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Insight on the inside

Phloem-based whitefly resistance in tomato

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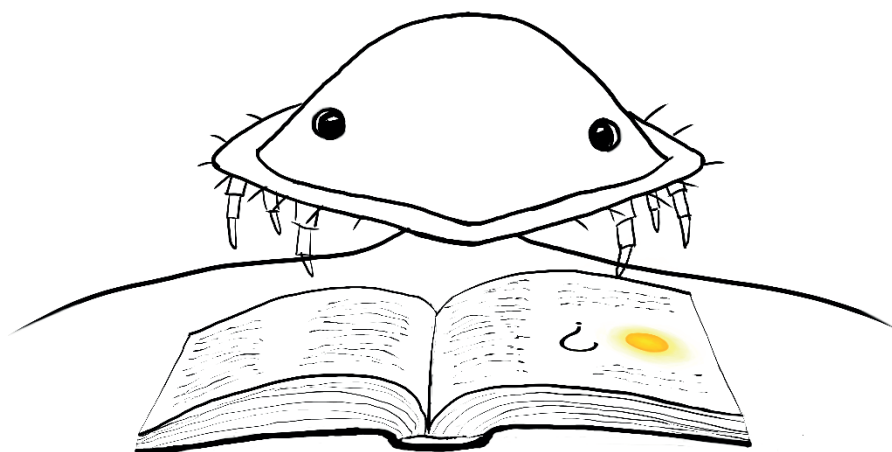
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Chapter 1

General introduction





The cultivated plants we use as crops or ornamentals are often highly vulnerable to insect infestations and diseases. For tomato (*Solanum lycopersicum*), infestations with whiteflies (*Bemisia tabaci*) are among the main contributors to crop losses, especially in (semi-)open field systems (Ramos *et al.*, 2018). Whiteflies are phloem-feeding insects with specialised mouthparts named stylets (Fig. 1.1). With their long and flexible stylets, whiteflies can manoeuvre around plant cells and reach the plant's vascular system without causing substantial damage to the plant, thereby circumventing most of the plant's defence mechanisms. This “stealthy” feeding style additionally makes whiteflies a good vector for plant viruses, which are transported from plant to plant via the insect's saliva, directly into the phloem. In this manner, the diseases caused by these whitefly-transmitted viruses spread fast and can lead to devastating crop losses (Navas-Castillo *et al.*, 2011; Polston *et al.*, 2014). This is especially the case for monocropping practices in which whitefly populations can grow explosively and all plants in the area are susceptible to the same viruses. Conventionally, whitefly infestations are prevented through the application of synthetic pesticides, but the non-target effects of these persistent compounds can be detrimental to terrestrial and aquatic ecosystems (Mamy *et al.*, 2023). Consequently, the increasing public and legal reservations against the use of conventional insecticides and the demand for more sustainable crop protection methods have spurred the interest in the development of crops with natural, or genetic, insect resistance. However, there is currently no tomato cultivar on the market with (sufficient) resistance against whitefly.

Many wild relatives of crop species have developed defence mechanisms to the stressors in their habitat, including herbivorous insects. During the breeding process, however, plants were selected based on traits that increase yield, facilitate the harvesting process or improve the market value, like the tastiest fruit (or the prettiest flower for ornamentals). As crops were, and still are, grown in

relatively protected environments, innate defence was not focussed on as a trait for breeding and, if any natural defence mechanisms were present in early domesticated tomato, this trait was in most cases lost. Nevertheless, wild tomato species have evolved intriguing defence mechanisms in their ongoing arms race with herbivorous insects, leading to a large variation between tomato accessions in levels of resistance to whitefly and other insects (Rick, 1973; Rick & Chetelat, 1995; Lucatti *et al.*, 2013; Rakha *et al.*, 2017; Kortbeek *et al.*, 2021). Once identified, such resistance mechanisms could be reintroduced into cultivars through classical breeding, and function as a sustainable alternative to the application of pesticides. However, we first need to elucidate these resistance mechanisms. What variation is there in resistances to whitefly in the wild tomato germplasm and what are the underlying mechanisms?

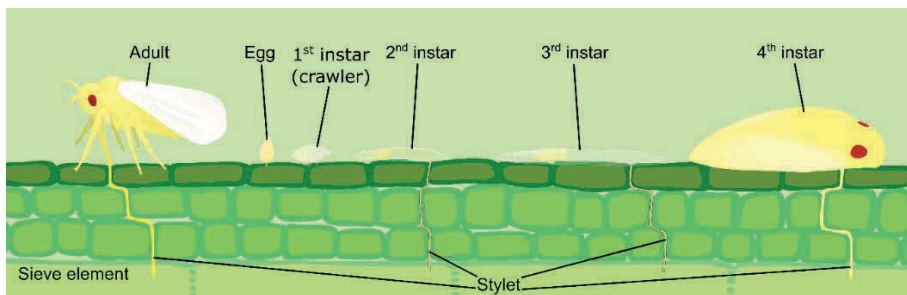


Figure 1.1 Illustration of *Bemisia tabaci* developmental stages. Adult and second to fourth instars are depicted with stylets inserted into the sieve elements.

The defence mechanisms of plants against herbivorous insects can vary widely, from physical barriers like thorns and cuticles (Lucas *et al.*, 2000; Zhao *et al.*, 2020), to several resistance (R) genes (Broekgaarden *et al.*, 2011; Kourelis & van der Hoorn, 2018; Zhang *et al.*, 2022), and constitutive and inducible chemical defences (Kaplan *et al.*, 2008; War *et al.*, 2012; Kessler, 2015). While all these types of mechanisms are interesting, chemical defence mechanisms are the most promising type of defence for crops, as physical defences like thorns also defend against crop growers and R-gene based resistance to insects is limited and



unstable in the field (El-Sappah *et al.*, 2019). Important for the chemical defence mechanisms are specialised metabolites, plant produced compounds that play a pivotal role in the interaction with other organisms. Specialised metabolites are incredibly divers, varying from small volatile terpenes to complexly decorated molecules, and are often produced and stored in, and excreted by, specialised tissue. An example of such specialised tissue are trichomes, hair-like structures on the leaves and stem that can be either glandular or non-glandular (Glas *et al.*, 2012). The chemical resistance of some wild tomato species partly stems from their glandular trichomes. The trichomes of these species produce and store specialised metabolites which function as a deterrent or toxin against pest-insects, with the concentration of specialised metabolites in wild tomato trichomes greatly exceeding that in cultivated tomato (Bleeker *et al.*, 2009; Balcke *et al.*, 2017; Kortbeek *et al.*, 2023).

Although trichome-based resistance mechanisms can be very efficient defence against whiteflies, it can also have non-target effects on beneficial insects such natural enemies of pest insects (Riddick & Simmons, 2014). Furthermore, not all wild tomato accessions exhibit this type of resistance. In previous work in our lab, a large set of tomato accessions was screened for trichome-based resistance, showing that there are large differences between accessions in their metabolites and level of resistance (Kortbeek *et al.*, 2021). When the collection sites of these accessions are plotted on a map, the resistance phenotypes do not cluster based on coordinates nor on highland versus lowland (Fig. 1.2). Furthermore, we had indications that on one of the tomato accessions on which whitefly adults' survival was equal to that on a susceptible cultivar, whitefly nymphs might not be able to develop successfully. We therefore hypothesised that some of the accessions without trichome-based resistance likely have encountered the same herbivorous insect species as some of the accessions with trichome-based resistance and, under the same selection pressure, have developed other chemical

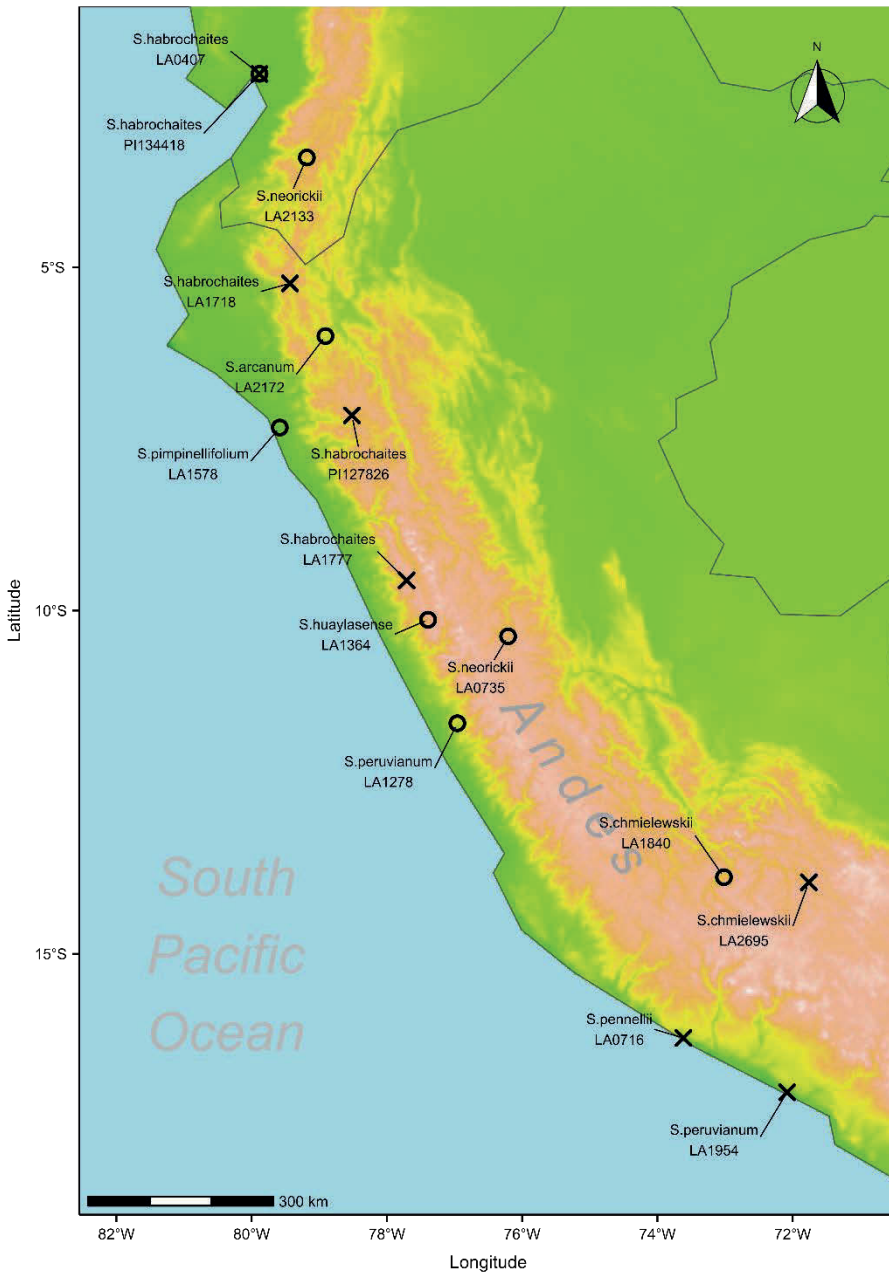


Figure 1.2 Geographical locations of wild tomato accessions used by Kortbeek *et al.* (2021), excluding accessions originating from the Galápagos Islands. Accessions are indicated with their trichome-based whitefly resistance, classified as resistant (×) or susceptible (○), based on adult whitefly survival according to Kortbeek *et al.* (2021).



resistance mechanisms which target other life stages of the insect. An alternative to the trichome-based would be a resistance mechanism on the inside, or more specifically, in the phloem. The phloem is the most important interaction site between plants and whiteflies, with developing nymphs continuously feeding from the phloem, and would therefore be a suitable location for a defence mechanism against these insects during their nymphal stages.

The phloem is part of the plant vasculature and transports compounds produced in the plant from source to sink, as opposed to the xylem which mainly transports water and minerals that are taken up by the roots to the shoot. The plant-produced compounds transported through the phloem include, among others, sugars, amino acids, organic acids, fatty acids and hormones, but also specialised metabolites (Broussard *et al.*, 2023). Although whitefly adults have been found to occasionally feed from the xylem, they feed predominantly from the phloem, especially during their nymphal stages (Jiang & Walker, 2003). Phloem-based resistance mechanisms could therefore be very efficient against whiteflies. Examples can be found in naturally occurring resistances such as in a resistant *Brassica oleracea* cultivar on which survival and oviposition of cabbage whiteflies (*Aleyrodes proletella*) are strongly reduced by a phloem-based resistance mechanism (Broekgaarden *et al.*, 2012). The efficiency of phloem-based defence is also utilised for the efficiency of pesticides against phloem-feeding insects. Neonicotinoid pesticides were based on xylem-mobile nicotine, which naturally occurs in tobacco (*Nicotiana spp.*). These persistent synthetic chemicals are taken up by the plant and transported throughout the plant via the xylem or the phloem, depending on the specific compound (Nauen *et al.*, 1999; Calvo-Agudo *et al.*, 2019). Although phloem-based resistance through neonicotinoid application is highly unfavourable due to the persistence of these pesticides, and their subsequent accumulation in ecosystems and harmful off-target effects (Donley *et al.*, 2024), naturally occurring phloem-based resistance

mechanisms are less likely to cause ecological harm. Not only would the compounds involved in the resistance mechanism be produced and accumulated inside the plant instead of being applied from the outside, but plant produced defence compounds are usually also unstable outside the plant, whereas pesticides like neonicotinoids have been designed to be persistent (Mullins *et al.*, 1993; Kagabu, 1999). A diverse set of specialised metabolites in the phloem of a variety of plant species has already been found to confer resistance against phloem-feeding insects. For example, benzoxazinoids in the phloem of Poaceae deter aphids from feeding and inhibit their growth (Givovich *et al.*, 1994; Ahmad *et al.*, 2011), castanospermine in the phloem of *Castanospermum australe* has a toxic and deterrent effect on aphids (Dreyer *et al.*, 1985; Molyneux *et al.*, 1990), and quinolizidine alkaloids in the phloem of Fabaceae repel aphids and deter them from feeding (Wink *et al.*, 1982; Wink & Witte, 1984; Dreyer *et al.*, 1985; Lee *et al.*, 2007; Otterbach *et al.*, 2019). However, such examples have not yet been found in the tomato phloem. Gaining insight into the phloem-based defence mechanisms in tomato and its wild relatives could therefore be an important step towards the development of more sustainable tomato production practices.



In several instances, indications have been found for the existence of phloem-based resistance mechanisms in tomato. For example, grafting a susceptible tomato cultivar onto resistant rootstocks cultivar led to resistance against the whitefly species *B. tabaci* (Žanić *et al.*, 2017) and *Trialeurodes vaporariorum* (Mandušić *et al.*, 2019) in the scion. This indicates that compounds involved in the resistance mechanism can be transported through the plant over long distances before reaching the whitefly during its interaction with the phloem. Furthermore, in the wild tomato species *S. galapagense*, a resistance against *B. tabaci* and *T. vaporariorum* was found that is not based on trichomes (Santegoets *et al.*, 2021). Based on the stylet probing and salivation behaviour of *T. vaporariorum*, studied using electrical penetration graph (EPG), it was concluded that there must be a



phloem-based defence mechanism in *S. pimpinellifolium* (McDaniel *et al.*, 2016). Despite the evidence for phloem-based resistance in wild tomato against whiteflies, the specialised metabolites content of wild and cultivated tomato remains to be explored. Consequently, no specific phloem-based specialised metabolites that influence the resistance against phloem-feeding insects have been identified in tomato as of yet.

To study the tomato phloem and its involvement in resistance mechanisms, there are several technical difficulties to overcome. Firstly, the trichomes on many of the wild tomato plants might interfere with the experiments and convolute the differentiation between phloem- and trichome-based resistance. Thus, measures should be taken to eliminate the effect of trichomes on the studied resistance phenotype. Secondly, the phloem is localised within other plant tissue and can, by humans, only be accessed through invasive methods that lead to wounding. Tomato plants have a rapid wound healing response, sealing off the cut surface before any phloem sap can be extracted. Therefore, an appropriate method would have to be set up to collect the phloem of wild tomato and prepare it for metabolomics analyses. Lastly, advances in LC-MS technology have let to increasingly large metabolic datasets but only a small portion of plant-produced metabolites is described in chemical libraries (Alvarez & Naldrett, 2021). Datasets resulting from untargeted metabolomics analyses often contain many more metabolic features than samples (high-dimensionality) and a large portion of the metabolic features is usually only present in a subset of the samples (sparsity). Moreover, there is typically a strong correlation between groups of metabolites, for example between metabolites that are part of the same pathway or that share a precursor (multicollinearity). Statistical models often perform poorly under this combination of challenges and the statistical analysis of these datasets and ensuing selection of metabolites of interests can therefore form a bottleneck in this type of research (Vinaixa *et al.*, 2012; Gorrochategui *et al.*,

2016). Over recent years, the methods used to analyse untargeted metabolomics data moved from more classical statistical models such as ANOVA and correlation analyses to Machine Learning models like Partial Least Squares Discriminant Analysis (PLS-DA), Principal Component Analysis (PCA), Random Forest (RF) and Neural Networks (NN) (Liebal *et al.*, 2020; Sen *et al.*, 2021; Chen *et al.*, 2022; Coler *et al.*, 2024; Chi *et al.*, 2024).



Thesis outline

The aim of this thesis is to explore the phloem of the wild tomato germplasm as a site for insect resistance through the identification of phloem-based specialised metabolites that affect the proliferation of *Bemisia tabaci*.

This was translated into the following objectives:

- Assess the developmental success of *B. tabaci* on a set of wild and cultivated tomato plants as a measure of resistance in the host plants.
- Analyse the specialised metabolites in the wild and cultivated tomato phloem and evaluate the natural variation in phloem-based specialised metabolites.
- Determine if there is a link between specialised metabolites in the phloem and the observed resistance phenotypes.

To this end, we first reviewed the current understanding of the role of phloem-based specialised metabolites in plant-insect interactions in **chapter 2**. Here we will see that specialised metabolites in the phloem can affect insects interacting with a plant, but those insects can, in turn, also influence the phloem composition.

In **chapter 3** we identified two *Solanum chmielewskii* accessions on which whitefly nymphs could not fully develop to the final instar stage. These accessions are promising candidates to study phloem-based resistance, because



S. chmielewskii was previously shown to have no trichome based resistance. With the use of grafting, metabolomics and inhibitor experiments, we were able to draw conclusions about the phloem/vasculature-mobile nature of the factor underlying the resistance phenotype and its link to riboflavin.

After finding this phloem-based resistance in *S. chmielewskii*, we hypothesised that there might be more wild tomato species with phloem-based resistance mechanisms that could be attributed to specialised metabolites. Assessing this natural variation would require screening many accessions in a large set of developmental bioassays and untargeted metabolomics analyses. The large developmental and metabolic datasets would need to be analysed and linked with suitable data analysis methods. For this purpose, we developed a Python package in **chapter 4** to analyse the data from the bioassays and metabolomics and link the two data types using machine learning.

In **chapter 5** we selected a plant panel consisting of a total of 12 accessions from the wild tomato species *S. chmielewskii*, *S. habrochaites*, *S. pennellii* and a susceptible *S. lycopersicum* cultivar. To omit the strong trichome-based resistance phenotypes of several of the included accessions, we performed developmental bioassays on susceptible cultivar scions grafted onto the wild plants. Next to the two resistant *S. chmielewskii* accessions identified in **chapter 3**, we also identify a *S. habrochaites* accession with a phloem-based resistance phenotype. We evaluated the diversity in metabolites in the phloem of all 13 genotypes using untargeted metabolomics analysis. With the data analysis package developed in **chapter 4** and a data analysis method based on Genome Wide Association Studies we linked the resistance phenotypes to the phloem metabolite data and selected metabolic features of interest.