Plant Science 252 (2016) 300–310

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Induced plant-defenses suppress herbivore reproduction but also constrain predation of their offspring

Livia M.S. Ataide[a,b], Maria L. Pappas[c], Bernardus C.J. Schimmel[a], Antonio Lopez-Orenes[a], Juan M. Alba[a], Marcus V.A. Duarte[b], Angelo Pallini[b], Robert C. Schuurink[d], Merijn R. Kant[a,*]

[a] *Department of Population Biology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands*
[b] *Department of Entomology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil*
[c] *Department of Agricultural Development, Laboratory of Agricultural Entomology and Zoology, Democritus University of Thrace, Pantazidou 193, 68 200, Orestiada, Greece*
[d] *Department of Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, The Netherlands*

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 22 July 2016
Accepted 8 August 2016
Available online 21 August 2016

Keywords:
–Tritrophic interaction
Jasmonates
Tetranychus
Phytoseiulus longipes

ABSTRACT

Inducible anti-herbivore defenses in plants are predominantly regulated by jasmonic acid (JA). On tomato plants, most genotypes of the herbivorous generalist spider mite *Tetranychus urticae* induce JA defenses and perform poorly on it, whereas the Solanaceae specialist *Tetranychus evansi*, who suppresses JA defenses, performs well on it. We asked to which extent these spider mites and the predatory mite *Phytoseiulus longipes* preying on these spider mites eggs are affected by induced JA-defenses. By artificially inducing the JA-response of the tomato JA-biosynthesis mutant *def-1* using exogenous JA and isoleucine (Ile), we first established the relationship between endogenous JA-Ile-levels and the reproductive performance of spider mites. For both mite species we observed that they produced more eggs when levels of JA-Ile were low. Subsequently, we allowed predatory mites to prey on spider mite-eggs derived from wild-type tomato plants, *def-1* and JA-Ile-treated *def-1* and observed that they preferred, and consumed more, eggs produced on tomato plants with weak JA defenses. However, predatory mite oviposition was similar across treatments. Our results show that induced JA-responses negatively affect spider mite performance, but positively affect the survival of their offspring by constraining egg-predation.

1. Introduction

Plants are constantly attacked by herbivores and have evolved a rich palette of traits associated with resistance, such as mechanical barriers, toxins and feeding deterrents to defend themselves against these attackers [1]. Many of the defense responses that enable plants to resist herbivores are inducible probably because displaying them constitutively would be energetically more costly and may cause auto-toxicity [2]. Moreover, induced defenses allow for a certain degree of tailoring of resistance to specific attackers [3]. Constitutive and induced defenses can operate directly or indirectly. While the direct defenses are established via toxins, deterrents or structural barriers, the indirect defenses require recruitment by the plant of an herbivore's natural enemy [2,4]. Together direct and indirect defenses make up the toolbox plants can use to combat herbivores, while the vulnerability of the herbivores to these defenses determine the extent to which plants can resist them. However, there is a potential conflict looming since direct defenses may not only decrease the performance of herbivores, but also of natural enemies that prey on these herbivores, and thereby ingest the same defensive substances [5–7].

Tomato plants are relatively hostile hosts for herbivores, where constitutive and inducible defenses have been documented in detail [8,9]. The inducible defensive system is orchestrated by several phytohormones of which jasmonic acid (JA) is a central player, given the fact that JA-biosynthesis or JA-perception mutants are highly vulnerable to herbivores and biotrophic pathogens [10,11]. For instance, the tomato JA biosynthesis-mutant *defenseless-1* (*def-1*) [10] is highly vulnerable to the larva of *Manduca sexta* [10] and *Spodoptera exigua* [12] and the spider mite *Tetranychus urticae*

---

[*] Corresponding author at: P.O. Box 94240, 1090, GE Amsterdam, The Netherlands.
E-mail address: m.kant@uva.nl (M.R. Kant).

http://dx.doi.org/10.1016/j.plantsci.2016.08.004

[13,14], while 35S:Prosystemin tomato, a transgenic plant primed to display exceptionally strong JA-defenses, is more resistant to herbivore attack [15]. In fact, not JA itself, but its isoleucine (Ile) conjugate, i.e. JA-Ile, binds to the receptors COI1 and JAZ protein and is the bioactive jasmonate responsible for the expression of defense genes [16,17] and concomitant downstream metabolic changes. Some of these defense genes are commonly used as JA-markers, i.e. as indicators of activated JA-defenses. In tomato, wound-induced proteinase inhibitors (PI), such as *PI-IIf*, are commonly used as JA-markers [13,14,18–21].

Tomato plants that may have a relatively high degree of disease and pest resistance yet suffer from several adapted pests and pathogens [22,23]. Among these pests are several species of herbivorous mites such the spider mites *Tetranychus urticae* and *Tetranychus evansi.* Performance of these mites on tomato is negatively correlated with PI transcript accumulation levels or enzyme activity [14,15,24] and with the presence of the terpene 7-epizingiberene [25] and methyl ketones [26]. In tomato PI-activity and 7-epizingiberene production can be induced by JA [13,14,27]. However, while these defenses may decrease the reproductive performance of maladapted spider mites significantly, they do often not prevent them from building a population [15]. In addition, spider mites were found to adapt to these defenses as well. Two distinct types of adaptations to tomato JA-defenses have been reported. The first type is resistance: although most populations of *T. urticae* initially perform poorly on tomato, they were found to often improve their reproductive performance when propagated on this plant for several generations [28,29]. The second type is suppression: some *T. urticae* genotypes can suppress inducible JA-defenses to intermediate levels [15,21], while *T. evansi* suppresses these defenses down to, [21] or below [24], the levels of uninfested plants. The fact that such adaptations appear to be relatively common in natural populations of *T. urticae* and *T. evansi* [21] suggests that JA-defenses pose a constant selective pressure on these mites.

Under laboratory conditions, JA-sensitive mites were found to benefit from residing on the same leaf as JA-suppressing mites [15,21] implying that defense suppression may backfire within herbivore communities by facilitating competitors. This notion is reinforced by the observation that suppressing JA-defenses was only beneficial to JA-defense sensitive mites when these could monopolize their feeding site [21,30]. These observations warrant to further explore the circumstances under which defense suppression is or is not beneficial for herbivores. It is well known that ingested plant defense compounds sometimes protect herbivores against their natural enemies [5,6,31,32]. This suggests that inducing defenses may actually be beneficial to resistant herbivores, since it may constrain predation pressure, while suppressing defenses may promote it. If so, we would expect the inducer spider mite *T. urticae* to experience less predation pressure than its competitor *T. evansi* that suppresses defenses. However, the opposite has been observed: most of the predators used in biological control of spider mites, such as the phytoseiids *Phytoseiulus persimilis* [33,34], *Neoseiulus californicus* [34], *Phytoseiulus macropilis* [35] and *Phytoseiulus fragariae* [36] are not efficient in controlling *T. evansi*, although yet another species, *Phytoseiulus longipes*, may be suitable for this purpose [37,38]. The reasons why *T. evansi* seems unpalatable to most predators are not known [39]. In addition, suppression of defenses may also impair the attraction of natural enemies and result in enemy free space, although the predators *P. macropilis* and *P. longipes* were found to prefer the odors of *T. evansi*-infested plants over uninfested controls despite suppression of several induced plant volatiles [24]. Taken together, much is still unclear about the costs and benefits of defense suppression and how this trait emerges and persists in natural populations.

We asked to which extent suppression of JA-defenses by spider mite *T. evansi* would affect performance of the mite itself, as well as predation by the predatory mite *P. longipes*. First, we manually induced the JA-response of the tomato JA-biosynthesis mutant *def-1* using exogenous JA + isoleucine (Ile) and used this assay to assess the relationship between *T. evansi* performance and the magnitude of the JA-defenses. Then, we tested to which extent these defenses modulate egg predation, since *P. longipes* rarely attacks adult spider mites and prefer to eat their eggs [40], in terms of prey-egg preference, prey-egg consumption rate and predatory mite oviposition. These same tests we performed using the eggs of a JA-defense inducer strain of *T. urticae* [15,21,30], as a benchmark for the impact of induction.

2. Materials and methods

2.1. Plants

Bean (*Phaseolus vulgaris* cv. Speedy), wild type tomato *Solanum lycopersicum* cv. Castlemart and mutant *def-1* (also in the cv. Castlemart genetic background [10]) plants were grown in a greenhouse with day/night temperatures of 18–25 °C, a 16:8 h (light: dark) regime and 50–60% relative humidity (RH). Cucumber plants (*Cucumis sativa* L. cv. Ventura) were grown in a climate room at 25 °C, 16:8 h (light:dark) and 60% RH (default settings). Bean and cucumber leaves were only used for propagating mites and tomato plants were used for experiments when they were 28 days old. Two days prior to the start of an experiment, all tomato plants were transferred to a climate room where the experiments were carried out.

2.2. Spider mites

The *T. urticae* strain used for this study was obtained from a natural population collected from spindle tree (*Euonymus europaeus*) in the Netherlands, referred to as “KMB” in Kant et al. [15] and as “Santpoort 2” in Alba et al. [21] in which it is described in detail. The *T. evansi* strain used for this study was originally collected from tomato plants in Brazil, referred to as *T. evansi* in Sarmento et al. [24] and as Viçosa-1 in Alba et al. [21] in which it and its interaction with tomato are described in detail. Both species were propagated on detached leaves (i.e. bean leaves for *T. urticae* and tomato leaves for *T. evansi*) laying flat on wet cotton wool inside plastic trays in a climate room. Fresh leaves were provided 3 times per week.

For all experiments we used adult female mites; 3 ± 1 days since their final molt. To obtain them we transferred adult females of *T. urticae* or *T. evansi* to detached bean leaves on wet cotton wool. After 48 h these adult females were removed and their eggs were left to hatch and mature to adulthood in a climate room. Since eggs to adult take about 14 days, we transferred the females to the bean leaves 17 days before the start of the experiments.

2.3. Predatory mites

The base colony of the predatory mite *P. longipes* had been provided by Koppert Biological Systems (Berkel and Rodenrijs, the Netherlands) in 2011 and had been maintained in a climate room on detached cucumber leaves infested with *T. urticae*. For the experiments, cohorts of predatory mite eggs were obtained by placing adult females on detached cucumber leaves (placed flat on wet cotton wool) and allowing them to produce eggs for 24 h. Subsequently, the adults were removed and the eggs were allowed to mature on these leaves under the same conditions as the base colony. From this cohort, adult predatory mite females were used when they were 8–9 days old since egg stage, i. e. 2 ± 1 days since their final molt.