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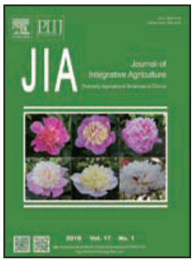

RESEARCH ARTICLE

The influence of *Tetranychus cinnabarinus*-induced plant defense responses on *Aphis gossypii* development

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Abstract
Carmine spider mites (*Tetranychus cinnabarinus*) and cotton aphids (*Aphis gossypii*) are both serious pests of cotton, and cause reductions in yields of this key agricultural crop. In order to gain insights into how plant defense responses induced by one herbivore species affect the behavior and performance of another, we examined how infestation with *T. cinnabarinus* influences the development of *A. gossypii* using cotton as a model. In this study, we measured the activities of several important biochemical markers and secondary metabolites in the leaves of cotton seedlings responding to infestation by *T. cinnabarinus*. Furthermore, the influences of *T. cinnabarinus* infestation on the development of *A. gossypii* in cotton were also examined. Our data showed that the activities of several key defense enzymes, including phenylalanine ammonia-lyase (PAL), peroxidase (POD), lipoxygenase (LOX), and polyphenol oxidase (PPO), were substantially increased in cotton seedlings responding to spider mite infestation. Further, the contents of gossypol and condensed tannins, key defensive compounds, were significantly enhanced in leaves of cotton seedlings following *T. cinnabarinus* infestation. Moreover, the *T. cinnabarinus*-induced production of defense enzymes and secondary metabolites was correlated with infestation density. The developmental periods of *A. gossypii* on cotton seedling leaves infested with *T. cinnabarinus* at densities of 10 and 15 individuals cm^{-2} were 1.16 and 1.18 times that of control, respectively. Meanwhile, the mean relative growth rates of *A. gossypii* on cotton leaves infested with *T. cinnabarinus* at densities of 8, 10 and 15 individuals cm^{-2} were significantly reduced. Therefore, these data suggested that the developmental periods of *A. gossypii* were significantly lengthened and the mean relative growth rates were markedly reduced when cotton aphids were reared on plants infested with high densities of spider mites. This research sheds light on the role that inducible defense responses played in plant-mediated interspecific interactions between *T. cinnabarinus* and *A. gossypii*.

Keywords: inducible defense, cotton, *Tetranychus cinnabarinus*, *Aphis gossypii*, interaction

1. Introduction

Inducible defense mechanism is an important strategy used by plants to counter the damaging effects of herbivorous invaders. The biochemical and molecular mechanisms used to defend against the herbivores are highly dynamic, and are classified as either direct or indirect, based on how they affect the herbivores. Direct defense responses influ-

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ence the herbivore by directly interacting with its biological processes. For example, morphological structures, such as plant cell walls, trichomes, and waxes form physical obstacles that directly affect the abilities of insects to feed (War *et al*. 2012). In addition to physical obstructions, plants also produce secondary metabolites and toxic, defensive proteins that act to reduce the palatability and digestibility of plants to their invaders. In contrast, indirect defense mechanisms enact influence over herbivores by promotion and/or attraction of their natural enemies (Dicke *et al*. 1999). Inducible direct defense responses are often initiated by damage to the plant, resulting in the induction and production of a range of secondary metabolites and defense-related enzymes that disrupt nutrient uptake by herbivores. These include inhibitors of insect digestive enzymes, proteases, lectins, amino acid deaminases and oxidases, as well as protective proteins that adversely affect herbivore physiology and metabolism (Chen *et al*. 2009). Inducible indirect defensive responses usually involve the release of plant volatiles that function to attract natural enemies of herbivores (Sabelis *et al*. 1999).

Phenylalanine ammonia-lyases (PALs), lipoxygenases (LOXs), polyphenol oxidases (PPOs), and peroxidases (PODs) are important biochemical markers for plant defense responses to herbivore infestation (Han *et al*. 2009). PAL is a key enzyme in the phenylpropanoid pathway, which is an important secondary metabolic pathway in higher plants (Koukol and Conn 1961; Karban and Myers 1989; MacDonald and D'Cunha 2007). LOXs, PPOs, and PODs also play important roles in plant defense against pests (Heitz *et al*. 1997; Chen *et al*. 2000; Chaman *et al*. 2001). LOXs are dioxygenase that catalyze the hydroperoxidation of polyunsaturated fatty acids forming fatty acid hydroperoxides that are degraded into unstable, highly reactive, toxic products. Further, the products catalyzed by LOX activity themselves, such as trauma hormones including the traumatin and jasmonic acid, may act as direct deterrents in response to mechanical trauma, pathogenesis and pests (Qin and Gao 2005; Sha *et al*. 2015). PPOs are plant metallo-enzymes that catalyze the formation of quinones from various phenolic precursors, leading to the formation of black or brown pigments that affect the quality and nutritional value of the plants under attack (Mayer 1987; Sha *et al*. 2015). PODs are monomeric hemoproteins involved in the synthesis of lignin and suberin, which form the main physical barriers against herbivore invasion (Ralph *et al*. 2004). Several studies have shown that plants increased the production of these enzymes in response to infestation with spider mites and other insects (Qin *et al*. 2005; Spence *et al*. 2007; Duan *et al*. 2012; Świątek *et al*. 2014; Sha *et al*. 2015).

Gossypol, a dimeric sesquiterpenoid aldehyde, and condensed tannins are the two key secondary metabolites that function as defensive compounds against herbivores due to their cytotoxicity (Williams *et al*. 2011; Zhou *et al*. 2013). The gossypol gland is responsible for gossypol biosynthesis and secretion, and both gland density and terpenoid content have been shown to be increased in young and developing leaves by herbivore attacking (Hagenbucher *et al*. 2013a). Condensed tannin content was dramatically increased in *Lygus lucorum*-infested cotton, thereby enhancing *L. lucorum* resistance (Liu *et al*. 2013).

Tetranychus cinnabarinus (Boisduval) (Acarina: Tetranychidae) and *Aphis gossypii* (Glover) (Homoptera: Aphididae) are both economically important pests that attack cotton seedlings, sharing the same ecological niche. Interestingly, in cases where herbivores share the same niche, automatic competition often occurs (Inbar *et al*. 1999b). Further, many studies have shown that plant-mediated interactions among herbivores plays an important role in interspecies competition. Changes in expression of defense enzymes and secondary metabolites induced by herbivory not only affect the herbivores that induce the original response, but also those in proximity sharing the same ecological niche (Inbar *et al*. 1999a). For example, jasmonate and other defensive metabolites were increased in caterpillar-damaged *Asclepias syriaca,* leading to a 50% decrease in milkweed aphid (*Aphis nerii*) population (Ali *et al*. 2014). Further, the population density and larval survival of *Liriomyza trifolii* was reduced by 41 and 26.5% in *Bemisia argentifolii*-infested tomato plants, respectively (Inbar *et al*. 1999b). During the course of our work, we observed that populations of *A. gossypii* were significantly reduced in *T. cinnabarinus*-infested cotton seedlings when compared to the uninfested controls. Further, we propose that this phenomenon may be attributed to the *T. cinnabarinus*-induced production of defensive enzymes and compounds in cotton seedlings.

2. Materials and methods

2.1. Plant and insect growth conditions

Cotton plants were grown in plastic pots filled with vermiculite and nutrient soil mix in a climate controlled growth chamber (16 h L:8 h D, (70–80)% RH, (28±2)°C). Thirty-day-old cotton plants with 4 true leaves were used for all experiments. *T. cinnabarinus* and *A. gossypii* were collected from cotton fields maintained at China Agricultural University and reared separately in a climate controlled growth chambers (16 h L:8 h D, (50–60)% RH, (28±2)°C) on mung bean and cotton seedlings, respectively.

2.2. Reagents

L-Phenylalanine, catechol, and guaiacol were purchased