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Induction of resistance to the whitefly *Trialeurodes vaporariorum* in tomato by external application of Jasmonic Acid (JA) and Benzothiadiazole (BTH).

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Introduction

Tomato cultivation inside the protected environment is threatened by numerous pests, among them the greenhouse whitefly, *Trialeurodes vaporariorum*, is one of the dominating species. In spite of many control measures (chemical, biological and physical methods) recommended and practiced inside greenhouses, still innovative measures need exploration since none of the methods singly can maintain the pest population below threshold level. Induction of resistance by exogenously applied synthetic jasmonic acid (JA) and salicylic acid (SA) analog Benzothiadiazole (BTH) could supplement biological control strategies. However, the response of the hosts as well as pests varies in different systems (Stout *et al.*, 1998). Thus, the influence of JA and BTH application on tomato plants (variety FMTT 260) in relation to its resistance induction against greenhouse whitefly *T. vaporariorum* (Westwood) was studied. Induction of resistance in the tomato plants due to exogenous application of JA and BTH was confirmed by the assessment of activated proteinase inhibitors and peroxidases by the enzyme assays.

Materials and Methods

Behavior and population development of *T. vaporariorum* on induced tomato plants were investigated in greenhouse and climate chamber experiments.

1. Preference of adult insects to either treated or untreated (control) leaves

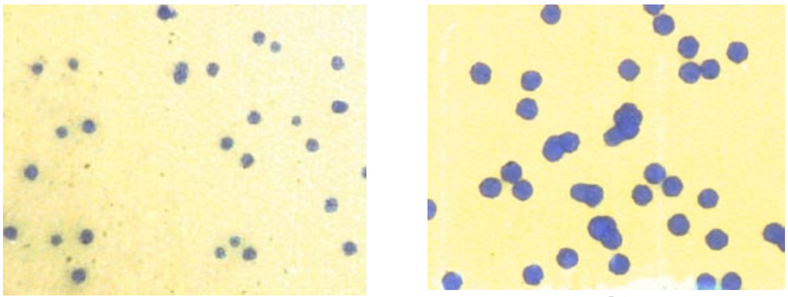
Two days after treatment, the treated (0.75 or 1 mM BTH; 1 or 1.5 mM JA) and control plants were arranged in a pair and the third leaves of each plant were confined inside a clip cage where 11-13 adult insects (irrespective of male or female) were released. The insects settling on either treated or control leaves were counted after 24 and 48 hours of their release. The total of number of eggs deposited on each leaf was counted at the end of experiment.

2. Performance of *T. vaporariorum* on induced tomato plants

2.1 Percentage of the eggs, larvae and pupae that developed to adult stages: The numbers of eggs deposited by ten adult *T. vaporariorum* on the third leaf of tomato plants were counted and before those eggs hatch, the plants were sprayed with 1.5 mM JA, 1 mM BTH or control solution, protecting the third leaf from any contamination due to treatment solutions. Two weeks after the first spray, treatments were repeated and number of eggs that developed to larvae, pupae and finally to adults were counted.

2.2 Fecundity of females developed on induced plants: Females that emerged from treated plants (1.5 mM JA, 1 mM BTH and control) were collected and allowed to feed on similarly treated plants. When the females were 3-5 days old, they were transferred for egg deposition on not treated plants, in order to estimate the fecundity of females. In addition to egg numbers, wing length and abdomen width and length of females were measured.

2.3 Feeding intensity of L1 and late instars: The number of honeydew droplets deposited by larvae was counted to estimate their feeding intensity. Strips of water sensitive papers were placed at the bottom of clip cage adjusted just below the third leaf of the treated plants where the developing larvae were feeding. Each honeydew droplet released by ten L1 and two late larval instars of *T. vaporariorum* while feeding on 1.5 mM JA, 1 mM BTH and control treated plants for six hours was visualized on the yellow paper as a distinct blue spot (**Figure 1**), and counted under a binocular.



(a) (b)

Figure 1: Visualization of blue spots indicating the honeydew droplets released by L1 (a) and late instars (b) of *T. vaporariorum* at 25X magnification

Data Analysis

The dichotomous results from the choice (preference) experiment were fitted into the generalised linear model with logit-link (logit is defined as the natural logarithm of the proportion of insects choosing the treated leaves, divided by the proportion of insects choosing the untreated leaves), and the mean differences between the treatment levels were tested at 5% significance level using Chi-square tests based on the estimates for means and variability on the logit scale. For all other performance experiments, differences between treatments were estimated by running ANOVA at 5% level of significance after appropriate transformation of the data.

Results and Discussion

1. Preference of *T. vaporariorum* on treated or control plants

The proportion of insects residing on treated or untreated (control) leaves evaluated at different times and the numbers of eggs are shown in **Figure 2**. There was no distinct preference between 0.75 mM treated and untreated (control) leaves but significantly more eggs were found on control leaves (p-value 0.04). After 48 hours insects showed preference for control leaves when compared with 1 mM BTH treatment, but there was no observable effect due to 1mM JA application for the preference of *T. vaporariorum*. Yet, adults avoided 1.5 mM JA treated leaves early after release and total number of eggs on treated leaves was significantly lower (p-value 0.026). These results corroborate descriptions of reduced preference of whiteflies for BTH treated plants under choice condition (Correa *et al*., 2005) and of reduced fecundity on treated plants. At the same time, JA also affected the preference and egg deposition by the adult *T. vaporariorum* on induced plants.

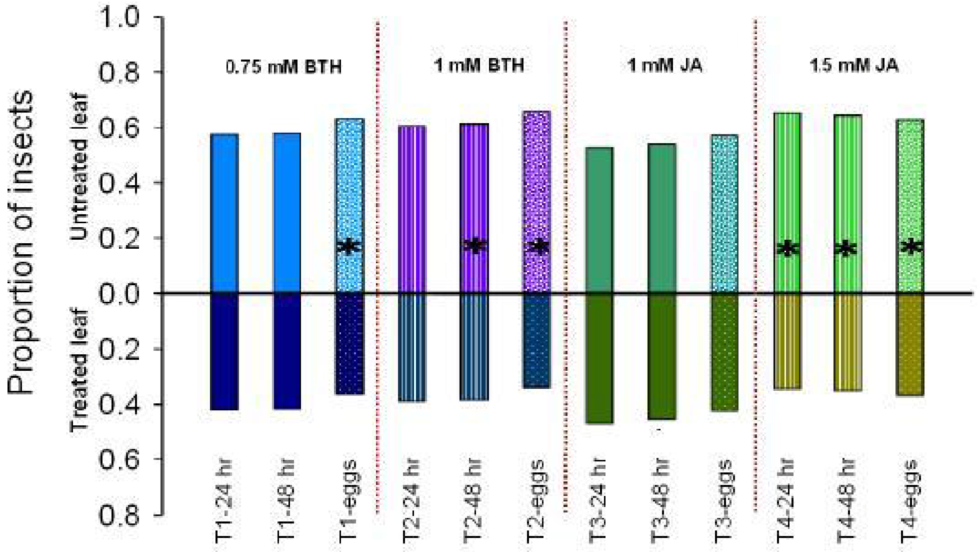


Figure 2: Proportion of *T. vaporariorum* on treated and untreated (control) leaves after 24 and 48 hours of release and eggs counted for different treatments. Asterisk sign (*) on bars indicate significant difference between inductor treatments and corresponding control for choice and egg laying by the female at 5% significance level.

2. Performance of *T. vaporariorum* on induced plants

2.1 Percentage of the eggs, larvae and pupae that developed to adult stages

21.9, 14.25 and 55.4 percent of the total eggs reached the adult stages on JA, BTH and control treated plants respectively (**Figure 3**). ANOVA between treatments reflected the highly significant effect of treatments for the mortality of eggs and subsequent development to the adult stage ($F_{(2, 27)}$-value 89.8; p-value <0.001) at 5% level of significance. Similarly, both of the treatments reduced the percent of larvae that successfully developed to adults when compared to control. However, the development from pupae to adult stage was not significantly affected by the different treatments ($F_{(2, 27)}$-value 2.64; p-value 0.09) at 5% significance level.

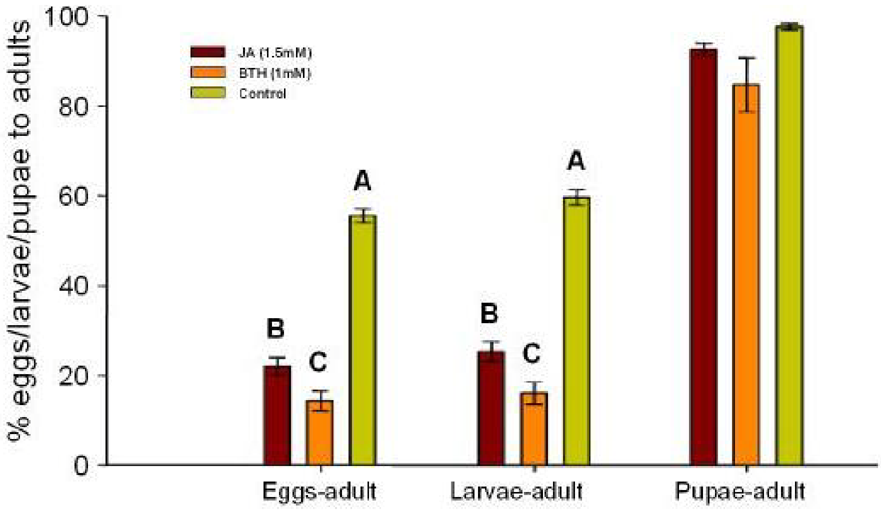


Figure 3: Percent of egg, larvae and pupae that developed to adult stage on JA, BTH and control treated plants. Different letters indicate significant difference between treatments within each group (eggs to adult, larvae to adult or pupae to adult) at 5% level of significance.

The results show that both JA and BTH treatments significantly impair the development of *T. vaporariorum*, since less eggs and larvae (compared to control) reached the adult stage. The unaffected pupal stage led to the speculation of induced antibiotic compounds taken up by the only feeding larval stages. Studies from other authors do not give a consistent picture about the consequences of induced JA and SA pathways against phloem feeding insects. Nombela *et al*., (2005) reported impaired egg hatch and delayed development of *B. tabaci*, preventing the L1 immatures from reaching the second or third developmental stage on BTH treated plants. Whereas, Zarate *et al*., (2007) reported that silver-leaf whiteflies that induce SA-associated genes actually enhance their success on Arabidopsis plants by failing to activate or suppressing the effectual JA-regulated defenses. Thus to clarify the mechanism of both JA and BTH induction processes against phloem feeders needs further investigation.

2.2 Fecundity of females developed on induced plants

Females emerged from BTH treated plants laid significantly fewer eggs (5.25 eggs/female/day) than females from JA (8.04 eggs/female/day) and control (8.23 eggs/female/day) treated plants. Thus, BTH significantly reduced the fecundity of the females compared to control and JA (p-values <0.001) at 5% level of significance. This result is consistent with the avoidance of treated plants by adult *T. vaporariorum* and reduced egg deposition by females on BTH treated plants in the preference experiment. At the same time, BTH application reduced the body size of the females. The reduced fecundity of *T. vaporariorum* due to BTH application might have a strong long term effect for the population development.

2.3 Feeding intensity of L1 and late instars

The counted numbers of honeydew droplets, 176.56, 218.85 and 204.05, released by L1 larvae feeding on plants treated once with JA, BTH and control solution respectively remained insignificant across treatments ($F_{(2, 26)}$-value 1.43; p-value 0.26) at 5% level of significance. Similarly, the difference between treatments for the feeding intensities of late instars larvae under both the single and repeated spraying of treatment solutions were insignificant. The similar feeding intensity of the growing larvae on treated and control plants but the significant differences in developmental parameters as shown above supports the hypothesis of antibiotic effects of induced resistance in tomato plants treated with JA and BTH against *T. vaporariorum.*

Conclusion

Adults of the greenhouse whitefly *T. vaporariorum* refused JA and BTH treated leaves of tomato plants in choice conditions. Moreover females avoided to lay eggs on treated plants. The reduced performance of larvae on treated plants but same feeding intensity strengthened the hypothesis of an antibiosis effect responsible for the reduction of whitefly performance on induced plants. Specially, the reduced fecundity of females developing on BTH treated plants might have long term effects for the population dynamics of *T. vaporariorum*.

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