Effects of exogenous Jasmonic acid and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of salicylic acid, on the egg production of a herbivorous mite *Tetranychus urticae* (Acari: Tetranychidae)

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(Received 7 July 2003; Accepted 19 January 2004)

Abstract

The effects of jasmonic acid (JA) and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of salicylic acid, treatment of lima bean plants on the egg production were investigated in a herbivorous mite, *Tetranychus urticae*. JA and BTH were applied at three concentrations for each treatment to soil in pots containing plants. Only the 0.1 mM JA-treatment significantly reduced the number of eggs laid by *T. urticae* over a three day period, whereas higher (1 mM) and lower (0.01 mM) concentrations did not affect the egg production of *T. urticae*. Fewer eggs were laid by *T. urticae* on lima bean plants treated with 1 mM and 10 mM BTH than with 0.1 mM BTH. These data suggest that JA and BTH are involved in the direct defense of the lima bean plant against *T. urticae*.

Key words: BTH; induced plant resistance; jasmonic acid; *Phaseolus lunatus*; systemic acquired resistance

INTRODUCTION

Jasmonic acid (JA) and salicylic acid (SA) are two endogenous signals implicated in eliciting plant resistance to herbivory. JA plays an important role in both herbivore resistance and pathogen resistance (Creelman and Mullet, 1997; Wasternack and Parthier, 1997). The exogenous application of JA to plants increases their defense against herbivores (Karban and Baldwin, 1997; Baldwin, 1998; Thaler et al., 1999; Omer et al., 2001; Redman et al., 2001). However, JA-treatment to uninfested plants may have a negative effect on plant fitness because the plant invests extra cost for JA-induced defense (Baldwin, 1998). SA is known to act as a signal for systemic acquired resistance in pathogen-infected plants (Ryals et al., 1996). Exogenously applied SA induces the defense genes that are activated systemically upon pathogen infection (Durner et al., 1997). The SA-related signaling pathway is also activated upon treatment with benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of SA (Kunz et al., 1997).

Ozawa et al. (2000) previously reported that

damage of lima bean plants by a spider mite, *Tetranychus urticae*, resulted in the expression of acidic and basic pathogenesis-related (PR) genes that were also induced by methyl salicylate and JA in leaves suggesting that both the SA- and JA-related signaling pathways are induced in response to *T. urticae* damage. They also found that both pathways are involved in the production of *T. urticae*-induced lima bean leaf volatiles that attract carnivorous natural enemies of *T. urticae* (Ozawa et al., 2000). The production of these volatiles is considered to mediate indirect defense of lima bean plants against the spider mites (Dicke et al., 1999). However, the involvement of JA and SA in the direct defense of lima bean leaves against *T. urticae* has not yet been studied except for a recent study by Li et al. (2002) in which they treated methyl-JA to a tomato mutant that was deficient in the biosynthesis of JA and found that the resistance of the treated plant to *T. urticae* feeding was restored and *T. urticae* reduced the egg production. In the present study, we investigated the level of direct defense against *T. urticae* in lima bean plants treated with various concentrations of JA and BTH.

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MATERIALS AND METHODS

Plants and mites. Lima bean (*Phaseolus lunatus*) plants were grown in pots (diameter: 8 cm, depth: 7 cm) containing soil in a greenhouse at $25\pm 2^\circ\text{C}$, 60–70% relative humidity (RH) and a photoperiod of 16 h. We used plants grown for 5–7 days after germination. These plants had two primary leaves and the first trifoliate leaf had just started to unfold. Herbivorous spider mites (*Tetranychus urticae*, green-form) were reared on lima bean plants in a climate-controlled room ($25\pm 2^\circ\text{C}$, 60–70% RH, 16L–8D). Adult females of these mites were used for all experiments.

JA and BTH-treatment of lima bean plants. Twenty milliliters of JA (0.01, 0.1, or 1 mM) or BTH (0.1, 1, or 10 mM) was added with a pipette to the soil in pots (diameter: 8 cm, depth: 7 cm) containing the lima bean plants. The concentrations used in this study were based on our previous report (Ozawa et al., 2000) in which 1 ml of 1 mM JA and SA were treated to a detached leaf. BTH, which is a potent synthetic mimic of SA and is used against several plant pathogens in the field (Thaler et al., 1999), was used in all experiments. Distilled water was used as a control (DW).

To study the effects of exogenous JA or BTH treatment on *T. urticae* activities, we placed one young adult female *T. urticae* randomly picked from the colony was placed on one of the primary leaves of a treated plant one day after treatment. The duration between treatment and inoculation of spider mites (1 day) was based on our previous study (Ozawa et al., 2000). The number of eggs laid was counted three days after inoculation. The

experiments were conducted in a climate-controlled room ($25\pm 2^\circ\text{C}$, 60–70% RH, 16L–8D). The data were analyzed using Scheffe's test.

Chemical analysis. One day after the application of JA to the soil, JA was extracted from the plants by the method of Weber et al. (1997) with slight modification. Dihydrojasmonic acid was used as an internal standard. The levels of JA and dihydrojasmonic acid were determined by GC-MS. GC was performed on a Hewlett-Packard 6890 equipped with an HP-5MS capillary column: i.d., 0.25 mm; length, 30 m; film thickness, 0.25 mm; injection temperature, 250°C . MS was performed on a Hewlett-Packard 5973 mass selective detector at an ionization of 70 eV. The temperature gradient was as follows: 60°C for 1 min, $60\text{--}120^\circ\text{C}$ at 20°min^{-1} , $120\text{--}180^\circ\text{C}$ at 3°min^{-1} , and $180\text{--}300^\circ\text{C}$ at 30°min^{-1} .

RESULTS AND DISCUSSION

In this study, only the 0.1 mM JA-treatments at the three concentrations significantly reduced the number of eggs laid by *T. urticae* over the three days as compared to the control. The remaining treatments had no effect on the fecundity of *T. urticae* (Fig. 1). However, the levels of JA in the lima bean leaves increased with increasing concentrations of JA (Fig. 2). Thus, JA treatment of plants does not always reduce the fecundity of herbivorous mites. It remains unclear as to why only the 0.1 mM treatment reduced the fecundity of *T. urticae*. These results suggest that JA-mediated lima bean defenses against spider mites may operate at an optimal dose response. Li et al. (2002) also re-

Fig. 1. Number of *T. urticae* eggs laid for 3 days on JA-treated plants (mean $\pm$ standard error). Fifty individuals were used as replicates. The letters above each bar indicate significant differences among treatments by Scheffe's test ($p < 0.01$).

Fig. 2. The amounts of JA in exogenously JA-treated lima bean plants (mean $\pm$ standard error). Four individuals were used as replicates. The letters above each bar indicate significant differences among treatments by Scheffe's test ($p < 0.05$).

Fig. 3. Number of *T. urticae* eggs laid for 3 days on BTH-treated plants (mean $\pm$ standard error). Fifty individuals were used as replicates. The letters above each bar indicate significant differences among treatments by Scheffe's test ($p < 0.05$).

ported that the treatment of a tomato mutant deficient in the biosynthesis of JA with methyl-JA restored resistance to *T. urticae* feeding and reduced the fecundity of *T. urticae*.

JA-treatments are usually accomplished by either exposure of plants to methyl jasmonate, or spraying of JA solution on plants (Thaler et al., 1999; Omer et al., 2001; Redman et al., 2001). Our data showed that simply adding JA to the soil is sufficient for accumulation of JA in plants.

When lima bean plants were treated with 1 mM and 10 mM BTH, the number of eggs laid by *T. urticae* declined. However, no significant difference was observed between the two concentrations (Fig. 3). No significant effects were observed upon treatment with 0.1 mM BTH. These results indicate the presence of a threshold between 0.1 mM and 1 mM in BTH-related defenses against *T. urticae*.

BTH treatment was previously reported to induce the SA-related signaling pathway (Kunz et al., 1997). However, BTH-induced plant defense was initially believed to be effective against pathogens but not against herbivorous insects (Thaler et al., 1999; Heil et al., 2000; Inbar et al., 2001). In the present study, we show that the BTH-treatment was as effective against *T. urticae* as JA-treatment.

ACKNOWLEDGEMENTS

This study was supported by CREST of JST (Japan Science and Technology Agency).

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