Consequences of russet mite-induced tomato defenses for community interactions
Glas, J.J.

Citation for published version (APA):
Glas, J. J. (2014). Consequences of russet mite-induced tomato defenses for community interactions

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
General introduction

In nature and agriculture plants are attacked by a suite of different plant parasites, including insects, mites and plant pathogens. For insects, it is estimated that of the approximately 1 million described species ca. 45% are phytophagous (Schoonhoven et al., 2005). Therefore, it is not surprising that plant-herbivore interactions are among the most ubiquitous of all ecological interactions (Jander & Howe, 2008). Yet, despite this herbivore pressure, plants under natural conditions often still appear relatively ‘green’ and one rarely encounters plants that are completely defoliated (Hairston et al., 1960).

This paradox may have an easy resolution as plants, rather than passively undergoing consumption, have evolved traits that allow them to respond to herbivory, for instance by inducing defense responses that reduce growth and/or survival of plant enemies (Howe & Jander, 2008). However, these defenses may in turn select for counter-adaptations at the herbivore side, for instance the ability to suppress defenses, and this can escalate into a so-called evolutionary arms race involving cycles of adaptation and counter-adaptation between plants and herbivores (Berenbaum, 1998). Getting a better understanding of how plant defenses function as well as the strategies that plant attackers have evolved to cope with these defenses is important as it can possibly help to improve crop resistance in agriculture.

In this thesis, I have specifically addressed the question whether herbivorous pest species that cause distinct plant defense responses can influence each other’s performance via their shared host.

Plant defenses: constitutive and induced, direct and indirect

Plant defenses are usually divided in constitutive defenses, i.e., those that are always present on a plant, and induced defenses, i.e., those that are activated only in response to a herbivore or pathogen attack. Examples of constitutive defenses include physical barriers, like cell walls lignified to prevent pathogens from entering the plants (Bhuiyan et al., 2009), waxy cuticle layers reducing suitable feeding sites per leaf surface (Eigenbrode & Shelton, 1990) or trichomes, i.e., specialized hairs found on aerial parts of a plant, which function as mechanical barriers (Pott et al., 2012) but also play important roles in induced defenses (Glas et al., 2012). Trichomes, which can be classified as either non-glandular or glandular, have been studied in
great detail, especially those of *Solanum* species because of their strong relationship with resistance against herbivores (Alba *et al.*, 2009). They play an important role in this thesis as well (see Chapter 5). Glandular trichomes are usually multicellular, consisting of differentiated basal, stalk and apical cells and they share the capacity to produce, store and secrete large amounts of different classes of secondary metabolites (Schilmiller *et al.*, 2008). On tomato, in total eight different types of trichomes are distinguished of which four (*i.e.*, type I, IV, VI and VII) are glandular, whereas the other four (*i.e.*, type II, III, V and VIII) are non-glandular (Figure 1.1). These trichome types differ in their morphology and the glandular types are known to differ as well in number of stalk and secretory cells and in their chemical contents (for a detailed description of the different tomato trichome types see Glas *et al.*, 2012).

Next to trichomes, also the herbivore-induced defense response of tomato plays a major role in this thesis (Chapters 2 & 4). Induced defenses are commonly subdivided into direct and indirect defenses. Direct defenses include the production of toxins, antifeedants and inhibitors of digestion that negatively affect the survival of herbivores and/or growth and development of individuals or populations (Howe & Jander, 2008). In contrast, indirect defenses refer to plant traits that enhance attraction of natural enemies of the herbivore, like predators and parasitoids, which can promote plant fitness (Van Loon *et al.*, 2000; Schuman *et al.*, 2012). In that sense, indirect defenses are on average more lethal to herbivores than direct defenses (see also section Induction and suppression of defenses of this introduction). Attraction of natural enemies is predominantly mediated via volatiles that are emitted by the plant in response to herbivore feeding and used by natural enemies to locate herbivores as prey (Kessler & Baldwin, 2001; Kant *et al.*, 2004). Other ways of plants to attract or arrest natural enemies is to provide them with food, for instance extrafloral nectar (Pemberton & Lee, 1996) and food bodies (Fischer *et al.*, 2002), or with shelter places, such as leaf domatia, *i.e.*, small hair-tufts or pockets that provide refuges for predatory arthropods (Walter, 1996).

How do plants recognize herbivores?

Plants are unable to run away from danger. This implies that they are strongly dependent on their constitutive and inducible defense system to protect themselves from being eaten by pathogens and herbivores. Notably, even before the herbivore starts consuming the plant, defensive responses can already be mounted. Evidence comes from the observation that the release of volatiles from herbivore-attacked plants ‘primes’ neighboring uninfested plants to elicit stronger and faster defense responses when these are attacked by herbivores (*e.g.*, Baldwin *et al.*, 2006; Heil & Bueno, 2007; Shiojiri *et al.*, 2012). Moreover, it was found that the mere walking of small insects or the deposition of eggs by insects can trigger defense responses (Peiffer *et al.*, 2009; Kim *et al.*, 2012).
Figure 1.1 Glandular trichomes in the section Lycopersicon. Wild tomato accessions have high densities of glandular trichomes that confer resistance to several pests. Panel A shows the leaflet surface of *S. habrochaites* acc. LA1777 with high densities of glandular trichome types IV, VI (B), and I (C). Surface of *S. pennelli* acc. LA716 is also covered by type IV trichomes (D, E) producing and secreting acyl sugars. This accession also has type VI trichomes but in low density (F). Panel G shows the surface of the cultivated tomato *S. lycopersicum* cv. Moneymaker. Cultivated tomato has low density of type VI trichomes (H) and type I trichomes. Sometimes type IV-like trichomes (I) are observed on stems, veins, and on the leaflet edges. White bars represent 500 µm in panel A, C, D, and G. In panels B, E, F, H, and I, bars represent 50 µm. Photos by Jan van Arkel, University of Amsterdam. Figure adapted from Glas et al., 2012.
Plant responses differ according to the type of herbivore that is feeding from the plant. This specificity in plant response can be achieved because plants respond to specific patterns of feeding damage (Erb et al., 2012). Second, plants are able to tailor defenses because they recognize elicitors, i.e., compounds that stimulate plant defense and which are present in the saliva or other secretions (Schwartzberg & Tumlinson, 2014) of herbivores and come into contact with the plant during feeding (Schmelz et al., 2009). Elicitors have been intensely investigated and at least five different groups have been described (Alba et al., 2012).

The first group comprises enzymes, such as \( \beta \)-glucosidase, originally found in the oral secretions of _Pieris brassicae_ caterpillars. Application of \( \beta \)-glucosidase to leaves resulted in the emission of a blend of herbivore-induced plant volatiles that was similar to the blend emitted by plants that were mechanically damaged and then treated with caterpillar regurgitant, and in both cases this resulted in the attraction of parasitic wasps (Mattiauci et al., 1995). A second group constitutes the so-called ‘Fatty Acid Conjugates’ (FACs), which are formed in the insect gut by a conjugation reaction between plant-derived fatty acids and insect-derived amino acids (Bonaventure et al., 2011) and come into contact with the plant during regurgitation. The first FAC elicitor identified, \( N \)-(17-hydroxylinolenoyl)-L-glutamine (also named volicitin), was isolated from oral secretions of beet armyworm caterpillars. Application of volicitin to corn (_Zea mays_) seedlings resulted in emission of a similar blend of volatiles as when attacked by caterpillars (Alborn et al., 1997). Then, a third group is the ‘caeliferins’, which are sulfated fatty-acids, originally identified in the regurgitant of grasshoppers (_Schistocerca americana_), which induced the release of volatiles when applied to damaged leaves of corn seedlings (Alborn et al., 2007). A fourth group of elicitors are derived from plant enzymes, like the ‘inceptins’, which are fragments from chloroplastic ATP synthase found in the oral secretions of _Spodoptera frugiperda_ larvae. Inceptins induced the accumulation of phytohormones, expression of defense genes and volatile emission in cowpea plants (Schmelz et al., 2006). Finally, elicitors can also be derived from cell wall fragments, such as oligogalacturonides which induce expression of defense genes in tomato plants (Doares et al., 1995) and it is thought that wound-inducible polygalacturonases play a role in the degradation of leaf pectin to generate oligogalacturonic acid fragments (Bergey et al., 1999). Not much is known about how plants perceive elicitors, but in maize (_Zea mays_) a plasma membrane protein has been identified that binds to the elicitor volicitin (Truitt et al., 2004).

**Regulation of induced defenses**

Induced defenses are mainly regulated by a set of three phytohormones: jasmonic acid (JA), salicylic acid (SA) and ethylene (ET). Herbivorous insects and necrotrophic pathogens typically induce JA-dependent defenses (Howe & Jander, 2008), whereas...
defenses against biotrophic pathogens (Glazebrook, 2005) and phloem-feeders (Kaloshian & Walling, 2005) are mediated by SA. ET is generally considered to function together with JA in the induction of defense responses (Lorenzo et al., 2003), whereas acting antagonistically with SA (Diezel et al., 2009). However, exceptions to these patterns are known as well (reviewed by Adie et al., 2007). Mutants defective in JA-biosynthesis or signaling are generally much more susceptible to insect attack (e.g., Howe et al., 1996), thereby demonstrating the importance of the JA-pathway for herbivore resistance. Similarly, plants unable to produce or accumulate SA are generally more susceptible to pathogen infections (e.g., Delaney et al., 1994).

**Jasmonic acid**

The JA-signaling pathway has been extensively reviewed (for a recent review see, e.g., Wasternack & Hause, 2013) and will be introduced only briefly here. In Chapters 2 and 4 the JA-pathway will be discussed in more detail. Biosynthesis of JA in tomato takes place in the chloroplast and peroxisomes of the phloem companion cells (Howe, 2004). Briefly, the first step comprises the formation of linolenic acid which is released from galactolipids of chloroplast membranes via the action of (a) phospholipase(s). Subsequently, linolenic acid is oxygenized by lipoxygenases (LOX), resulting in the formation of hydroperoxylinolenic acid, which is then converted by allene oxide synthase (AOS) and allene oxide cyclase (AOC) to produce oxophytodienoic acid (OPDA). In a next step, OPDA is exported from the chloroplast and imported into the peroxisomes where it is converted by OPDA reductase3 (OPR3) into cyclopentane-1-octanoic acid, followed by three cycles of β-oxidation to yield the final product JA. In the cytosol JA is conjugated to isoleucine, by the enzyme jasmonate resistant 1 (JAR1) (Suza et al., 2010) resulting in the formation of JA-Ile, which is the bioactive form of JA (Fonseca et al., 2009). Perception of JA-Ile occurs in the nucleus by the SCFCOI1 (Skip/Cullin/F-box) receptor complex, which ubiquitinates transcriptional suppressors [i.e., the Jasmonate ZIM domain (JAZ) proteins] after which these are degraded by the proteasome and downstream defense responses are activated, including, for instance, the synthesis of proteinase inhibitors (PIs) (Gimenez-Ibanez & Solano, 2013; Figure 1.2).

**Salicylic acid**

Similar to the situation for JA, there is wealth of literature describing the role of SA in plant disease resistance (reviewed by Vlot et al., 2009) and only the most important points will be introduced here. SA in plants regulates defenses against pathogens, including bacteria, fungi and viruses. These defenses can be triggered locally but also systemically leading to enhanced resistance in uninfected parts of the plant, a phenomenon which is called Systemic Acquired Resistance (SAR). SAR includes the up-regulation of defense genes, including the Pathogenesis-Related proteins (PR-pro-
CHAPTER 1 | GENERAL INTRODUCTION

teins), which play a role in resistance against microbes (Van Loon et al., 2006). In addition, the SA and JA signaling pathways operate together with reactive oxygen species (ROS) in the activation of the plant hypersensitive response (programmed cell death) (Bostock, 2005).

SA is an aromatic compound derived from chorismate in the phenylpropanoid pathway and its biosynthesis has been proposed to occur via two distinct routes. The first route occurs via isochorismate synthase (ICS), which is responsible for the production of the SA-precursor isochorismic acid. In the second route, chorismic acid is converted via phenylalanine into cinnamic acid and then into SA via either benzoic acid or coumaric acid (Boatwright et al., 2013). Yet, it is good to note that the biosynthesis of SA has not been completely resolved in plants and some steps are still hypothetical in tomato (indicated by dashed lines in FIGURE 1.2). Accumulation of SA leads to the translocation of the regulatory protein NONEXPRESSOR OF PR GENES1 (NPR1) to the nucleus (Koornneef & Pieterse, 2008). Subsequently, in the nucleus, NPR1 interacts with the TGA transcription factors, resulting in the induction of PR-genes. Via which exact protein SA is perceived is still a matter of an ongoing debate: Wu et al. (2012) reported that SA binds to NPR1 but in another study it was found that SA, instead of NPR1, binds to NPR3 and NPR4 in Arabidopsis (Fu et al., 2012).

SA is not only important for pathogen-induced responses but also for indirect plant defense, as it can be converted by the JA-induced enzyme SAMT into methyl salicylate (MeSA), a volatile of which the emission increases in response to spider mite feeding. Together with other herbivore-induced plant volatiles, such as terpenes, MeSA plays a role in the attraction of predatory mites to spider mite-infested tomato plants (Ament et al., 2010).

Other phytohormones

Even though JA and SA are known as the primary regulators of plant defense, several others play regulatory roles as well, including not only ET (Adie et al., 2007) but also abscisic acid (ABA) (Dinh et al., 2013), auxin (Kazan & Manners, 2009), cytokinins (Choi et al., 2011), gibberellins (Yang et al., 2012) and brassinosteroids (Nakashita et al., 2003). This means that the defense signaling pathways that are primarily regulated by JA and SA can be modulated by additional signals as well. This implies that defense signaling pathways do not stand on their own but are connected to each other, forming complex networks, and with ample cross-communication occurring between them, leading to responses that can be either antagonistic or synergistic (Pieterse et al., 2009).

Probably the best documented example of crosstalk is that between the SA and JA pathway, which have been frequently observed to antagonize each other (e.g., Niki et al., 1998). To date, several regulators mediating the antagonistic effect of SA on JA
have been identified in Arabidopsis, like NPR1, which was shown to be required for the SA-mediated suppression of JA-dependent responses (Spoel et al., 2003). Yet, NPR1 has diverse functions and its role may be different in different plant species as indicated by the finding that in tobacco (Nicotiana attenuata) NPR1 was found to down-regulate SA production, thereby preventing JA/SA-crosstalk and protecting the plant from becoming very susceptible to herbivores (Rayapuram & Baldwin, 2007). A second mediator of JA/SA-crosstalk is OCTADECANOID-RESPONSIVEARABIDOPSIS59 (ORA59), a transcription factor whose accumulation was shown to be negative-

**FIGURE 1.2 Schematic overview of the JA- and SA-signaling pathway as summarized in the text. Solid arrows indicate confirmed steps in tomato; dashed lines indicate multiple hypothetical or indirect steps. Red inhibition lines indicate the antagonistic effects between the SA and the JA-pathway. Abbrevations: Ch = chorismate; Ich = isochorismate; Phe = Phenylalanine; TF = Transcription Factor; other abbreviations are explained in the text.**
ly regulated by SA in Arabidopsis (Van der Does et al., 2013). Additional regulators have been identified as well in Arabidopsis including WRKYs (e.g., WRKY70, WRKY50/51) as well as TGA transcription factors (e.g., Li et al., 2004; Zander et al., 2010; Pieterse et al., 2012). In contrast, regulators that mediate the antagonistic effect of JA on SA have not been identified yet (Pieterse et al., 2012).

At present, the biological function (or the necessity) of JA/SA-crosstalk is not well understood. On the one hand, it has been suggested that crosstalk allows plants to ‘choose’ the best defensive strategy depending on the type of attacker encountered (Kunkel & Brooks, 2002) and to prioritize energy flows but, on the other hand, it clearly creates problems as well as it may, for example, also be abused by pathogens and herbivores (Zarate et al., 2007; El Oirdi et al., 2011). Hence, why plants benefit from the JA/SA-antagonism (assuming natural selection would otherwise have selected against this trait – if physiologically possible) remains a question to be addressed in the future (Thaler et al., 2012).

Induction and suppression of defenses

Herbivores deal with induced plant defenses in distinct manners. First, they may avoid induced plants or plant parts, as observed, for instance, for Manduca sexta larvae and leafhoppers, which preferentially fed on plants that were impaired in JA-defenses (Paschold et al., 2007; Kallenbach et al., 2012). Second, herbivores may become tolerant or resistant to induced plant defenses, as reported for instance for spider mites (Kant et al., 2008) and Manduca sexta caterpillars (Wink & Theile, 2002). Third, herbivores may suppress induced defenses (reviewed by Alba et al., 2011). Since defense suppression is a major theme in this thesis, this phenomenon will be discussed in more detail here.

Defense suppression is well known from plant pathogens, and many examples are known from plant pathogenic bacteria (e.g., Zhao et al., 2003), fungi (e.g., Weiberg et al., 2013), viruses (e.g., Hanley-Bowdoin et al., 2013) as well as nematodes (e.g., Haegeman et al., 2012). However, evidence is accumulating that also insects and mites can manipulate plant defenses. Herbivorous arthropods whose feeding activities are associated with concomitant suppression of plant defenses include phloem-feeders, i.e., whiteflies (Zarate et al., 2007; Zhang et al., 2009, 2013), aphids (Soler et al., 2012; Schwartzberg & Tumlinson, 2014) and mealybugs (Zhang et al., 2011), cell-content feeders, i.e., spider mites (Kant et al., 2008; Sarmento et al., 2011), as well as chewing insects including lepidopteran caterpillars (Weech et al., 2008; Consales et al., 2012; Savchenko et al., 2013) and beetles (Chung et al., 2013). Finally, it has been reported that also caterpillar eggs may suppress plant defenses (Bruessow et al., 2010).

Defense suppression may occur in different manners. Some herbivores have found ways to make good use of the antagonistic effects between the JA- and SA-
pathway. Probably the best known example is that of whiteflies which suppress the
JA-dependent responses, to which they are susceptible, by inducing SA-defenses
(Zarate et al., 2007; Zhang et al., 2013). However, in whitefly-infested bean plants that
had been co-infested with spider mites not only JA but also SA accumulation was
reduced, suggesting that in this system suppression of JA-defenses is not due to ele-
vated SA levels (Zhang et al., 2009). Other herbivores, like spider mites, were found
to suppress both the JA- and SA-defense signaling pathway (Sarmento et al., 2011).
Suppression may also occur independent from the JA- and SA-pathway as observed
for Spodoptera littoralis caterpillars (Consales et al., 2012).

Much remains to be learned about the causal mechanisms underlying defense
suppression by herbivores. However, similar to plant pathogens and nematodes, her-
bivores have been found to produce and secrete effector molecules that suppress
induced plant defenses (Walling, 2009). Notably, effectors have identified in evolu-
tionary distant species, including pathogens, spider mites, insects and nematodes.
As an example, spider mites belong to the chelicerates and insects to the unirami-
ans and is estimated that these two groups have diverged already early in the evolu-
tion of arthropods, probably over 400 million years ago from a common aquatic
ancestor (Weygoldt, 1998). Hence, this suggests that effectors may have evolved
independently in different lineages of plant parasites.

The first reported example was glucose oxidase (GOX), a salivary enzyme that,
depending on the host plant, can counteract JA-dependent accumulation of nicotine
induced by herbivore feeding (e.g., Eichenseer et al., 1999, 2010; Musser et al.,
2002). However, this response may well benefit the host plant rather than the herbi-
vore and hence may not be adaptive from the herbivore’s point of view (Voelckel et
al., 2001). Following the discovery of GOX, more examples of herbivore effectors
have been found, which are present in the saliva and eggs of herbivores. Effectors
functionally characterized to date have been isolated from lepidopteran caterpillars
(Schmelz et al., 2012) as well as from aphids (e.g., Bos et al., 2010; Atamian et al.,
2013) and nematodes (Mitchum et al., 2013). In spider mites, effectors have been
identified as well which are currently being characterized (C. Villarroel, University of
Amsterdam, unpublished data). With the increasing availability of genome and tran-
scriptome sequencing data I expect that more examples of herbivore effectors will
become available in the near future.

Finally, an alternative strategy of herbivores to circumvent plant defenses is to
vector pathogens, like viruses or bacteria, which also may cause suppression of
induced defenses. For example, the western flower thrips (Frankliniella occidentalis)
was found to transmit the tomato spotted wilt virus, which exploits the antagonism
between the JA and SA pathway and thereby benefits its vector (Abe et al., 2012).
Viruses transmitted by whiteflies have been reported to suppress JA-defenses as
well, implying that is still unclear whether suppression of defenses is caused by the whiteflies themselves (Zarate et al., 2007; Zhang et al., 2009) or by the viruses they transmit (Zhang et al., 2012). In addition, oral secretions of the Colorado potato beetle (Leptinotarsa decemlineata) were reported to contain bacteria that induce SA accumulation thereby suppressing JA-responses to which the beetles are susceptible (Chung et al., 2013). Hence, it seems that most herbivores that suppress defenses via the JA/SA- antagonism seem to do this with the help of microbes. Finally, an effector protein called SAP11 which suppresses JA-defenses in Arabidopsis was identified from a phytoplasma, and this enhanced the reproduction of its insect vector, the leafhopper Macrosteles quadrilineatus (Sugio et al., 2011).

Experimental system
For this thesis research, I have focused on the interaction between the cultivated tomato (Solanum lycopersicum) and one of its main herbivorous pests, the tomato russet mite (Aculops lycopersici). Experiments I did early during my PhD project showed that these mites suppress tomato defenses, which was the major reason to start this project. This suppression and its consequences for the russet mite’s natural community are the main topics of this thesis.

The cultivated tomato belongs to the family of the Solanaceae, which have their origin in South-America. For my experiments I used the tomato S. lycopersicum cultivar Castlemart as well as Moneymaker. Castlemart is the genetic background of two plants altered in the JA-defense pathway, i.e., the mutant defenseless-1 (def-1), which is deficient in the induced expression of JA-dependent defense genes (Howe et al., 1996), and 35S::prosys+, in which the prosystemin gene is overexpressed resulting in a constitutively activated JA-dependent defense response (Chen et al., 2006). I also used transgenic tomato plants that express a gene of the bacterium Pseudomonas putida, called salicylate hydroxylase (nahG), which converts SA into catechol. Hence, nahG plants are unable to display SA-mediated defense and exhibit increased susceptibility to viral and bacterial pathogens (Brading et al., 2000). nahG plants are in the genetic background of the S. lycopersicum cultivar Moneymaker.

In several key-experiments in this thesis, I have used three other important pests of tomato: (1) the two-spotted spider mite (Tetranychus urticae), (2) its relative the red tomato spider mite (Tetranychus evansi) and (3) the plant pathogenic bacterium Pseudomonas syringae pv. tomato DC3000. Hence, these species will be briefly introduced as well here. The reason to include these species is that they have been well characterized in terms of the defenses they elicit, as well as with respect to their sensitivity to these defenses, and, hence, they were included as a benchmark (or reference) in my experiments. In addition, T. urticae and P. syringae were used to investigate the ecological consequences of russet mite-induced defenses.
Tomato russet mites
Tomato russet mites belong to the family of Eriophyidae (superfamily Eriophyoidea). Figure 1.3 shows a picture of a russet mite residing together with an adult female of *T. urticae* on a tomato leaflet. It is thought that the natural host of the tomato russet mite was a wild solanaceous plant somewhere in South-America and that its association with the cultivated tomato is only recent (Oldfield, 1996). When tomato was domesticated is unknown but the oldest records date from 500 BC from southern Mexico (Smith, 1994).

Approximately 3000 species of eriophyoids have been described, but it is estimated that this number is less than 10% of the actual number of species existing in this family (Lindquist *et al.*, 1996). Eriophyoid mites are the smallest arthropods that feed on plants, and they cannot be seen without magnification. Their worm-like bodies are between 140-300 μm long (Sabelis & Bruin, 1996). Eriophyoids are yellow to white in color and do not seem to accumulate chlorophyll from plants, like spider mites. They disperse primarily by means of the wind but some species also by attaching themselves to larger animals, like aphids, for phoretic transport (Sabelis & Bruin, 1996). Adult eriophyoid mites have only two pairs of legs, unlike tetranychid mites, which possess four pairs of legs. Eriophyoid mites have a female based sex ratio and an
arrhenotokous parthenogenic mode of reproduction, meaning that males are haploid and females are diploid. Hence, unfertilized eggs develop into males, whereas fertilized eggs can develop into either males or females. Sperm transfer in eriophyoid mites occurs via spermatophores: these are little sacks on a stick which contain sperm cells and which are placed by the males on the plant surface after which they are picked up by the females. The life-cycle of most eriophyoids is rather simple: it consists of the egg, two nymphal stages and the adult. Eriophyoid mites are considered as the second most important family of mite plant pests after the Tetranychidae (Lindquist et al., 1996). Plants of economic importance that are damaged include citrus, apple, pear, grapes, tomato, wheat, sugarcane, tea, coffee, olive, coconut as well as ornamental plants. Eriophyoid mites are often host specific and damage occurs on all the above-ground plant parts, including the reproductive tissues and fruits. For this reason, eriophyoids are sometimes used as biological agents for control of weeds or invasive plants (Sabelis & Bruin, 1996).

Many eriophyoids cause the formation of galls in which they live and which can have various shapes (for a description see Westphal & Manson, 1996). Other plant malformations induced by eriophyoids include abnormal development of hairs (‘erinea’), blisters, rolling of the leaves, swelling of buds or excessive branching (‘witches’ brooms’) (Westphal & Manson, 1996). Eriophyoid mites are cell-content feeders: they possess a stylet (typically about 7-30 µm long), with which they pierce plant cells to ingest the watery cytoplasm (Royalty & Perring, 1996). Due to their small size, the mites are usually not able to penetrate the cells below the epidermis. Unlike many tetranychid mite species, which usually kill their host plant, eriophyoids generally leave their host intact (Sabelis & Bruin, 1996), even though they do cause damage by destroying the epidermal cell layer, thereby reducing gas exchange and photosynthesis (Royalty & Perring, 1988).

Remarkably, eriophyoid mites are the only Acari known to vector plant pathogens, mostly viruses (Oldfield & Proeseler, 1996). A well-known example is the wheat curl mite (Aceria tulipae), which transmits the wheat streak mosaic virus. However, transmission of plant diseases has never been reported for the tomato russet mite.

Tomato russet mites occur worldwide and they feed on solanaceous plants only (Table 1.1, Figure 1.4). Feeding occurs on the foliage, the stem and also the inflorescence and young fruits. Early indications of russet mite damage include silvering of the underside of the leaves. In later stages of the infestation, the leaves turn bronze colored (‘russet’) after which they dry out and drop from the plant. Furthermore, the stem loses its trichomes, becomes rusty brown and sometimes develops small cracks. Just like other eriophyoids, russet mites usually don’t kill their host, with the exception of the cultivated tomato on which they cause severe damage, including wilting and death of the plant (Perring, 1996) (Figure 1.5).
Death of the plant is thought to occur because of the destruction of the epidermis which leads to the collapse of the underlying mesophyll cells and desiccation of the plant (Royalty & Perring, 1988). Cells adjacent to the destroyed cells typically have thickened, lignified, cell walls (Royalty & Perring, 1988), which might be an effective plant response to prevent desiccation. However, it is clear that in several cultivated

<table>
<thead>
<tr>
<th>Latin name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Browallia americana</td>
<td>-</td>
</tr>
<tr>
<td>Capsicum annuum</td>
<td>Bell pepper</td>
</tr>
<tr>
<td>Convolvulus sp.</td>
<td>Morning glory</td>
</tr>
<tr>
<td>Convolvulus arvensis</td>
<td>Bindweed</td>
</tr>
<tr>
<td>Datura innoxia</td>
<td>Downy thornapple</td>
</tr>
<tr>
<td>Datura stramonium</td>
<td>Jimsonweed</td>
</tr>
<tr>
<td>Ipomoea batatas</td>
<td>Sweet potato</td>
</tr>
<tr>
<td>Lycopersicon peruvianum</td>
<td>-</td>
</tr>
<tr>
<td>Lycopersicon pimpinellifolium</td>
<td>Currant tomato</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>Tobacco</td>
</tr>
<tr>
<td>Petunia hybrid</td>
<td>-</td>
</tr>
<tr>
<td>Physalis minima</td>
<td>Sunberry</td>
</tr>
<tr>
<td>Physalis peruviana</td>
<td>Cape gooseberry</td>
</tr>
<tr>
<td>Solanum lycopersicum</td>
<td>Tomato</td>
</tr>
<tr>
<td>Solanum melongena</td>
<td>Aubergine</td>
</tr>
<tr>
<td>Solanum muricatum</td>
<td>Melon pear</td>
</tr>
<tr>
<td>Solanum nigrum</td>
<td>Black nightshade</td>
</tr>
<tr>
<td>Solanum pseudocapsicum</td>
<td>Jerusalem-cherry</td>
</tr>
<tr>
<td>Solanum tuberosum</td>
<td>Potato</td>
</tr>
</tbody>
</table>

**Figure 1.4** Distribution map of the tomato russet mite (*Aculops lycopersici*). Black dots = Present, no further details; blue dot = Widespread; red dots = Localised; yellow dot = Occasional or few reports [source: http://www.cABI.org; accessed February 2014].
novel host plants this defense mechanism does not prevent the plant from dying (Figure 1.5; Sabelis & Bruin, 1996).

Serious reductions in yield in cultivated tomatoes have been reported due to russet mite infestations with losses of up to 50% in South Africa (Perring, 1996). In contrast to most vagrant eriophyoids, which feed only on the abaxial side of the leaves, russet mites feed on all green surfaces as well as the fruits, suggesting that they are not harmed by direct sunlight (Bailey & Keifer, 1943).

Under optimal conditions of 27°C and 30% RH, russet mites can develop from egg to adult in just 6 or 7 days. Each female produces around 30-50 eggs during her lifetime, which is between 17 and 30 days, depending on temperature (Kawai & Haque, 2004).

Upon death of the host plant or at the end of the tomato growing season, mites disperse by wind to nearby alternative host plants where they overwinter. Several natural enemies have been reported to feed and reproduce on russet mites in the laboratory (see, e.g., Park et al., 2010) but on whole plants biological control is often unsuccessful (Trottin-Caudal et al., 2003; Fischer et al., 2005; Van Houten et al., 2013).

**Figure 1.5** Russet mites (*Aculops lycopersici*) cause death of tomato (*Solanum lycopersicum*) plants. Habitus of tomato plants at different time-points after infestations. Plants were 21 days old when infested with russet mites (ca. 1500 mixed stages) at the basis of the main stem and photos were taken on day 0, 4, 7, 11, 14 and 18 after infestation. Pictures by J. van Arkel (University of Amsterdam).
Spider mites
Spider mites are small herbivorous arthropods (the adult females are ca. 0.5 mm long) belonging to the family of the Tetranychidae, which consists of about 1200 species in total. The two-spotted spider mite *T. urticae* is a polyphagous pest species, with an extremely broad host range consisting of 1100 different plant species (Dermauw *et al.* 2012). *Tetranychus urticae* is a worldwide pest of greenhouse and field crops, including tomatoes, potatoes, beans, maize, cucumbers, peppers, strawberries, apples, grapes and citrus as well as ornamental plants such as roses and gerbera (Helle & Sabelis, 1985).

From the same genus is the red tomato spider mite *T. evansi* which is a specialist on solanaceous plants. *Tetranychus evansi* originates in Brazil but has been introduced in Africa somewhere in the 1970s and since about 20 years the species has made its entry into southern Europe as well (Boubou *et al.*, 2012; Navajas *et al.*, 2013). In Spain, *T. evansi* is replacing native *T. urticae* populations on several host plant species (Ferragut *et al.*, 2013).

Like russet mites, spider mites are cell-content feeders. The adult females possess ca. 150 µm long stylets which they use to lacerate-and-flush feed from the spongy parenchyma and palisade parenchyma cells (Park & Lee, 2002). Symptoms of spider mite feeding are visible as small chlorotic lesions on the leaf surface. Depending on the temperature, adult females produce on average 5-12 eggs per day on tomato, which develop in reproducing adults themselves within a period of 2 weeks (Sarmento *et al.*, 2011; Alba *et al.*, submitted). Similar to eriophyoid mites, spider mites possess an arrhenotokous parthenogenic mode of reproduction but males transfer sperm directly to the female instead of using spermatophores and under favourable conditions mite populations increase exponentially to very high densities overexploiting the host.

Spider mites produce silken web, which at high mite densities covers the leaf surface and protects them from predation (Lemos *et al.*, 2010). Spider mites are difficult to control chemically because they quickly develop resistance to acaricides (Van Leeuwen *et al.*, 2012). Biological control is applied successfully in most crops for *T. urticae* using the predatory mite *Phytoseiulus persimilis*, whereas *Phytoseiulus longipes* has been identified as an effective predator of *T. evansi* (Ferrero *et al.*, 2011). Note, however, that in general biological control using predatory mites on tomato remains troublesome because of the trichomes which hinder not only the spider mites, but also the predators (Van Haren *et al.*, 1987; Van Houten *et al.*, 2013).

**Pseudomonas syringae**
The hemibiotrophic pathogen *P. syringae* pv. *tomato* DC3000 is a bacterial plant pathogen that is widely used as a model organism to understand pathogenesis in
plants (reviewed by Xin & He, 2013). *Pseudomonas syringae* causes bacterial speck disease on tomato as well as on *Arabidopsis* (Xin & He, 2013). Symptoms of *P. syringae* infection are visible as small, dark lesions on leaves which sometimes can be surrounded by chlorotic halos (Hirano & Upper, 2000). Bacteria enter the plant through stomata or wounds and multiplication occurs in the apoplast of the leaf. All aerial parts of the plant, such as leaves and fruits, can be infected (Xin & He, 2013). Notably, DC3000 is known to produce the toxin coronatine, which a mimic of the phytohormone JA-isoleucine (JA-Ile). By producing coronatine, *P. syringae* induces JA-dependent defenses thereby repressing SA-dependent defenses to which the bacterium is sensitive (Glazebrook, 2005).

**General outline of this thesis**

Russet mites have an extremely destructive effect on their tomato host, as evidenced by rapid degradation of the trichomes, strong stress responses and mortality of the plant (FIGURE 1.5). Why russet mites, unlike spider mites, cause these severe disease-like symptoms and what the consequences are for the mites and its community is not known. A great deal of the experiments described in this thesis were performed with the aim to shed light on this question.

First, in CHAPTER 2, tomato defense responses induced by russet mites are described and compared with those induced by the spider mite *T. urticae*, as they have been well characterized for the latter species. Second, in CHAPTER 3, the results of performance experiments are presented that were aimed at revealing the effectiveness of tomato's inducible defense system against russet mites. These experiments were done using mutants that are impaired in the JA- and SA-signaling pathway. In addition, the consequences of russet mite-induced responses for the performance of the spider mite *T. urticae*, and for the bacterial phytopathogen, *P. syringae*, are described here, as well as the reciprocal effects of spider mites and bacteria on russet mite growth.

Subsequently, in CHAPTER 4 transcriptome-wide responses induced by russet mites are compared with those induced by *T. urticae* and *T. evansi*, which have been identified as, respectively, ‘inducers’ and ‘suppressors’ of plant defenses. Then, in CHAPTER 5, a description is given of the degradation of glandular trichomes that is associated with russet mite herbivory as well as the consequences this has for biological control of the russet mite. Finally, the switch is made from the ‘ask-the-plant’ to the ‘ask-the-mite’ approach and a brief overview is presented of some results that have been obtained from sequencing the russet mite's genome and transcriptome. From the sequencing data, a list has been compiled of putative secreted proteins, of which some could function as elicitors and/or effectors of plant defense responses. These results are discussed in the general discussion (CHAPTER 6) in the
context of the other findings described in this thesis. In this chapter, I also discuss the implications of my findings as well as some possible directions for future research.

References


Alba, J.M., Schimmel, B.C.J., Glas, J.J., Pappas, M.L., Schuurink, R.C., Sabelis, M.W. & Kant, M.R. Spider mites suppress both jasmonate and salicylate defenses to their benefit but this may backfire when living in communities. Submitted.


CHAPTER 1 | GENERAL INTRODUCTION


cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15, 760-770.


