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Host Plant Preference Study for *Ceratothripoides claratris* (Shumsher) (Thysanoptera: Thripidae) and CaCV (Genus *Tospovirus*; Family Bunyaviridae) in Bangkok, Thailand

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ABSTRACT

Host plant preference of *Ceratothripoides claratris* and Capsicum chlorosis virus (CaCV) were conducted in Bangkok, Thailand. More information about the host plants of CaCV and its thrips vector is needed, which could help working out cultural control measures that might hinder spread of the virus and pest population growth. The potential relevance of the results for crop rotation approaches that would reduce tospovirus epidemics is discussed and common pea is suggested to be integrated in crop rotation. Fourteen crop plants were potted and placed in a greenhouse, which was covered with non-thrips proof net to allow natural infestation. Thereafter, the number of adult thrips and larvae was counted in order to determine host preference. The susceptibility of plants to CaCV was tested by ELISA. *Ceratothripoides claratris* was the predominant thrips species that infested all offered plant species except for angled luffa and common pea. As scarce other thrips species were recorded, the high numbers of larvae were assumed to be *Ceratothripoides claratris* larval stages. *Ceratothripoides claratris* had a clear and significant preference to hosts in the families Solanaceae and Cucurbitaceae. Tospovirus could be detected in tomato, pepper and tobacco, which all belong to the family of Solanaceae.

Key words: Thrips, tospovirus, solanaceae, crop rotation, common pea

INTRODUCTION

Herbivorous thrips are of economic interest, as some species damage their host plants by piercing and sucking in parenchyma cell contents, which leads to substantial yield losses (Premachandra *et al.*, 2005a). Vegetable, legume and ornamental plants are preferred host groups (Pappu *et al.*, 2009). In Bangkok, Thailand, *Ceratothripoides claratris* Shumsher (Thysanoptera: Thripidae) is the predominant pest species on tomatoes *Lycopersicon* sp. (Solanaceae) both in fields and in greenhouses (Murai *et al.*, 2000; Rodmui, 2002; Premachandra *et al.*, 2004). Premachandra *et al.* (2004) showed that *C. claratris* is well adapted to high temperatures (i.e., 30-35°C) within greenhouses in the tropics.

Besides the direct damage caused by feeding, a few thrips species transmit tospoviruses (Family Bunyaviridae) that cause serious epidemics. With estimated global yield losses of up to US \$ 1 billion in a wide range of crops, tospoviruses are considered as the most aggressive emerging plant viruses (Naidu, 2007). The thrips/tospovirus relationship is very specific as only a few of the many known thrips species are able to acquire and transmit the virus (Adkins *et al.*, 2005). Ten

thrips species are accepted and confirmed vectors for tospovirus under experimental and natural conditions (Jones, 2005; Pappu *et al.*, 2009). So far, there are 19 distinct tospovirus species (Pappu *et al.*, 2009). Premachandra *et al.* (2005a) demonstrated that *C. claratris* is a vector of *Capsicum chlorosis* virus (isolate AIT) (CaCV-AIT) on tomato. For the survival of the pathogen it is essential that the vector lays its eggs on the CaCV-infected plants. The immobile first instar larvae acquire the virus and start to transmit as adults. Second instar larvae can transmit but are not that infectious. Thus, only some of the young thrips larvae are able to transmit the tospovirus and mainly as adults. The ability of thrips to acquire the tospovirus is dependent on the thrips developmental stages (Moritz *et al.*, 2004) as well as on the thrips genetics (Halaweh and Poehling, 2009).

The thrips *C. claratris* invaded the greenhouses shortly after transplanting the crop and after lengthy periods of empty greenhouses. That is to say, other crops in the vicinity of the greenhouses could act as an alternative host and hence a reservoir for the thrips/tospovirus complex. Premachandra and Borgemeister (2006) determined the infestation of selected plant species in Thailand and defined tomatoes as the most preferable species for *C. claratris*. Other infested food crops were eggplant, water melon, cucumber and pumpkin. Thus far, however, there is no investigation about preferred host plant species for both *C. claratris* and CaCV.

Thrips are difficult to control due to their short life cycle and a narrow window of time for control possibilities (eggs in leaf tissue, pupation in the soil and resistance against insecticides) (Lewis *et al.*, 1995; Naidu *et al.*, 2006). There are no curative methods for virus infected plants. Preventive control methods therefore are predominantly designed to reduce population growth and spread of the virus. The objective of this investigation is to learn more about the host plants of the vector and the virus and thereby find preventive cultural methods, e.g., crop rotation and intercropping.

This study was part of a larger research project aiming to develop sustainable vegetable production under protected cultivation in the humid tropics.

MATERIALS AND METHODS

Host plants: The experiment was performed at the Asian Institute of Technology (AIT) in greater area of Bangkok (Pathumthani), Thailand, during January February 2006, as part of a collaborative project (Protected cultivation-an approach to sustainable vegetable production in the humid tropics) with the Faculty of Horticulture, Leibniz University of Hannover, Germany. Host plant seeds were sown at different times depending on their germination time in peat compost. To prevent the immigration of pests the plants were kept in a sealed greenhouse. The greenhouse was equipped with an evaporative fan and pad cooling system and was maintained at 28-30°C with a Relative Humidity (RH) of 90-100%. When seedlings were about 20 cm high, thereafter, they were planted coevally in 7 L containers and transferred into an experimental greenhouse where they were placed on one meter high tables. During the experiment a local soil/compost substrate (supplier: Dinwondeekankasat, Ayutthaya, Thailand, soil texture: 30% sand, 39% silt and 31% clay, organic matter: 28%, pH 5.3) was used. The experimental greenhouse was 2×4×0 m and was covered with a non thrips-tight 40-mesh screen (Econet M, Ludvig Svensson Inc., Kinna, Sweden) which allowed natural infestation of thrips from outside. Plants were daily irrigated in the morning with tap water at temperatures of 28-30°C and RH of 70-80%.

Counting of thrips: Specimens of *C. claratris* discovered in the experiments were identified by R. zur Strassen and voucher specimens were deposited at the Senckenberg Museum, Frankfurt, Germany (Premachandra *et al.*, 2005b).

To determine levels of thrips infestation and hence host preference, number of adults and larvae were counted 5 weeks after transplanting in February 2006 (dry season). Whole plants were carefully collected in plastic bags and sealed before transferring them to the laboratory, where the leaves and other plant parts were placed into PVC containers (15×9 cm) containing 70% ethanol, this easily dislodged the insects. After five minutes, the plant materials were discarded, while the liquid was poured through a filter with thrips proof nylon gauze (64-microns open mesh size). The gauze was dried in the air and then examined under a stereomicroscope Olympus SZ30 (Olympus, Japan). Adults of *C. claratris*, *Thrips palmi* Karny (Thysanoptera: Thripidae) and other thrips species were counted. As there is no clear taxonomic key for the classification of larvae their absolute number was recorded. The thrips were determined by morphological characteristics following the key in Jangvitaya (1993).

Virus detection by enzyme-linked immunosorbent assay: The susceptibility of the plants to tospovirus (genus *Tospovirus*; family Bunyaviridae) was tested by DAS-ELISA (Double Antibody Sandwich-Enzyme-linked immunosorbent assay). Earlier experiments showed that it takes about 10-14 days until the virus can be detected in the whole plant (tomato) using DAS-ELISA (Premachandra *et al.*, 2005b). Plants showing symptoms were tested with DAS-ELISA after five weeks, plants without symptoms were tested after nine weeks.

The compound direct ELISA for Watermelon silver mottle virus (WSMoV) and Groundnut bud necrosis virus (GBNV) (AGDIA® Inc., Elkhart, IN, USA. Cat. No. SRA 61500) was used, following the manufacturer's instructions, for detecting CaCV in the plants (Premachandra *et al.*, 2005b). CaCV-Infected leaves of tomato, tobacco and pepper were used as a positive control reference. And, as negative controls, tomato, pepper and melon leaves were used in addition to the sample puffer. The absorbance was carried out at OD₄₀₅ nm (A₄₀₅) using an EL 312 ELISA-reader (BIO-Tek Instruments, Inc, Vermont, USA). The results were evaluated as Rek (1987). To ensure the virus (CaCV), samples of the plant leaflets, infected with CaCV, were tested with PCR using specific primers (Knierim *et al.*, 2006) at the Institute of Plant Diseases and Plant Protection, Hannover University.

Data analysis: The experimental design included five replicates per plant species and within the greenhouse the pots were arranged in a randomized complete block design. Thrips counts were analyzed using Kruskal-Wallis test via the SAS option proc npar1way. If the test result was significant it was followed by Bonferroni (Dunn) test ($p = 0.05$).

RESULTS

Data from the counting of the thrips and the ELISA-test with antibodies to serogroup IV are given in Table 1.

From the total of 653 counted adults Thrips, only 11 *T. palmi* and 5 other unidentified thrips species were counted, which clearly demonstrates the preponderance of *C. claratris* adults over other available thrips species in the experimental area. Almost no *T. palmi* or other thrips species occurred on the offered plants in the greenhouse. In addition, *C. claratris* adults and larvae had a similar distribution; therefore, it is accepted that the larvae counts represent *C. claratris* larvae.

Adults of *C. claratris* had significantly preferred certain hosts among others for feeding ($H = 48.69$, 13 d.f., $p = 0.0001$) as well as breeding, as determined from the larvae counts ($H = 51.11$, 13 d.f., $p = 0.0001$). Adults of *C. claratris* were collected from all tested crop plants

Table 1: Adult and larvae counts of the thrips *Ceratothripoides claratris* and *Thrips palmi* as well as CaCV incidence

Host plant			Thrips species counts (Mean±SD)			
Family	Species	Common name	<i>C. claratris</i>	<i>T. palmi</i>	Larvae	CaCV incidence
Asteraceae	<i>Lactuca sativa</i> L.	Lettuce	0.60±1.34 ^d	0.40±0.55 ^a	5.20±4.32 ^e	0/5
Cucurbitaceae	<i>Cucumis melo</i> L.	Honey melon	18.60±10.33 ^{abcd}	0.00±0.00 ^a	49.00±30.08 ^{abc}	0/5
	<i>Cucumis sativus</i> L.	Cucumber	9.20±14.24 ^{abcd}	0.00±0.00 ^a	23.60±42.83 ^{abc}	0/5
	<i>Cucurbita moschata</i> (Durch.) Poir	Pumpkin	0.80±1.09 ^d	0.40±0.89 ^a	0.40±0.89 ^f	0/2
	<i>Luffa acutangula</i> L.	Angled luffa	0.00±0.00 ^d	0.00±0.00 ^a	1.50±1.73 ^f	1/5
	<i>Momordica charantia</i> L.	Bitter gourd	9.00±4.64 ^{abcd}	0.00±0.00 ^a	22.80±15.97 ^{abc}	0/5
Fabaceae	<i>Phaseolus vulgaris</i> L.	Common bean	2.60±1.95 ^{cd}	0.20±0.45 ^a	11.60±14.43 ^{bc}	0/5
	<i>Pisum sativum</i> L.	Common pea	0.40±0.89 ^d	0.00±0.00 ^a	0.20±0.45 ^f	0/4
	<i>Vigna unguiculata</i> (L.) Walp	Yard long bean	2.60±2.41 ^{cd}	0.00±0.00 ^a	3.80±6.38 ^f	0/5
Solanaceae	<i>Nicotiana tabacum</i>	Tobacco	4.00±3.32 ^{bcd}	0.80±0.84 ^a	0.80±1.30 ^f	3/4
	<i>Solanum melongena</i> L.	Eggplant	27.80±12.07 ^a	0.40±0.89 ^a	86.80±45.71 ^a	0/4
	<i>Capsicum annuum</i> L.	Pepper var. Chili	0.75±0.96 ^d	0.00±0.00 ^a	1.00±2.00 ^f	2/4
	<i>Solanum esculentum</i> L.	Tomato var. FMTT 260	26.00±13.8 ^{ab}	0.00±0.00 ^a	73.20±30.88 ^{ab}	5/5
		var. King Kong 2	25.20±25.19 ^{abc}	0.00±0.00 ^a	59.00±62.90 ^{abc}	5/5

Thrips counts were analysed using Kruskal-Wallis test, followed by Bonferroni (Dunn) test ($p = 0.05$). Means followed by different letters in columns indicate significant difference

except for angled luffa and were very low on pumpkin, common pea, lettuce and pepper Chili. The highest infestation levels of *C. claratris* were on the eggplants and the two tomato varieties. For the larvae, the preferred hosts were similar to those of the adults. Moderate, though not significant, infestation of larvae and adults was found on honey melon, cucumber and bitter gourd.

The presence of CaCV isolate was confirmed by the PCR tests except in the angled luffa specimen (data not shown). CaCV was detected by DAS-ELISA and PCR in three repetitions of tobacco (3/4), two of pepper Chili (2/4) and all ten tomato samples (Table 1). The infested plant species all belong to the family of Solanaceae. Exceptionally one repetition of angled luffa (Cucurbitaceae) had a positive ELISA-result. Hence, susceptibility for tospovirus could not be excluded for this plant species. Noteworthy, some crops, though, were a preferred host of *C. claratris*, it was not a good host of the tospovirus.

DISCUSSION

The results clearly show that *C. claratris* is the predominant thrips species in the greenhouses at the AIT as described by Premachandra *et al.* in 2004. No more than one *T. palmi* on only a few vegetable species could be collected. Previous studies showed that *T. palmi* is not as well adapted to the high temperatures in the tropics and in the greenhouses as *C. claratris* (Murai, 2001; Premachandra *et al.*, 2004).

Premachandra and Borgemeister (2006) already described tomatoes and eggplants as suitable plants for feeding and reproduction of *C. claratris*. From our results, moreover, honey melon, cucumber, common bean and bitter gourd were preferred host plants for *C. claratris*. Lettuce could not be excluded as a suitable host with more than five larvae in average. CaCV systemic infected lettuce has also been described (McMichael *et al.*, 2002).

Despite the high thrips infestation, the host preference of *C. claratris* is actually selective. Almost no thrips specimens were found on pumpkin, angled luffa and common pea. In contrast, Premachandra and Borgemeister (2006) described pumpkin as a good host plant for *C. claratris*.

Common pea had the lowest infestation level with only two adults and one larva on all the five replicates. This investigation, to our knowledge, is the only host plant preference study for *C. claratris* on common pea. Therefore, the common pea could be excluded as a host plant for *C. claratris* and thus may be considered for crop rotation control approaches.

Although, pepper had a very low infestation rate, CaCV could be detected in half of the plants. Pepper has already been described as a host of CaCV (McMichael *et al.*, 2002; Premachandra *et al.*, 2004; Persley *et al.*, 2005). Despite the low infestation by *C. claratris*, the plants were actually infected with CaCV, which shows that vectors can lead to virus epidemics regardless of the size of their population (Kucharek *et al.*, 2000).

All of the tested tomato plants were infected with CaCV. This confirms tomato as the optimal host for both CaCV and its vector *C. claratris* and corroborate results of (Premachandra *et al.*, 2005b). Although the infestation of tobacco by *C. claratris* was low, the tospovirus infection level was high. *Nicotiana* sp. was described as an important host mainly to the tospoviruses (Premachandra *et al.* 2004).

The other plant species were not susceptible to tospovirus. Crops that are resistant to CaCV could be a dead end for the tospovirus (Pappu *et al.*, 2009). In other words, as CaCV has a smaller host range and seems to prefer plants in the family Solanaceae, such important information may be useful when crop rotations are considered in protected cultivation in order to reduce tospovirus epidemics.

CONCLUSION

Although, *C. claratris* has colonised the majority of the offered host crops, it selectively and significantly prefers only a few of them. In contrast to its vector, CaCV has less host range, which was mostly within the Solanaceae family. In protected cultivation crop rotation could reduce thrips population and the spread of the tospovirus. Therefore a rotation with tomato and common pea could be a preventive control method against thrips population growth and the consequent tospovirus spread. Moreover, Crops that are resistant to CaCV, such as cucumber, pumpkin, common bean, common pea or eggplant, could reduce the spread of the tospovirus when included in crop rotations with tomato culture.

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