droplets by an industrial mixer (3). The solution can then be sprayed on plants (4). The result is a plant covered in sticky oil droplets that is protected against arthropod pests such as thrips (5).

The Natural Plant Defense project features a consortium of several companies: Van Iperen B.V. (plant growth specialist), Holland Biodiversity B.V. (agricultural research and consultancy), Holland Green Machine B.V. (agricultural spray technology), and a multidisciplinary team of researchers with expertise in various disciplines including physical and soft matter chemistry, polymer sciences, ecology, plant sciences, and entomology, working together at multiple institutions including Wageningen University & Research, Leiden University, Aeres University of Applied Sciences Almere, and the University of Groningen. The main pest species and model crop of the project are western flower thrips (*Frankliniella occidentalis*) and *Chrysanthemum morifolium* plants (more information on these organisms is provided later at the end of the introduction). The end goal of the Natural Plant Defense project is the successful development of a sustainable, environmentally friendly alternative to conventional chemical pesticides, contributing to the broader goal of reducing harmful chemical inputs in agriculture.

Testing for effects of plant-oil-based adhesives on plant physiology

In addition to developing the sprays with adhesive droplets and testing their effects on target pests, the Natural Plant Defense project also investigates the effects of the adhesive droplets and their carrier solutions on plant physiology, specifically on plant growth, phytotoxicity, and on the induction of metabolomic responses in relation to simultaneous abiotic stress and herbivore infestation. When applied to plants, certain oils can interact with plant tissues in ways that modify leaf surface properties, disrupt cell membranes, interfere with gas exchange, reduce plant growth, or trigger stress and defense responses (Werrie et al. 2020). For instance, the application of *Cymbopogon citratus* (lemongrass) oil may reduce may inhibit germination, seedling growth and decrease chlorophyll a, b and carotinoid contents, indicating impaired photosynthesis (Poonpaiboonpipat et al. 2013). Other oils have been shown to cause phytotoxic effects such as chlorosis, necrosis, or impaired growth, depending on the plant species, oil type, and concentration used (Loke et al. 1992; Verdeguer et al. 2020; Casella et al.

2023). At the same time, plant oils can also stimulate defense-related pathways, leading to the accumulation of metabolites involved in resistance against herbivores or pathogens (Colpas et al. 2009; Ben-Jabeur et al. 2015; Werrie et al. 2022). Such physiological changes may be particularly relevant in crops such as *Chrysanthemum*, where plant chemistry is thought to be closely linked to herbivore resistance (De Jager et al. 1995a; Leiss et al. 2009b; Chen et al. 2020). To better understand the physiological effects of the application of plant-based adhesives and their consequences for plant defense against pests, we monitored growth and employed a metabolomics approach to investigate how the application of plant-based oils affects the leaf metabolome of *Chrysanthemum*.

GC-MS and ^{1}H NMR and their power for plant metabolome analysis

Metabolomics techniques allow for the comprehensive profiling of a diverse range of metabolites and can reveal subtle yet functionally very important shifts in plant biochemistry. Gas chromatography–mass spectrometry (GC-MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy are two of the most powerful and widely used analytical techniques for the chemical profiling of plants (Wolfender et al. 2013). In the context of crop protection research, these techniques are increasingly used to study how plants respond metabolically to herbivorous pests such as western flower thrips and to the application of biological pesticides (Leiss et al. 2011; Shahid et al. 2023). Thrips feeding induces complex metabolic changes in host plants, including in the levels of phenolics, carbohydrates, amino acids, and other metabolites such as terpenoids and green leaf volatiles, which may play roles in direct defense or in signaling pathways (Zhang et al. 2021; Kutty and Mishra 2023; He et al. 2022; Delphia et al. 2007; Leiss et al. 2009b). GC-MS is particularly suited for the detection and identification of such volatile and semi-volatile compounds. Meanwhile, ^{1}H NMR enables a broader, unbiased quantification of a broad range of primary metabolites such as sugars, amino acids, and organic acids, providing insight into systemic physiological changes, including plant responses against biotic and abiotic stress (Fan et al. 1986; Ryan and Robards, 2006). Combined, GC-MS and ^{1}H NMR offer a holistic view of the plant's biochemical state, allowing the distinction between the metabolic effects of herbivory, biopesticide-induced resistance, and their combined effects. This approach is particularly valuable in agriculture for understanding how treatments with natural materials influence plant nutritional contents, overall plant growth, plant defense pathways and resilience, as well as being useful for the identification of new thrips resistance biomarkers and for guiding the correct implementation of more sustainable pest management strategies (Leiss et al. 2011; Mouden and Leiss 2021; Shahid et al. 2023).

Combined thrips control effectiveness of plant-based adhesives and predatory arthropods

As mentioned before, biological control agents are often essential components of integrated pest management strategies to help regulate pest populations and reduce the need for chemical interventions. For example, parasitoid wasps and predatory arthropods such as true bugs, and predatory mites are widely used as biological control agents, mostly in greenhouses but also in open field crops (Mouden et al. 2017; Knapp et al. 2018). The combined use of plant-based adhesives and arthropod predators may potentially have additive effects and therefore together lead to a more successful control of thrips. However, many botanical oils and extracts such as those from neem, clove and citrus, have been shown to exhibit toxic or sublethal effects on non-target organisms, including beneficial predators and pollinators (Biondi et al. 2012; Monsreal-Ceballos et al. 2018; Toledo et al. 2020; Ghasemzadeh et al. 2022). These plant oils may interfere with predator survival, development, reproduction, and / or behavior through mechanisms such as the disruption of the nerve system, growth and molting or by having other toxic and repellent properties (Guedes et al. 2024; Costa et al. 2025; Lisi et al. 2025), highlighting that the term 'natural' does not always equal 'safe'. In this context, assessment of the effects on non-target arthropods is vital for any plant-based pest control product, particularly when these products are designed for use within integrated pest management programs. Therefore, in chapter 6, bioassays were performed to investigate the potential side effects of the plant-oil-based adhesive formulations on two common biological control agents: the true bug *Orius laevigatus* and the predatory mite *Transeius montdorensis*. (More details on the predatory arthropods used in the research are provided at the end of the introduction.)

Thesis objectives per chapter

This PhD thesis is one of the core outputs of the Natural Plant Defense project and presents the results of the experimental investigations regarding the efficacy of the solutions with plant-oil-based adhesive droplets against western flower thrips, their effects on plant growth and plant metabolomic contents, the interaction of these sticky sprays with predatory arthropods, and the overall potential of this method as an integral component of sustainable pest management. Here, the specific objectives of the research in this thesis are further explained for each chapter.

The thesis features a total of seven chapters (including the introduction). To start, **chapter 2** presents a review on sticky plants and the role of stickiness and plant-based adhesives in agriculture. This review was undertaken to understand the role of plant stickiness in defense against arthropods, the factors involved in successful trapping of arthropods by sticky plants, and to create an overview of the ways in which plant stickiness and plant-based adhesives are already and can potentially be applied in agriculture. In **chapter 3**, the first proof of concept results are presented of testing the effectiveness of two solutions with adhesive droplets, RGO (made with rice germ oil) and MIX (made using a mixture of sunflower oil, linseed oil and olive oil), against western flower thrips in a series of laboratory assays. In **chapter 4**, the effect of solutions containing adhesive rice germ oil droplets on thrips damage and on the growth and metabolome of sprayed leaves of *Chrysanthemum* plants with and without thrips is investigated over time. In **chapter 5** this work is continued and the effects of the MIX adhesive droplets and the solution with natural chemicals on thrips and *Chrysanthemum* are tested in a similar plant assay. This time the aim was to more deeply investigate both local effects in sprayed plant parts and systemic effects in newly grown (unsprayed) leaves and the interaction with thrips-induced effects on the metabolome of *Chrysanthemum*. **Chapter 6** then presents the results of several plant assays to test the compatibility of two predatory arthropods that are commercially used to control thrips and other pests, *Orius laevigatus* (a true bug) and *Transeius montdorensis* (a predatory mite), with the aforementioned solutions with adhesive droplets and another adhesive spray made from rice germ oil and olive oil (RGO-OLO). The central question answered here is whether the use of these predatory arthropods can be combined with the sprayable adhesives, and if doing so improves overall thrips control. Finally in **chapter 7** (the general discussion), the broader agricultural and societal implications of the experimental results of the previous chapters are discussed and an outlook is given on the next steps required in product development to close the gap between research and application. Fig. 1.3 on the next page presents a flow diagram showing how the chapters of this thesis are connected.

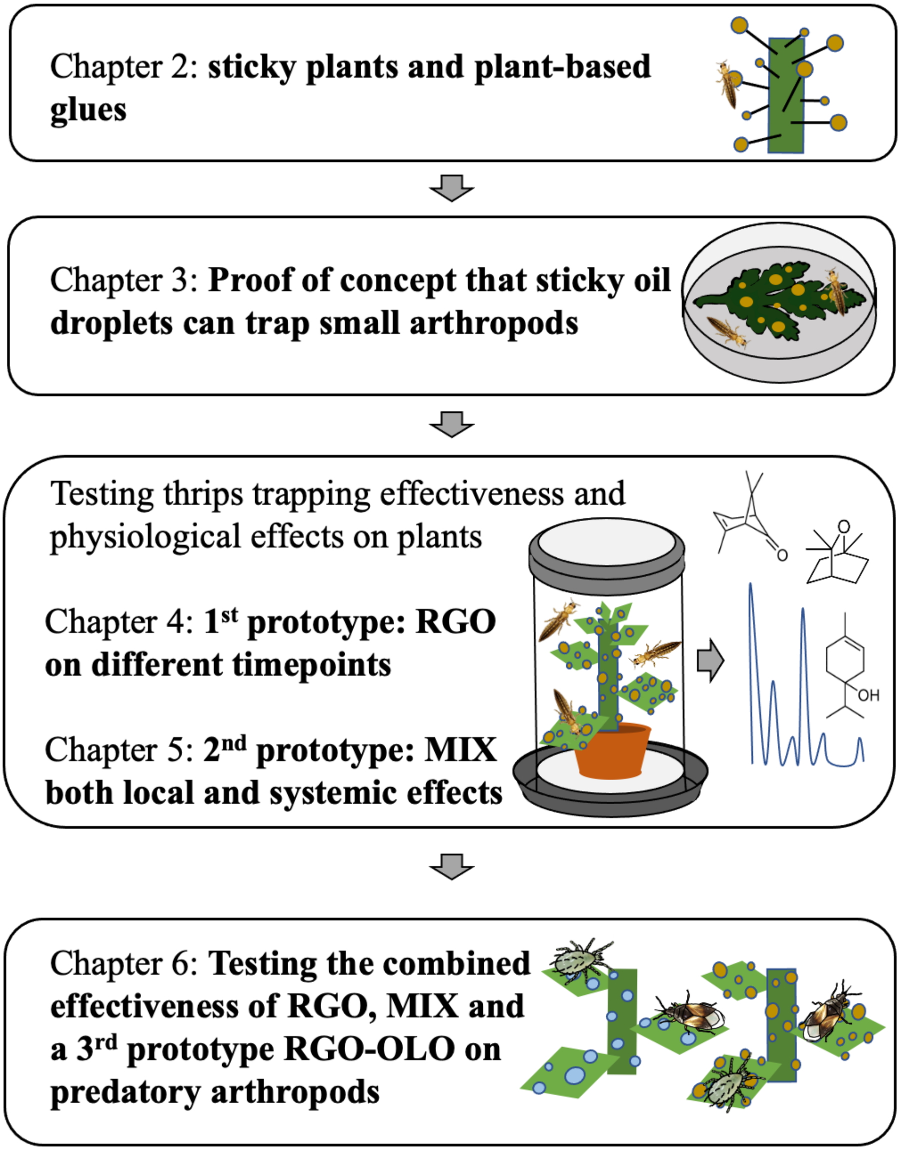

Fig. 1.3 overview of the chapters of the thesis

Extra information on the model organisms

The following paragraphs provide some more detailed information on the organisms used in the studies that make up this thesis.

The crop: *Chrysanthemum*

Cultivated *Chrysanthemum* (Asterales: Asteraceae, L.) is an ornamental crop of significant cultural, and economic value (Fig. 1.4a). After rose, it is the second most popular flowering plant in terms of global trade. Through breeding efforts, many different varieties of *Chrysanthemum* with different flower shapes, colors and sizes have been created (Spaargaren and van Geest 2018). In this thesis, *Chrysanthemum* × *morifolium* (Ramat), specifically the cultivar "Baltica", was used as a model species (Fig. 1.4b). This cultivar is mainly grown as a cut flower and as potted plants. As for many chrysanthemums, propagation is mainly done asexually via cuttings. The plant maintains extended vegetative growth under long day conditions and flowers more quickly under short day conditions (Cockshull 1976; Cockshull and Hughes 1972). Like many commercially grown chrysanthemums, Baltica is susceptible to western flower thrips (Fig 1.4c). However, variability in thrips resistance exists within and among *Chrysanthemum* cultivars, with resistance being linked to both morphological traits, such as flower color and shape (Rogge and Meyhöfer, 2021), and biochemical traits of the plant, including the content of metabolites such as phenolics (Leiss et al. 2009b), and isobutylamides in the leaves (Tsao et al. 2005). Understanding how the variation in the contents of specific metabolites in the tissues of *Chrysanthemum* is linked to natural product application and herbivore resistance will be an important step towards sustainable thrips management in commercial *Chrysanthemum* production.

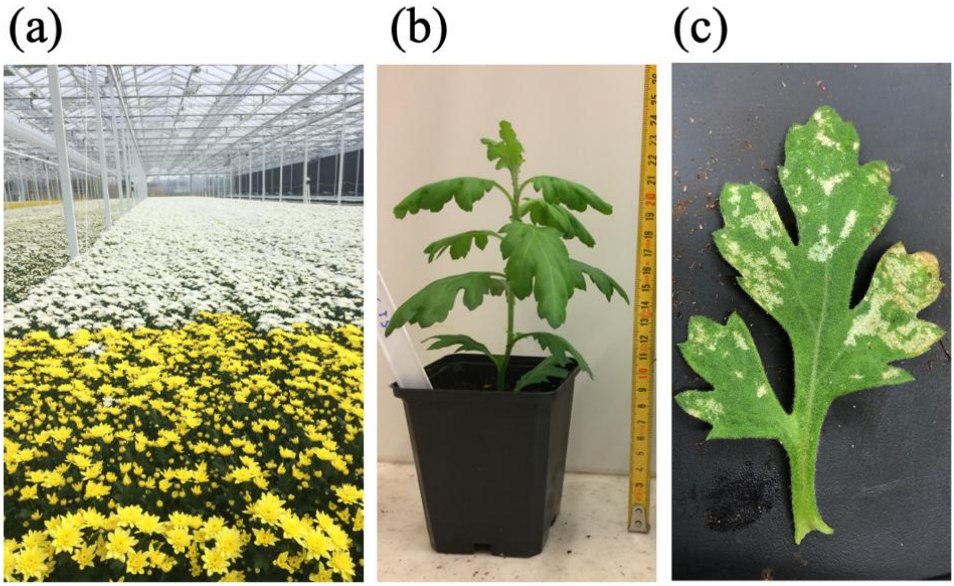

Fig. 1.4 *Chrysanthemum* greenhouse (a), young *Chrysanthemum* cv. Baltica plant (b), typical thrips feeding damage to chrysanthemum (c), - pictures by T.V. Bierman.

The pest: Western flower thrips

Thrips (Thysanoptera: Thripidae, Stevens) are small plant-feeding insects, recognizable by their slender bodies and fringed four wings. They occur in many habitats including tropical, subtropical, and temperate environments and quite frequently in greenhouses (Kumar and Omkar 2021). Out of the 7700 reported species of thrips only around 1% are currently causing issues in agriculture (Morse and Hoddle 2006). The western flower thrips (WFT) (*Frankliniella occidentalis*, Pergande) (Fig. 1.5a) is the most notorious of the problematic thrips. Originally from California, this species likely invaded Europe via the Netherlands in 1983, via trade (Kirk 2002). Like many other thrips, *F. occidentalis* is polyphagous and feeds on different plant species, including many field and horticultural crops (Jensen 2000). Next to causing direct damage via feeding and oviposition to leaves, flowers and fruits, which is visually unappealing and can lead to malformed growth (Cloyd 2009), WFT is a transmitter of several plant (tospo)viruses including chrysanthemum stem necrosis virus, groundnut ring spot virus, impatiens necrotic spot virus, tomato chlorotic spot virus, and tomato spotted wilt virus (Riley et al. 2011). Infestation by these viruses may affect growth and product quality significantly, often causing large yield losses. Furthermore, thrips-infected materials are unfit for export. Together, economic losses due to WFT may amount to millions if not billions of dollars annually, although the exact cost is hard to estimate (Goldbach and Peters 1994; Kirk 2002).

The biology and behavior of WFT play a huge role in its pest status. The species is haplodiploid. Fertilized eggs produce females whereas males emerge from non-fertilized eggs (Moritz et al. 2004). Under optimum temperatures (25 – 30 °C) and conditions (humid enough, plenty of food), the hemimetabolous lifecycle from egg to adult can be completed within two weeks (Kumar and Omkar 2021). WFT does not go into diapause and reproduces continuously (Morse and Hoddle 2006). The females are typically bigger (1.5 mm length) and live longer (up to c.a. 28 days at 30 °C, c.a. 78 days at 15 °C) than the males (1 mm length, lifespan half as long). Females can lay 150-300 eggs in their lifetime (Cloyd 2009; Kumar and Omkar 2021). If left unmanaged, populations can quickly increase. Control is difficult since WFT quickly develops pesticide resistance and shows thigmotactic behavior, meaning that the insect prefers to hide in tight spaces such as within flowers and buds, which makes them difficult to reach by pesticide sprays and for predatory arthropods alike (Steenbergen et al. 2018). Current management approaches for thrips include the use of thrips-proof mesh, yellow and blue sticky traps, behavioral manipulation via pheromones, allelochemicals and UV-reflective screens, the release of arthropods that are predators of thrips and treatment of plants with entomopathogenic nematodes, fungi, bacteria and viruses, chemical control using synthetic and naturally derived compounds, and breeding for metabolome-based host plant resistance (Reitz et al. 2020; Mouden et al. 2017; Mouden and Leiss 2021; Kumar and Omkar, 2021; Steenbergen et al. 2018). So far, these techniques have not been sufficient to solve the western flower thrips problem.

The predators: *Orius laevigatus* and *Transeius montdorensis*

Orius laevigatus (Hemiptera: Anthocoridae, Fieber) (Fig. 1.5b) is a generalist true bug, that is widely used as a predator in greenhouse crops for the control of *F. occidentalis* and other arthropod pests (Weintraub et al. 2011). The species is highly effective due to its aggressive feeding behavior, high mobility, and ability to prey on both the larvae and adults of thrips. Typically, between 2 and 3 mm in size (Mouratidis et al. 2022), *O. laevigatus* is well adapted to hunt thrips in various crops. *Transeius montdorensis* (Mesostigmata: Phytoseiidae, Schicha) (Fig. 1.5c), a predatory phytoseiid mite that natively occurs on islands situated in the South Pacific Ocean and Australia, has also emerged as a promising candidate for thrips control (Labbé et al. 2019).

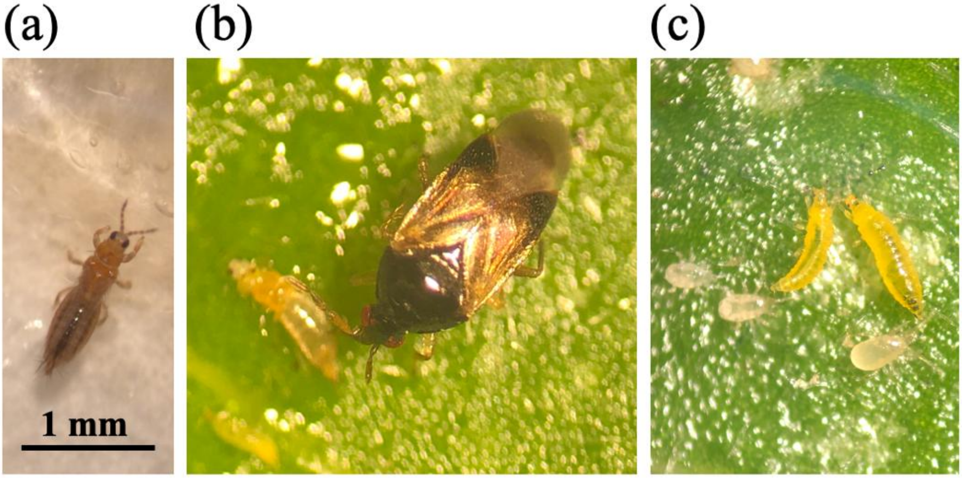

Fig. 1.5 A female adult of western flower thrips, (*Frankliniella occidentalis*) (a). Commercially used thrips predators: true bug *Orius laevigatus* adult female eating a thrips stuck in RGO-OLO sample (live observation from pilot experiment not included in thesis) (b) and several predatory mite *Transeius montdorensis* individuals and two *F. occidentalis larvae* of presumably 2nd instar (c), - pictures by T.V. Bierman

T. montdorensis exhibits a broad prey range and preys mostly upon the eggs and early larval stages of *F. occidentalis* (Nguyen et al. 2025). *T. montdorensis*' small size (c.a. 500 microns) allows it to access thrips in concealed areas such as flower buds and leaf folds, where other predators may be less effective (Sabelis and van Rijn 1997). Both *T. montdorensis* and *O. laevigatus* can be mass-reared efficiently and are commercially available for use in a range of cropping systems. Typically, these predators are released preventively or at the onset of infestation (Mouden et al. 2017; Rodíguez and Coy-Barrera 2023). In the absence of prey, populations of these predators can survive on alternative food sources such as pollen (Coll and Guershon 2002). True bugs and predatory mites can be integrated successfully with each other (Farkas et al. 2016), but both predators are commonly negatively affected by chemical control methods, including by botanicals and biopesticides (Bonsignore and Vacante 2012). As such it is crucial to investigate the compatibility of these thrips predators with plant-based adhesive sprays for thrips control before these adhesives are implemented on a commercial scale.