Effect of Host Plant Resistance on Thrips Development

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Abstract

Thrips (*Frankliniella occidentalis* and *Thrips parvispinus*) are a major pest in pepper cultivation. Control of thrips is difficult because of their polyphagous and cryptic behaviour as well as their increasing resistance to many insecticides. Host plant resistance is therefore urgently needed. We characterized 32 accessions from 4 pepper species for resistance to thrips, resulting in the identification of 9 accessions with contrasting levels of resistance (highly resistant, medium resistant and susceptible). Adult and pre-adult survival, developmental time and reproduction rate were assessed. Our results show that resistance had no effect on adult survival, but that oviposition rate and larval mortality are significantly affected. In the resistant accessions the development of thrips larvae was blocked. Using gas chromatography – mass spectrometry, we could identify several compounds that correlate with the level of resistance to thrips. Some of them have already been shown to have an effect on insects. Also, some specific secondary metabolites were shown to be induced by thrips infestation.

Keyword: *antibiosis, larval mortality, oviposition, solanaceae, secondary metabolites*

Introduction

Pepper (*Capsicum*) is one of the most produced vegetables in the world based on data released by the World Food and Agriculture Organization (FAO, 2012). Unfortunately the production of pepper both in the greenhouse and in the field is still constrained by the high infestation of insect pest in which thrips (*Frankliniella occidentalis*) are among the most damaging, both in greenhouse and field cultivation (Siemonsma & Piluek, 1994). *F. occidentalis* can cause large losses in pepper production through direct damage by feeding on leaves and fruit and indirect damage by transferring viruses (Jones, 2005). Thrips control is difficult because of their polyphagous nature, high reproductive rate, their facultative parthenogenic mode of reproduction, their ability to develop resistance to pesticides, and their cryptic habit: larvae hide in closed bud and pupate in soil (Jensen, 2000; Bielza, 2008).

Therefore resistance to thrips is a desirable trait in pepper. However, while resistant accessions have been found (Fery & Schalk, 1991; Maris et al., 2004; Maharijaya et al., 2011), not much is known about the mechanism of resistance to thrips in pepper. The objective of our study was to unravel possible mechanisms of thrips resistance in pepper. Preference, adult and pre-adult survival, developmental time, and reproduction rate were assessed. We also explored the relationship between some morphological characters and metabolites content of the leaves to thrips resistance.

Materials and methods

Plant and thrips

Three highly resistant, three medium resistant and three susceptible (*C. chinense* PI281428, *C. chinense* PI315023, *C. chinense* no 4661) accessions were chosen based on the results of a previous screening for thrips resistance (Maharijaya et al., 2011). An F_2 population consisting of 196 F_2 plants was developed from a cross between *C. annuum* AC 1979 as female parent and *C. chinense*

4661 as male parent. Thrips was reared on the susceptible Chrysanthemum cultivar Spoetnik® (Fides, De Lier, the Netherlands) in a growth chamber at 25°C, 16/8 hr day/night, and 70% relative humidity. Thrips larvae (L1 stage) were obtained by allowing female thrips to lay eggs in small cucumber fruits for one day, after which the adult thrips were brushed off and fruits were kept at 25°C for four days, when the new larvae emerged.

Resistance test

Five newly emerged *F. occidentalis* L1 larvae were placed on a single fresh fully opened leaf that was placed with the abaxial side downwards in a sterile 50 x 9 mm petri dish with lid (BD Falcon®). Leaves and larvae were incubated in a climate chamber at 25°C, 16 h light, 70% RH. Damage caused by larvae was scored after two days using a visual scale ranging from 0 (no damage) to 3 (severe damage) as described in Maharijaya (2011). Survival of L1 larvae into the L2 stage was assessed by counting the number of L2 larvae and dividing this by the total number of larvae placed on the leaf.

Preference test

A preference test was conducted through choice test in a petridish system. 2 leaf discs (∅ 4cm) of resistant and 2 leaf disc of susceptible accessions were placed in opposite direction with abaxial side upward in a petridish with agar (15 g/l agar). Five female thrips were placed in the middle of the petridish covered with air permeable plastic (Fresh Cling®). Number of female thrips stay on each leaf was counted after 15 and 30 minutes carefully.

Developmental and reproduction test

The effects of resistance factor in pepper to the developmental stages of thrips were assessed by adult survival test and developmental study. Adult survival was studied by placing 10 females on a single leaf disc taken from new fully opened leaf using a leaf punch (∅ 4cm) that was placed with the abaxial side downwards on 1.5 % agar in a Petri dish, covered with air permeable plastic (Fresh Cling®). After four days the numbers of living and dead females were counted. Thrips development was studied by placing one individual synchronized L1 (first instar) larva on a leaf disc (∅ 4cm). Sixty leaf discs were used for each accession. The number of individuals developing through successive developmental stages was determined by daily observation. For reproduction rate ten females were placed on a single leaf disc (∅ 4cm). After allowing 24 hours for oviposition all females were removed. Every day the newly emerged larvae were counted under a stereo microscope and removed.

Morphological characters test

Leaf color, trichome density (hairiness), cuticula thickness, and leaf toughness were assessed in F_2 population. New fully opened leaves were used and subjective scoring from 0 to 3 was applied for each character except for cuticula thickness. For cuticula thickness the leaf blade was inserted in polystyrene foam and excised as thin as possible with a razor. The sections were put on a glass slide and coloured with 0.1% safranin in glycerin-water (1:1) and then covered. The sections were observed and cuticle thickness was measured under a binocular microscope equipped with a micrometer. Correlation analysis was done for each morphological character versus resistance scores.

Metabolites test

Metabolite content of the pepper leaves were measured using Gas-chromatography-mass-spectrometry (GC-MS). To select candidate metabolite compounds related to thrips resistance, Pearson correlation analysis followed by False Discovery Rate correction were applied.

Result and Discussion

Antibiosis is the dominant resistance mechanism in pepper

In this study we found no significant difference between the number of female adult of *F. occidentalis* on leaves of resistant and susceptible accessions. This indicates that preference (antixenosis) might not influence much in resistance mechanism against thrips in pepper. This is supported by previous study showing that there is highly significant correlation between damage score of thrips on pepper leaves in a non-choice (leaf assay) versus choice situation (greenhouse test) (Maharijaya et al., 2011).

Our study showed that resistance factor in pepper strongly affected the development of thrips from larvae to pupae as well as reproduction rate. There was a clear and significant difference in survival of larva reared on leaves of resistant, medium resistant and susceptible accessions. The F2 population also showed a high correlation between damage caused by larvae with the survival of larva. The resistance factor in pepper also affect the oviposition rate as shown as the less number of egg produced by female adult thrips reared on resistant accessions. Because the resistance factors in leaves can affect strongly the biology of thrips i.e. survival of the larvae and suppress reproduction rate; it is more likely that the resistance mechanism is antibiosis (Smith, 1989).

No effect of leaf color, hairiness, cuticula thickness and toughness to resistance against thrips in pepper

Any leaf characters that interfere with suppression to thrips life-cycle and reproduction are potential resistance factors which may contribute to mechanism of defense against thrips. Some mechanisms of defense against insect in pepper have been reported such as tolerance to *F. Occidentalis* (Fery & Schalk, 1991), trichomes against *Scritothrips dorsalis* (Yadwad et al., 2008), against whitefly (Firdaus et al., 2011) and against aphid (Bosland & Ellington, 1996). However, we found no evidence for a role of morphological characters in resistance since we found no significant correlation for those morphological characters with resistance level to thrips in this study.

Metabolites correlated with resistance in pepper

In our study, we detected several metabolites that significantly correlated with level of resistance in pepper. Unfortunately, some of these metabolites could not be tentatively annotated. Some of these detected metabolites have been reported earlier to be involved in insect resistance in several crop species. This finding suggested the possible role of metabolites in the resistance mechanism against thrips in pepper. The identification of metabolic compounds with a relationship to thrips resistance in pepper may help to elucidate the resistance mechanism and to identify the genes involved.

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