Biology, population dynamics, vector potential and management of *Ceratothripoides claratris* on tomato in central Thailand

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FOR

My parents

My husband, Chandima

My son Dilina and daughter Suvini

Abstract

Biology, population dynamics, vector potential and management of _Ceratothripoides claratris_ on tomatoes in central Thailand

W.T.S. Dammini Premachandra

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae) is a serious pest species attacking field- and greenhouse-grown tomatoes in Thailand, especially in the greater Bangkok area. Under laboratory and greenhouse conditions, investigations were conducted to study its life cycle, life-fertility, population dynamics, vector competence for Capsicum chlorosis virus (CaCV) (isolate AIT) and susceptibility to the two bio-pesticides neem and spinosad.

In depth life cycle and life-fertility studies demonstrated that temperature has a profound effect on the development, fecundity and longevity of _C. claratris_. As estimated by the modified Logan model, the optimum temperature for egg-to-adult development was 32-33°C. At 30°C, high level of pre-adult survivorship, net reproductive rate (R_0), intrinsic rate of increase (r_m) and shorter mean generation time (G) and doubling time (t) and female biased sex ratios were recorded for _C. claratris_, indicating that it had the best performance at this temperature. At 40°C, both egg development and reproduction of _C. claratris_ were inhibited. _Ceratothripoides claratris_ showed arrhenotokous parthenogenesis. Longest longevity of female and male _C. claratris_ was recorded at 25°C and 30°C, respectively. Both male and female longevity was shortest at 40°C.

On tomatoes leaves were the most preferred plant part for both larvae and adults of *C. claratris*. However, the thrips can also colonise flowers and fruits. Differential sex ratios of adult *C. claratris* were detected on these different plant parts. It was noticeable that *C. claratris* infestation always developed from the bottom to the top of the tomato plants. Substantial fluctuations in larval and adult densities of *C. claratris* were observed on tomatoes over time. As estimated by Taylor's power law this thrips species showed an aggregated distribution on tomato leaves.

Studies on the vector competence of *C. claratris* elucidated that it was highly competent (up to 87%) to transmit CaCV (isolate AIT) to tomatoes. However, the transmission efficiency was influenced by the larval stage at which the virus was acquired. Higher transmission rate in *C. claratris* was observed when the virus was acquired as first instar larvae (L1) compared to second instar larvae (L2). Both adults and L2 had the ability to transmit the virus when acquired as L1. The transmission efficiency of *C. claratris* was not influenced by the sex of the thrips. In net-house trials, virus incidences on tomatoes were initiated by the invasion of *C. claratris* from the outside. In addition, the density of thrips seemed to influence the rate of virus spread inside the net-house.

Two neem products affected *C. claratris* through direct and residual contact toxicity, i.e., NeemAzal-TS® with 1% azadirachtin, as well as systemic activity, i.e., NeemAzal-MD 5 with 5% azadirachtin, by inhibition of moulting of L1 to L2. Maximum mortalities recorded with direct, residual and systemic activities were 76, 100 and 81% at the highest dose rate tested. With 18% a topical application of

NeemAzal-TS® had no strong ovicidal effects on eggs of *C. claratris*; however with 65%, it greatly reduced the survival of L1 emerging from the neem-treated eggs. Reproduction of *C. claratris* was not affected by both neem products. Spinosad caused 100% mortality in all foliar-dwelling stages of *C. claratris*, i.e., L1, L2 and adults, both through direct and residual contact toxicities, indicating that spinosad is more effective against *C. claratris* than neem.

Zusammenfassung

Biologie, Populationsdynamik, Vektorpotential und Kontrolle von *Ceratothripoides claratris* an Tomate in Thailand

W.T.S. Dammini Premachandra

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae) ist ein bedeutender Schädling, der insbesondere in der Umgebung von Bangkok, Thailand, erhebliche Schäden an Feld- und Gewächshaustomaten verursacht. In Labor- und Gewächshausversuchen wurden Untersuchungen zum Entwicklungszyklus, der Fertilität und Populationsdynamik, zur Übertragung des CaCV (isolate AIT) und zur Wirksamkeit der Bio- Pestizide Neem und Spinosad durchgeführt.

Detaillierte Studien zum Entwicklungszyklus und der Fertilität zeigten, daß die Temperatur einen erheblichen Einfluß auf die Entwicklung, Fekundität und Lebensdauer von *C. claratris* hat. Basierend auf Berechnungen des Logan Modells beträgt die optimale Temperatur für die Entwicklung vom Ei bis zum Adulten 32-33°C. Bei einer Temperatur von 30°C wurden für *C. claratris* eine hohe Überlebensrate von prä-adulten Stadien, eine hohe Reproduktionsrate (R_0), spezifische Wachstumsrate (r_m) und eine kürzere mittlere Entwicklungszeit (G) und Verdopplungszeit (t) und ein weiblich dominiertes Geschlechterverhältnis gemessen, was darauf hinweißt, daß der Thrips an diese Temperatur am besten angepaßt ist.

Ab einer Temperatur von 40°C wird sowohl die Eientwicklung als auch die Reproduktion von *C. claratris* gehemmt. *Ceratothripoides claratris* weißt eine arrhenotoke Parthenogenese auf. Die maximale Lebensdauer für weibliche und männliche *C. claratris* wurde bei 25°C bzw. 30°C erreicht. Sowohl weibliche als auch männliche Tiere hatten die kürzeste Lebensdauer bei 40°C.

Sowohl Larven als auch Adulte von *C. claratris* bevorzugen Blätter im Vergleich zu anderen Pflanzenteilen an Tomaten. Der Thrips kann allerdings auch Blüten und Früchte besiedeln. Auf den verschiedenen Pflanzenteilen wurden unterschiedliche Geschlechterverhältnisse der adulten *C. claratris* beobachtet. Auffällig war, daß der Befall von *C. claratris* sich immer vom unteren Teil der Tomatenpflanze zum oberen entwickelte. Im Zeitablauf wurden beträchtlichen Schwankungen in den Dichten von *C. claratris* Larven und Adulten verzeichnet. Basierend auf Berechnungen mit der Taylorschen Verteilungsformel konnte eine aggregierte Verteilung von *C. claratris* auf Tomatenblättern ermittelt werden.

Untersuchungen zum Vektorpotential von *C. claratris* zeigten, daß der Thrips mit bis zu 87% ein hohes Potential hat den Virus CaCV (isolate AIT) auf Tomaten zu übertragen. Die Effizienz der Virusübertragung wurde jedoch stark durch das Larvenstadium beeinflußt in welchem das Virus aufgenommen wurde. Im Vergleich zum zweiten Larvenstadium (L2) wurde eine höhere Übertragungsrate ermittelt wenn der Virus im ersten Larvenstadium (L1) aufgenommen wurde. Sowohl Adulte als auch L2 haben die Fähigkeit zur Virusübertragung, wenn dieser im L1 Stadium aufgenommen wurde. Das Vektorpotential von *C. claratris* wurde

nicht durch das Geschlecht der Thripse beeinflußt. In Netzhausversuchen wurde festgestellt, daß das Auftreten des Virus an Tomatenpflanzen durch einen von außerhalb verursachten Thripsbefall ausgelöst wurde. Auch die Thripsdichte scheint das Ausmaß der Virusausbreitung im Netzhaus zu beeinflussen.

Neem Produkte können *C. claratris* sowohl durch direkten Kontakt und Kontakt mit Rückständen auf behandelten Pflanzenoberflächen (hier NeemAzal-TS® mit 1% Azadirachtin), als auch über systemische Effekte (hier NeemAzal-MD 5 mit 5% Azadirachtin) durch Hemmung der Häutung von L1 zu L2 beeinträchtigen. Bei der höchsten in diesen Versuchen verwendeten Konzentration wurden maximale Mortalitätsraten durch direkte, Rückstands- und systemische Effekten von 76, 100 und 81% verzeichnet. Eine Topikale Applikation von NeemAzal-TS® hatte mit 18% keine starken Effekte auf die Entwicklung der Eier von *C. claratris*. Jedoch wurde die Überlebensrate der aus den mit Neem behandelten Eiern geschlüpften L1 mit 65% stark reduziert. Die Reproduktion wurde durch keines der beiden Neem Produkte beeinflußt. Spinosad bewirkte 100% Mortalität bei oberirdisch aktiven Stadien (d.h. L1, L2 und Adulten) von *C. claratris*, sowohl durch direkten Kontakt als auch durch Kontakt mit den Rückständen. Demnach ist Spinosad gegenüber *C. claratris* deutlich wirksamer als die beiden untersuchten Neempräparate.

Schlagworte: *Ceratothripoides claratris,* Temperatur, Verteilung, tospovirus, bio-pestizide

Contents

Abbreviations

AAP	Acquisition access period
AIT	Asian Institute of Technology
ANOVA	Analysis of variance
CaCV	Capsicum chlorosis virus
d.f.	Degree of freedom
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
F	Statistical F-value
F1	First generation
G	Mean generation time
GBNV	Groundnut bud necrosis virus
g / lw	Grams per litre water
h	Hours
IAP	Inoculation access period
INSV	Impatiens necrotic spot virus
IPM	Integrated pest management
L : D	Relation of light to darkness
L1	First instar larva

L2	Second instar larva
LSD	Least significant difference
min	Minutes
ml / lw	Mili-litres per litre water
P	Statistical probability value
r	Statistical co-relation co-efficient in correlation analysis
R^2	Statistical R^2-value in regression analysis
r_m	Intrinsic rate of increase
R_o	Net reproductive rate
s	Seconds
SAS	Statistical analysis system
SE	Standard error
t	Statistical t-value
t	Doubling time
WAP	Weeks after planting
WPP	Weeks post-planting
WSMV	Watermelon silver mottle virus
Wt/Vol	Weight to volume
λ	Finite rate of increase

1 General introduction

Tomato, *Lycopersicon esculentum* (Mill) (Solanaceae), is native to tropical South America (Taylor, 1986). All tomato varieties in Europe and Asia originated from seeds obtained in South America by Spanish and Portuguese merchants during the 16th century (Villareal, 1978). Thereafter, in less than a century tomato has become a major world food crop (FAO, 2000). It is an important source of lycopene and vitamin C (Madhavi and Salunkhe, 1998). In Thailand, tomatoes are grown in all regions of the country but major production areas are found in the central and north-eastern regions. They are consumed as fresh fruits and, in addition, processed for export as canned fruits, concentrated juice, and dried fruits, and generating export incomes of over one billion Thai Baht annually (Anonymous, 2004). In 2002 world production of tomatoes was estimated at 108 million metric tons of which 242,000 metric tons were produced in Thailand (FAO, 2004).

Insect pests, mites as well as nematodes cause serious economic losses in tomato production in Thailand. They often not only cause direct damage to the plants, but also indirect, especially through vectoring important viral diseases of tomatoes (Deang, 1969; Gomaa *et al.,* 1978; Berlinger *et al.,* 1988; Kakar *et al.,* 1990; Talekar, 1991; Berlinger, 1992; Jinping, 1994).

Thrips (Thysanoptera: Thripidae) are polyphagous insects and most of them are worldwide serious pests of vegetables. Crop damage is caused directly by mechanical damage through feeding and ovoposition and indirectly by transmitting

tospoviruses (Tommasini and Maini, 1995). Within the pest complex of tomatoes in Asia, thrips are some of the most destructive pests. *Thrips palmi* Karny, *Scirtothrips dorsalis* Hood, *Megalurothrips usitatus* Bagnall, *T. tabaci* Lindeman, *T. flavus* Schrank, *Frankliniella occidentalis* (Pergande) and *F. schultzei* (Trybom) are the most commonly occurring thrips species on tomatoes in Southeast Asia (Bansiddhi and Poonchaisri, 1991; Bernardo, 1991; Chang, 1991). However, in Thailand the thrips fauna on tomatoes is not well documented. So far only *T. flavus* and *Ceratothripoides claratris* (Shumsher) have been recorded on tomatoes (Bansiddhi and Poonchaisri, 1991; Murai *et al.,* 2000; Rodmui, 2002). *Ceratothripoides claratris* is a key pest attacking tomatoes and the predominant thrips species on field- and greenhouse-grown tomatoes in central Thailand, especially in the greater Bangkok area (Murai *et al.,* 2000; Rodmui, 2002). In addition to tomatoes *C. claratris* has been recorded on egg plants, *Solanum melongena* L. (Solanaceae), cucumbers, *Cucumis sativus* L., pumpkins, *Curcurbita moschata* (Duch.) (both Cucurbitaceae), *Vigna sinensis* (L.) Savex-Hass and *V. unguilulata* (L.) Walp (both Fabaceae), and bird pepper, *Capsicum annuum* L. (Solanaceae) (Jangvitaya, 1993; Mound and Kibby, 1998). Apart from Thailand, *C. claratris* has also been recorded in India (Jangvitaya, 1993) and Malaysia (S. Okajima cited in Murai *et al.,* 2000). The closely related *C. dianthi* (Priesner) is one of the most harmful thrips species found in greenhouses in southern Italy (Marullo, 1998).

Like all thrips, *C. claratris* has six developmental stages, i.e., egg, first (L1) and second instar larvae (L2), prepupa, pupa and the adult. Female *C. claratris* insert the bean-shaped eggs (average size 0.13 mm) into the plant tissue. Leaves, stems and fruits are the susceptible plant parts of tomatoes for *C. claratris* (Murai *et al.,* 2000). The newly laid eggs are pale white in colour. Immediately before hatching the red pigmented eyes appear. The small white L1 starts to feed on the plant tissue right after emergence. The average size of a L1 is approximately 0.10 and 0.40 mm in width and length, respectively. The second larval instar is considerably bigger in size (width and length 0.16 and 0.8 mm, respectively) (Rodmui, 2002). The late L2 drop from the foliage to the soil or leaf litter, where they develop into the non-moving and non-feeding prepupae and pupae. Adults are dark brown in colour (Jangvitaya, 1993; Mound and Kibby, 1998; Rodmui, 2002). The average size of the female is approximately 0.20 mm in width and 1 mm in length. With 0.18 and 0.80 mm in width and length, respectively, males are smaller than females (Rodmui, 2002). Feeding of larvae and adults as well as oviposition by female *C. claratris* cause physical damage and in severe infestations tomato plants start to dry out (Figure 1.1-A). Dark spots of excrements are often visible on the leaves. At high population densities, thrips also feed and oviposit on young fruits, causing scarification and malformation (Figure 1.1-B).

A	**B**

Figure 1.1 Feeding damage caused by *Ceratothripoides claratris* on tomatoes A - Whole plant B - Fruit.

Under laboratory conditions at 25°C, development time of larvae, pupae and egg to adult are 4, 3 and 10 days, respectively (Rodmui, 2002). However, additional life table data, e.g., development and reproduction at different temperatures, for *C. claratris* is lacking.

Ceratothripoides claratris is competent for the tospovirus, Capsicum chlorosis virus (CaCV) (see results). Since, 2002 these tospovirus infections were observed in the greenhouses at Asian Institute of Technology (AIT), causing serious problems on tomato plants. Disease outbreaks were always associated with severe *C. claratris* infestations. Later research has indicated that this virus resembles CaCV (Pissawann Chiemsombat, Edgar Maiss, unpublished data) recently described in Queensland, Australia (McMichael *et al.,* 2002). Yet, no

research has been conducted for determining the vector competence of *C. claratris* for this CaCV.

The direct damage of *C. claratris* (Murai *et al.,* 2000; Rodmui, 2002) coupled with its virus transmission lead to high losses in tomato production in Thailand. Therefore, suitable management strategies against *C. claratris* are urgently needed. Presently, the predominant plant protection strategy in the vegetables in Asia is chemical control. Between 1980 and 1999 the quantity of pesticides imported to Thailand has increased from 9,855 to 33,969 tons, at an annual growth rate of 6.7% (Anonymous, 2002). This heavy use of pesticides resulted in emerging problems such as pest resistance (Talekar and Shelton, 1993; Williams and Dennehy, 1996), and resurgence, deleterious effect on natural enemies, contamination of water sources, and direct health hazards to both farmers and consumers (Saha, 1993). For pest control use of resistant varieties (de Jager and Butot, 1993; Shelton *et al.,* 1998), habitat management (Suzuki and Miyara, 1984; Riddell-Swan, 1988) and biological control (Gillespie, 1989; Gilkeson *et al.,* 1990; Wittmann and Leather, 1997) have been proposed as alternatives to chemical control. In *C. claratris* so far two eulophid parasitoids, i.e., *Ceranisus menes* Walker and *Goethena shakespearei* Girault, and a mirid predator have been identified as natural enemies (Murai *et al.,* 2000; Rodmui, 2002). However, presently little is known on their potential to combat *C. claratris* outbreaks in greenhouses. In general, thrips are an increasing pest problem in tropical and sub-tropical climates, though few investigations have been conducted on their

integrated control in vegetables and especially on tomatoes (Bansiddhi and Poonchaisri, 1991; Talekar, 1991; Okojima *et al.,* 1992). In order to avoid the detrimental effects of an abusive use of synthetic pesticides, integrated pest management (IPM) strategies, mainly based on biological control measures, need to be developed and implemented against *C. claratris*. Several case studies have demonstrated the feasibility of IPM in Asia (Krishnaiah *et al.,* 1981; Berlinger, 1992; Kather, 1995). In recent years, neem-based insecticides derived from neem tree, *Azadirachta indica* Juss. (Meliaceae) and spinosad, a fermentation product of the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao have acquired popularity in IPM programs because of their rather species-specific activity, limited effects on beneficials, have a low-mammalian toxicity and fast rate of metabolism (Jacobson, 1989; Miles and Dutton, 2000; Schoonejans and Van Der Staaij, 2001). Previous studies indicated that neem and spinosad can efficiently control thrips (Ascher *et al.,* 2000; Jones *et al.*, 2002; Nawrocka, 2002; Thoeming *et al.,* 2003). Moreover, a prerequisite for the successful development and subsequent implementation of IPM is the sound knowledge of the biology and ecology of the target pest. Hence, some of the research work presented in this study focused on in-depth studies on the biology, population dynamics of and the virus transmission by *C. claratris*.

The main objectives of the chapter 2 were to study the effects of temperature on the development, reproduction and survival of *C. claratris* on tomatoes. In addition, basic thermal requirements of different life-stages of *C. claratris* were established.

Finally, the data on reproduction were used to construct the life-fertility tables for *C. claratris*.

In chapter 3, the spatial and temporal distribution of *C. claratris* on tomatoes were determined under greenhouse conditions. These data will provide basic information for future monitoring and sampling programs of *C. claratris*.

Chapter 4 presents studies of the vector competence of *C. claratris* for the tospovirus, CaCV isolated from tomato plants in greenhouses at AIT. The ability of acquisition of the virus by first and second instar larvae and the transmission efficiencies of second instar larvae and adult *C. claratris* were tested. In addition, efforts were made to correlate the thrips infestation and spread of the virus on tomatoes under greenhouse conditions.

In the last chapter, chapter 5, feasibility of two bio-pesticides, i.e., neem and spinosad, for the management of *C. claratris* were determined on tomatoes under laboratory and greenhouse conditions. Effects of these products were tested on the survival of foliar-dwelling life stages of *C. claratris*, i.e., larvae and adults, and their reproduction. Neem products were tested for topical, residual and systemic toxicity, while spinosad were tested for topical and residual toxicity, only.

All experiments were carried out in laboratories and greenhouses of AIT during 2001-2004, and were part of a larger study which aims to establish sustainable and environmentally friendly vegetable production systems under protected cultivation in the humid tropics.

2 Influence of temperature on the development, reproduction and longevity of *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes in Thailand[1]

2.1 Abstract

Ceratothripoides claratris (Shumsher) is a serious pest attacking tomatoes in Thailand. Temperature-dependent development of *C. claratris* was studied at seven constant temperatures, i.e., 22, 25, 27, 30, 34, 35 and 40ºC. Pre-adult survivorship was greatest (95%) at 25 and 30°C and shortest at 22ºC. Egg-to-adult time decreased within a range of 20 to 30ºC and at 34ºC it started to increase. The lower thermal threshold for egg-to-adult development was estimated at 16 and 18ºC by linear regression and the modified Logan model, respectively. The optimum temperature for egg-to-adult development was estimated at 32-33ºC by the modified Logan model. The influence of temperature on reproduction and longevity of *C. claratris* was determined at 25, 30 and 35 and 40ºC. Both inseminated and virgin females failed to reproduce at 40ºC. Virgin females produced only male offspring, confirming arrhenotoky.

The sex ratio of the offspring of fertilized females was strongly female-biased, except at 25ºC. Mean total fecundity per female and mean daily total fecundity per female were highest for both virgin and inseminated females at 30ºC. Female

longevity was longest at 25ºC and shortest at 40ºC. Male longevity was longest at 30ºC and shortest at 40ºC. The net reproductive rate (R_0) and intrinsic rate of natural increase (r_m) was greatest at 30ºC while, mean generation time (G) and the doubling time (t) were highest at 25ºC. The finite rate of increase (λ) was fairly constant (1.1–1.5 days) over the three temperatures tested. The pest potential of *C. claratris* for tropical Asia is discussed.

2.2 Introduction

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae), previously described as *Taeniothrips claratris* Shumsher and *Mycterothrips moultoni* Seshadri and Ananthakrishnan, was first detected in 1987 attacking melons, *Cucumis melo* L. (Cucurbitaceae), in northern Thailand (Okajima *et al.*, 1992). In the same region it was later also found on egg plants, *Solanum melongena* L. (Solanaceae) (Jangvitaya, 1993). Moreover, Mound and Kibby (1998) recorded *C. claratris* infestations of cucurbits in Thailand. On tomatoes in Thailand *C. claratris* was first detected in 1999 (Murai *et al.*, 2000). Murai *et al.* (2000) reported that no other thrips species were found on tomatoes in central Thailand. In addition to *C. claratris* also *Thrips palmi* Karny (Thysanoptera: Thripidae) occur in fields and greenhouses in the greater Bangkok area, though in very low numbers. *Ceratothripoides claratris* has also been found on tomatoes in Malaysia in 1999 (S. Okajima, cited in Murai *et al.*, 2000). Jangvitaya (1993) reported that *Luffa acutangula* L. (Cucurbitaceae) and *Clitoria ternatea* L. (Fabaceae) are also host plants of *C. claratris* in Thailand. In a host plant preference study, *C. claratris*

heavily attacked and successfully reproduced on cucumber, pumpkin, cowpea, yard long bean and chili (D. Premachandra, unpublished data). Moreover, *C. claratris* is apparently vectoring *Tomato necrotic spot virus* (TNSV) on tomatoes (D. Premachandra, unpublished data).

Rodmui (2002) studied the life cycle of *C. claratris*. As in other thrips species, *C. claratris* has six development stages, i.e., eggs, which are embedded in the plant tissue, two active larval instars, two inactive pupal instars, i.e., prepupa and pupa, and the adult. Adults and the two larval instars feed on the foliage. The late second instar larvae drop off the plants and pupate in the soil or on leaf litter. Adults are dark brown in colour and females have an average width of about 0.20 mm and a length of 0.98 mm, and males are slightly smaller (Rodmui, 2002).

Under greenhouse and field conditions in Thailand *C. claratris* preferentially attacks leaves of tomatoes, and to a lesser extent stems and fruits (Murai *et al*., 2000; Rodmui, 2002). The damaged leaves initially appear bleached and finally dry out. Under severe infestations in greenhouses tomato plants die seven weeks after initial infestation, i.e., prior to the first fruit setting. Hence, *C. claratris* can cause a total loss in greenhouse tomatoes in Thailand.

Although *C. claratris* is an important vegetable pest in Thailand, to date only one study has addressed some aspects of its biology and reproduction (Rodmui, 2002). However, at present no information on the impact of temperature on the development, reproduction and survivorship of *C. claratris* is available. In-depth life cycle studies are essential for developing integrated pest management strategies,

e.g., for determining the accurate timing for releases of natural enemies and/or application of selective insecticides. Temperature-dependent developmental rates of an insect are important to explain and predict the fluctuations in population densities. Moreover, parameters generated from life-fertility studies are crucial components for a better understanding of the population dynamics of a species (Southwood, 1978). This study is part of a larger project that seeks to develop sustainable vegetable production systems under protected cultivation in the humid tropics, where temperatures in greenhouses are of paramount importance for plant growth, as well as for incidence of, and damage caused by pests and diseases. Hence, the objectives of this study were to investigate the temperature-dependent development, reproduction and longevity of *C. claratris*.

2.3　Materials and Methods

Thrips source and the host plant

Adult *C. claratris* were initially collected from tomato plots at the Asian Institute of Technology (AIT), Bangkok, Thailand. Thereafter, thrips were reared on potted tomato plants *Lycopersicon esculentum* Mill. (Solanaceae), cv. King Kong II in a small net-house (10 × 20 m, mesh size of the net approximately 400 μm). *Ceratothripoides claratris* was identified based on its morphological characteristics, and voucher specimens were deposited at the Division of Entomology, Department of Agriculture, Bangkok, Thailand and the Senckenberg Museum, Frankfurt, Germany. All experiments were carried out on tomato plants (cv. King Kong II).

Experimental conditions

The development of *C. claratris* was studied in a temperature controlled climate chamber at 22, 25, 27, 30, 34, 35 and 40 ± 1ºC, 75 ± 5% relative humidity (RH) and a 12L:12D photoperiod. Experiments on reproduction and longevity of the thrips were conducted at 25, 30 and 35 and 40 ± 1ºC, 75 ± 5% RH and a 12L:12D photoperiod.

Development

Cohorts of eggs were obtained by allowing females of *C. claratris* (approx. 250) to oviposit on excised tomato leaflets for 4 h at the desired temperature. Leaflets were kept in a Petri dish (8.5 × 1.5 cm) lined with a thin layer (approx. 1.5 cm thickness) of a mixture of Plaster of Paris and charcoal (ratio 9:1). In order to keep the leaflets viable, the petioles were wrapped in wet cotton. Two holes (2 cm diameter, covered with a 64 µm mesh nylon tissue) were cut into the lid of the Petri dish to facilitate ventilation. After introducing the adults for oviposition, the Petri dish was thoroughly sealed, using modelling clay, to prevent thrips from escaping. After 4 h, the adults were removed from the leaflet with a fine camel hair brush, and the eggs were incubated. In preliminary experiments at 40ºC, excised tomato leaflets started to deteriorate after one day. Hence, at 40ºC, instead of leaflets, a whole tomato plant was used to obtain eggs and for further rearing of the thrips larvae.

For the experiments, leaf discs (2 cm diameter) were punched out from fully grown tomato leaflets. The discs were placed abaxial surface uppermost on water agar

(0.9%) (1.5 cm thickness) in a small plastic container (5 × 4 × 3 cm), in the following referred to as "larval container". One hole (1 cm diameter, covered with a 64 μm mesh nylon tissue) was cut into the centre of the lid. An individual newly emerged first instar larva was placed on a leaf disc with a fine camel hair brush. Every two days larvae were transferred to fresh leaf discs. The second larval instar was determined by the occurrence of an exuviae. In preliminary experiments late second instar larva started to leave the leaf discs for pupation. Hence, 12 h after moulting the second instar larva were transferred to a "pupal container" and reared there until emergence of the adults. Pupal containers were identical to larval containers except that the base was lined with a mixture of Plaster of Paris and charcoal (9:1). To facilitate pupation, the leaf disc with the second instar larva was placed in a sandwich-manner between two fresh leaf discs. Thereafter, the space between the lid and the pupal container was sealed with modelling clay. Development duration and survival of immature stages, i.e., eggs, first instar larva, second instar larva, prepupae and pupae, were determined at 12 h intervals under a stereo microscope. At least 30 individual larvae were observed at each temperature tested. Moreover, the sex of the emerged adults was determined and the development time of males and females were calculated separately. The data on the developmental time was used to establish the basic thermal requirements of the different life stages of *C. claratris*.

Reproduction and longevity

Reproduction was quantified both for inseminated and virgin females. To obtain synchronized-aged females, first second instar larva from the rearing unit were kept on tomato leaves in a Petri dish (8.5 × 1.5 cm) until they reached the pupal stage. Then female pupae were kept separately for generating virgin females. For studies with inseminated females, a virgin female and a male were simultaneously introduced into the assay arena, and after 24 h the male was removed. In these experiments instead of leaf discs excised tomato leaflets with petioles were used. The petiole was inserted into a small glass vial (1.4 × 1.5 × 4.4 cm) filled with water agar (0.9%). The vial was placed in a plastic container (7.5 × 5.5 × 7 cm), and the base of the vial (approximately 1 cm in depth) was embedded in a layer of Plaster of Paris and charcoal (9:1 ratio, 3 cm thickness). One hole (1 cm diameter, covered with a 64 μm mesh nylon tissue) was cut into the centre of the lid. For a precise determination of the pre-oviposition period, females were transferred to fresh tomato leaflets every 6 h until day 3 of the experiment. Thereafter, tomato leaflets were replaced every 24 h until the death of the females. Leaflets bearing eggs were incubated. Fecundity estimates, i.e., total and daily fecundity, were based on the number of emerged first instar larvae on leaflets. At least 100 first instar larva produced by virgin and inseminated females were randomly selected and individually reared to adulthood for sex determination at each temperature regime tested. In addition, survival of virgin and inseminated females was recorded daily. Longevity of male *C. claratris* was studied in assay arenas similar

to the ones used for females. Newly emerged males were individually placed on a single tomato leaflet in a plastic container. Every three days males were transferred to a fresh tomato leaflet, and survival was recorded daily. Data on pre-adult survival, daily fecundity of individual, inseminated females and the sex ratio of their offspring at each temperature tested, was used to construct the life-fertility tables.

Data analyses

Data on development time of the different life stages and egg-to-adult time, adult longevity and fecundity of virgin and inseminated females were compared across temperatures using analysis of variance (ANOVA) (GLM procedure; SAS Institute, (1999)). In case of significance, means were separated using LSD (P = 0.05). For estimation of the lower developmental threshold (To = intercept/slope) and the thermal constant (K = 1/slope = the number of day-degrees to complete the pre-reproductive phase; Campbell *et al*., 1974), a simple regression over the linear range, of the relationship between temperature (T) and developmental rates was used (Campbell *et al*., 1974). The modified Logan model (Logan *et al*., 1976) by Lactin *et al*. (1995),

$$R(T) = e^{\rho T} - e^{[\rho Tmax - (-T)/\Delta]} + \lambda,$$

where, T is the temperature in degree Celsius (°C), ρ, Tmax, Δ and λ are fitted coefficients, was used to describe temperature-dependent development rates of adult *C. claratris.*

The life-fertility table parameter estimates, i.e., the intrinsic rate of increase (r_m), net reproductive rate (R_o), mean generation time (G), doubling time (t) and the finite rate of increase (λ), were calculated using the jackknife program (Hulting *et al.*, 1990). Differences of these estimates over the temperatures tested were compared using the Newman-Keuls sequential test (Sokal and Rohlf, 1995) on the basis of jackknife estimates of variance (Meyer *et al.*, 1986).

2.4   Results

Development

All life stages, i.e., first instar larva, second instar larva , prepupa, pupa and adults of *C. claratris* developed at the temperatures tested except at 40ºC where eggs did not hatch (Table 2.1). At 25 and 30°C, pre-adult survivorship was 95%. At 27, 34 and 35°C pre-adult survivorship ranged from 72-90%, and the lowest survivorship (43%) was recorded at 22°C. Egg-to-adult time differed significantly across temperatures (F = 858; d.f. = 5, 291; *P* < 0.0001) with the longest and shortest duration at 22 and 30ºC, respectively. Egg-to-adult developmental time decreased within a range of 22 to 30ºC; thereafter it started to increase (Table 2.1). Total developmental time of males and females did not differ significantly at the six temperatures tested. However, the development time of the immature stages of *C. claratris* was significantly influenced by the temperatures tested (Table 2.1).

Table 2.1 Mean (± SE) developmental time (in days) of different life stages of *Ceratothripoides claratris* at seven constant temperatures.

Life-stage	Temperatures (°C)						
	22	25	27	30	34	35	40
Egg	6.64 ± 0.04a[1]	4.20 ± 0.04b	4.12 ± 0.09b	3.07 ± 0.02c	2.61 ± 0.03e	2.81 ± 0.05d	Did not
	(67)[2]	(97)	(60)	(74)	(59)	(86)	hatch
Larva I	3.16 ± 0.04a	2.57 ± 0.03b	2.05 ± 0.04c	2.01 ± 0.01c	2.00 ± 0.00c	1.98 ± 0.01c	-
	(67)	(97)	(60)	(74)	(59)	(86)	
Larva II	4.32 ± 0.11a	3.74 ± 0.05b	1.63 ± 0.21d	1.15 ± 0.07e	2.23 ± 0.08c	2.28 ± 0.08c	-
	(67)	(97)	(56)	(73)	(59)	(78)	
Prepupa	1.75 ± 0.06a	0.78 ± 0.03b	0.77 ± 0.05b	0.78 ± 0.04b	0.80 ± 0.04b	0.77± 0.04b	-
	(34)	(93)	(55)	(70)	(52)	(70)	
Pupa	3.82 ± 0.20a	3.27 ± 0.05b	2.76 ± 0.09c	1.79 ± 0.04d	1.71 ± 0.08d	1.88 ± 0.05d	-
	(32)	(92)	(54)	(70)	(48)	(64)	
Egg-adult	19.55 ± 0.31a	14.55 ± 0.07b	11.37 ± 0.18c	8.80 ± 0.09e	9.42 ± 0.10d	9.76 ± 0.08d	-
	(29)	(92)	(54)	(70)	(48)	(62)	

[1] Means followed by the same letter within rows are not significantly different (P = 0.05, LSD multiple range test [SAS Institute, 1999]); [2] numbers entering each life stage.

Duration of egg, larval and pupal stages of *C. claratris* was significantly longer at 22°C than at all other temperatures tested (eggs - F = 1258; d.f. = 5, 364; *P* < 0.0001;first instar larva - F = 301.0; d.f. = 5, 356; *P* < 0.0001; second instar larva - F = 216.3; d.f. = 5, 320; *P* = 0.0001; prepupa - F = 69.4; d.f. = 5, 307; *P* < 0.0001; pupa - F = 126.9; d.f. = 5, 292; *P* < 0.0001). No significant differences in development time of first instar larva and prepupa and pupa were recorded above 25 and 27°C, respectively (Table 2.1).

In terms of total development time of *C. claratris*, the egg stage lasted longest (range 28-36%) and the prepupal stage shortest (range 5-9%) at all temperatures. For all life stages of *C. claratris* significant relationships between the development rate and the temperatures tested were recorded (Table 2.2). For egg and egg-to-adult time the relationships between development rates and temperatures were strongly linear (R^2 = 0.91, *P* < 0.0001) at 22-34°C and 22-30°C, respectively, while weaker relationships were recorded for the second instar larva (22-35°C) and pupa (22-34°C). Linear regressions did not yield a good fit for development of the first instar larva and prepupal. The modified Logan model provided a good fit for data on egg-to-adult time within a range of 22-35°C (R^2= 0.90, *P* < 0.0001) (Figure 1.1). The fitted parameters were estimated as ρ = 0.00813, T_{max} = 43.5, Δ = 2.921 and λ = -1.151. Based on this model, the lower, optimum and maximum temperatures were estimated as 18, 32-33 and 38°C, respectively.

Table 2.2 Estimates of the linear regression analyses and lower thermal thresholds and the thermal constants for egg, second instar larvae, pupae and egg-adult stages of *Ceratothripoides claratris*.

Life stages	Linear range (°C)	Regression equations [a]	R^2	F values	*P*>F	To [b]	K [c]
Egg	22-34	Y = –0.2571 + 0.0192X	0.91	3021	0.0001	13.36	51.90
Larva II	22-30	Y = –2.9220 + 0.1345X	0.43	172.2	0.0001	21.70	7.44
Pupa	22-34	Y = –0.4997 + 0.0340X	0.53	275.8	0.0001	14.70	29.41
Egg - adult	22-30	Y = –0.1354 + 0.0083X	0.91	2078	0.0001	16.37	120.92

[a] Calculated after Campbell *et al*. (1974), where X is the temperature (°C) and Y is the developmental rate (1/developmental time).

[b] Lower development threshold (°C).

[c] Thermal constant (in day degrees).
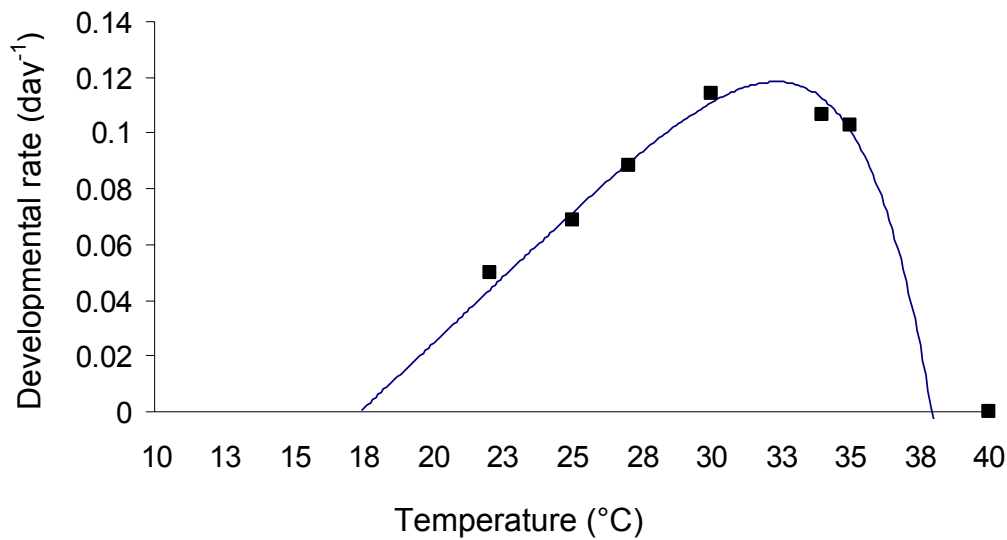
Figure 2.1 Developmental rate of *Ceratothripoides claratris* expressed as a temperature (°C) function using the modified Logan model.

Reproduction and longevity

Both inseminated and virgin females failed to reproduce at 40ºC. At 25ºC, the pre-oviposition period was significantly longer for both inseminated (F = 26.84; d.f. = 2, 36; $P < 0.0001$) and virgin females (F = 18.28; d.f. = 2, 34; $P < 0.0001$) than at the other two temperatures tested (Table 2.3). Except at 35ºC, duration of pre-oviposition varied significantly between inseminated and virgin females. At 25 ($P < 0.004$, t test) and 30ºC ($P < 0.0001$, t test), the pre-oviposition period of inseminated females was longer than that of virgin females.

Mean total fecundity per female and mean daily total fecundity per female were significantly higher for both virgin (for mean total fecundity per female F = 4.36; d.f. = 2, 44; $P < 0.0187$; for mean daily total fecundity per female F = 31.4; d.f. = 2, 44;

 P < 0.0001) and inseminated females (for mean total fecundity per female F =

14.03; d.f. = 2, 43; P < 0.0001; for mean daily total fecundity per female F = 34.15;

d.f. = 2, 43; P < 0.0001) at 30ºC than at the other two temperatures tested (Table

2.3). Mean total fecundity of inseminated and virgin females did not differ at 25

and 30ºC.

Virgin females produced only male offspring, whereas inseminated females had

both male and female offspring. The F1 sex ratio of mated females was strongly

female biased at 30 and 35°C with 71% and 65% female progeny, respectively. In

contrast, at 25°C, the F1 of inseminated females was male biased, with a mean

proportion of 62% males (Table 2.3).

The pattern of reproduction and survivorship of inseminated and virgin females of

C. claratris at three temperatures are shown in Figures 2.2 A-E. Survivorship, as

well as progeny production declined as females aged at each temperature tested.

Post-oviposition periods were short (1-3 days) for inseminated and virgin females

across the temperatures tested. For both types of females, the pattern of

oviposition was erratic at 25ºC (Figure 2.2 A and B). In contrast, an ovipositional

peak was evident at 6 (Figure 2.2 C) and 4 days (Figure 2.2 E) after emergence in

inseminated females at 30ºC and in both inseminated and virgin females at 35Cº

(Figure 2.2 E and F), respectively. Moreover, at 30ºC, peaks in oviposition

occurred in virgin females at 7 and 9 days after emergence (Figure 2.2 D).

Table 2.3 Mean (± SE) pre-oviposition period (days), total fecundity, daily fecundity per female, sex ratio and longevity of inseminated females and males (days) of *Ceratothripoides claratris* at different constant temperatures.

Parameters	Temperatures (°C)			
	25	30	35	40*
Pre-oviposition period of virgin females	2.5 ± 0.17a	1.38 ± 0.04c	1.82 ± 0.01b	-
Pre-oviposition period of inseminated females	3.3 ± 0.19a	1.75 ± 0.06b	1.76 ± 0.14b	-
Total fecundity of virgin females	69.8 ± 13.95b	110.07 ± 11.16a	62.71 ± 11.33b	-
Total fecundity of inseminated females	44.9 ± 6.53c	116.50 ± 11.65a	70.06 ± 9.78b	-
Daily fecundity/virgin female	3.17 ± 0.41a	9.22 ± 0.80b	6.08 ± 0.66c	-
Daily fecundity/inseminated female	2.37 ± 0.39a	10.30 ± 1.54b	5.39 ± 0.55c	-
Sex ratio (% female progeny)	38.0 ± 5.72b	71 ± 7.72a	65 ± 5.90a	-
Female longevity	18.5 ± 1.51a	12.2 ± 0.73b	10.9 ± 0.90b	2.79 ± 0.25c
Male longevity	14.1 ± 2.12ab	17.1 ± 1.14a	11.0 ± 1.54b	1.21 ± 0.20c

* No progeny production - Within rows, means followed by the same letter are not significantly different ($P = 0.05$, LSD multiple range test).
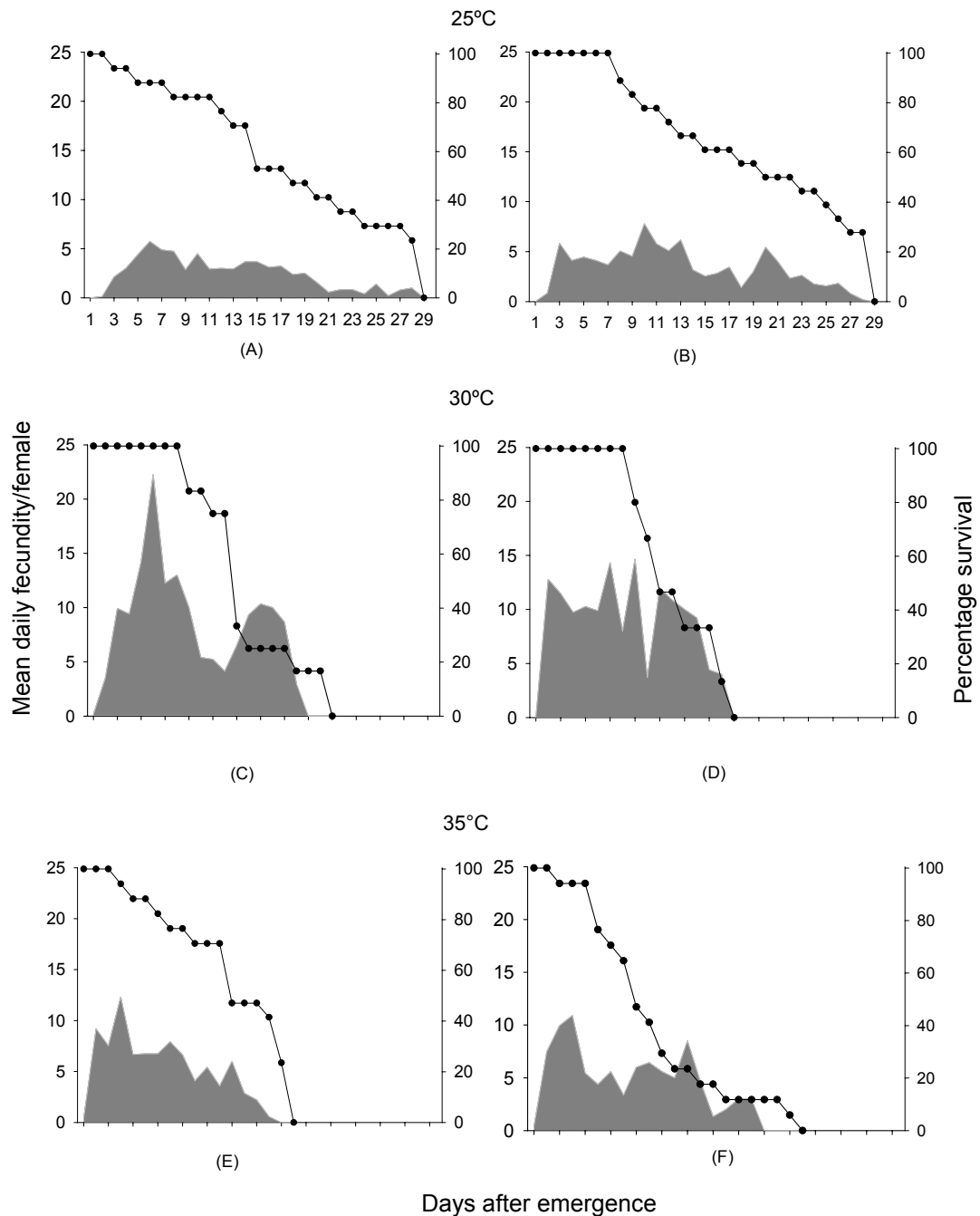
Figure 2.2 Age-specific reproduction and survivorship of inseminated and virgin females of *Ceratothripoides claratris* at 25, 30 and 35°C. (A) – inseminated, 25ºC; (B) – virgin, 25ºC; (C) – inseminated, 30ºC; (D) – virgin, 30ºC; (E) – inseminated, 35ºC; (F) – virgin, 35ºC.

Life-history parameters of inseminated thrips females varied significantly with temperatures (Table 2.4). The net reproductive rate (R_0) was significantly greatest at 30ºC; it was twice as high as at 35ºC and five times higher than at 25ºC. The intrinsic rate of natural increase (r_m) was significantly highest and lowest at 30 and 25ºC, respectively. With 20.67 and 5 days the maximum mean generation time (G) and the doubling time (t), respectively, were recorded at 25°C. The finite rate of increase (λ) remained fairly constant over the three temperatures tested.

Table 2.4 Mean (± SE) population growth parameters of *Ceratothripoides claratris* at three constant temperatures.

Parameters	Temperatures (°C)		
	25	30	35
r_m	0.139 ± 0.007c	0.347 ± 0.010a	0.281 ± 0.010b
Ro	17.60 ± 2.682c	84.39 ± 8.450a	38.45 ± 5.390b
G	20.67	12.80	13.00
λ	1.15	1.41	1.32
t	5.00	2.00	2.47

Means followed by the same letter within rows are not significantly different (P = 0.05, Student-Newman-Keul sequential test). r_m - intrinsic rate of natural increase; R_o - net reproductive rate; G - mean generation time (days); λ - finite rate of increase; t - doubling time (days).

Mean longevity of inseminated females and males *C. claratris* differed significantly among the three temperatures tested. Female longevity was significantly longer at 25ºC (F = 26.84; d.f. = 3, 109; P < 0.0001) than at the other three temperatures.

 Male longevity was longest at 30ºC, and shortest for both females and males at 40ºC. No significant differences in longevity of male and female thrips were found at 25 and 35ºC. In contrast, at 30ºC, male longevity was significantly longer than that of females (P < 0.0005, t test), while at 40ºC female longevity was significantly longer than that of males (P < 0.0001, t test).

2.5   Discussion

Temperature is a key factor for the development, survival and reproduction, of poikilothermic organisms (Andrewartha and Birch, 1954; Sharpe and DeMichele, 1977). Information on temperature-dependent development, survivorship and reproduction of different insects, including thrips (Kawai, 1985; Shibao, 1996; Murai, 2001; Hoddle, 2002) is well documented. This is the first report on development, reproduction and longevity of *C. claratris* at different constant temperatures. Temperature had a profound effect on the development, fecundity and longevity of *C. claratris*. At 22ºC, development of all thrips life stages was considerably prolonged and the pre-imaginal mortality was highest (57%). At 40ºC, development of *C. claratris* eggs was inhibited. Estimates of the modified Logan model indicate 38ºC as the maximum temperature for development of *C. claratris*. Best performance of *C. claratris* was recorded at 30ºC, as shown by the high level of pre-adult survivorship, coupled with the respective estimates of the different life-history parameters, i.e., the higher net reproductive rate (R_0), intrinsic rate of increase (r_m) and shorter mean generation time (G) and doubling time (t).

Moreover, estimates of the modified Logan indicate values an optimum temperature for the development of *C. claratris* at 32-33ºC.

Inhibition of egg development at the highest temperature tested (i.e., 40ºC) was most likely due to desiccation under heat stress. Low or no mortality was recorded for the first instar larva at all temperatures. Possibly emerging young larvae still sustained some nutritive reserves from the egg stage. In a life table study with *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae), however, Hoddle (2002) reported highest mortality in first instar larva at the lowest temperature (i.e., 15°C) tested. A possible reason for this discrepancy might be the fact that *S. perseae* is more a thrips of sub-tropical to temperate climates, whereas *C. claratris* is obviously a species well adapted to tropical conditions. In our experiments, high mortality of second instar larvae (87%) occurred at the lowest temperature (22ºC), indicating that second instar larvae are the most susceptible pre-imaginal life stage of *C. claratris* to low temperatures. Considerably lower mortalities at all temperatures tested were recorded in the pupal stages. In general, temperature greatly affected the development time of *C. claratris* larvae. For instance, at 22° compared to 30ºC, a four times longer developmental time was recorded in second instar larvae. According to Thrichilo and Leigh (1988), factors like temperature that delay the development times will act primarily on the active moving larvae, possibly through a reduced food intake (Bakker, 1961). In the present study, temperature had little to no effect on the development time of prepupae and pupae.

Rodmui (2002) reported a slightly shorter egg-to-adult time of *C. claratris* at 25ºC than in this study, possibly due to differences in methodology. More data on the effect of temperature on development are available for several important thrips pests in the tropics and subtropics, such as *T. palmi*, *S. dorsalis* Hood, *T. tabaci* Lindeman and *T. hawaiiensis* (Morgan) (all Thysanoptera: Thripidae). For instance, temperatures exceeding 30ºC have detrimental effects on the egg development in *T. hawaiiensis* (Murai, 2001). Similar effects have been reported in *T. tabaci*, *Frankliniella intonsa* (Trybon) and *F. occidentalis* (Pergande) (all Thysanoptera: Thripidae) (Katayama, 1997; Murai, 1988, 2000), corroborating results of this study. Under laboratory conditions on cucumbers, the lower threshold temperature for development of *T. palmi* was estimated at 11.6ºC (Kawai, 1985) which is markedly lower than that of *C. claratris*, indicating that *C. claratris* has a greater potential for development at higher temperatures than *T. palmi*. Likewise, *T. hawaiiensis*, *T. tabaci* and *S. dorsalis* have lower threshold temperatures for development from egg to adult (Tatara, 1994; Murai, 2000, 2001) than *C. claratris*. Thus, it appears that *C. claratris* in comparison to other important thrips pests in the tropics and subtropics is less tolerable to cooler but better adapted to higher temperatures.

Reduced longevity of both males and females and the inhibition of progeny production by both inseminated and virgin females at 40ºC emphasize the adverse effects of high temperatures on reproduction and longevity in *C. claratris*. At 25ºC, even though females lived longer, they were less fecund. The significantly lower longevity of females compared to males at 30ºC was most likely associated with

the increased reproductive capacity of the females at this temperature (Gaum *et al.*, 1994). *Ceratothripoides claratris* reproduces by arrhenotokous parthenogenesis. Except at 25ºC, the sex ratios of inseminated females were strongly female-biased, leading to a rapid increase in thrips density. The male-biased sex ratio at 25ºC can be possibly explained by less matings, sperm depletion and reduced sperm viability. In addition, at 25ºC females lived longer than at the other temperatures tested. In western flower thrips and in *Spalangia cameroni* Perkins (Hymenoptera: Pteromalidae) the proportion of daughters in the progeny decreased with age, presumably as a result of sperm depletion (Higgins and Myers, 1992; King, 2000). The high net reproductive rate at 30ºC resulted from a significantly higher fecundity compared to the other temperatures tested and a high proportion of females in the F1. The pronounced ovipositional peaks during the early reproductive cycle at higher temperatures are possibly associated with a higher metabolic rate (Sharpe and DeMichele, 1977). The small r_m value at 25ºC mainly resulted from the reduced fecundity of inseminated females at lower temperatures. In addition, the doubling time and mean generation time were also longer at 25ºC, contributing to a lower r_m value. The life-history estimates of *C. claratris* at 25ºC reported by Rodmui (2002) correspond well with our data, except for the net reproductive rate which was two times higher in the previous study. Once again, methodological differences and the different models used for the analysis of life-history parameter estimates may account for this difference. The net reproductive rate (R_0) of *T. palmi*, *T. hawaiiensis* and *T. tabaci* at 25ºC are higher than that of *C. claratris*. In contrast, at 30 and 35ºC the net reproductive

rate of *C. claratris* is four and two times higher than that of *T. palmi*, respectively. Likewise, at 30ºC the intrinsic rate of increase of *C. claratris* is three times higher than that of *T. palmi* (Kawai, 1985).

The data on development, reproduction and longevity of *C. claratris* indicate that this species is better adapted to high temperatures (i.e., 30-35ºC) than other important tropical thrips species like *T. palmi* and *S. dorsalis*. Moreover, the relatively short life cycle of *C. claratris*, coupled with its high reproductive potential, female biased sex ratio and long lifespan can lead to a rapid population build up, both in the field and under greenhouse conditions in the tropics. At present, little is known on the host plant spectrum and the geographic distribution of *C. claratris*. However, our results clearly show that *C. claratris* has the potential to become a serious constraint for tomato production in tropical Asia. In ongoing studies we are investigating the host plant preference of and virus transmissibility by *C. claratris*.

3 Distribution and population dynamics of *Ceratothripoides claratris* (Thysanoptera: Thripidae) on tomatoes in Thailand[2]

3.1 Abstract

Ceratothripoides claratris (Shumsher) is one of the most important thrips pest of tomatoes in central Thailand. Hence, we conducted studies to determine the intra- and inter-plant distribution of *C. claratris* on tomatoes in two types of greenhouses, i.e., open-plastic and closed net-house. The experiments were conducted on the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand. Both adults and larvae of *C. claratris* showed foliage-biased distribution with no niche separation between adults and larvae. Sex ratios of adult *C. claratris* did not significantly differ on flowers and leaves, whereas on fruits males significantly outnumbered females. On flowers, no diurnal periodicity of occurrence of *C. claratris* was detected. Infestation of *C. claratris* gradually spread from the bottom to the top of the tomato plants over time irrespective of the stem systems, i.e., single and double, and house type. In the net-house, infestations of *C. claratris* commenced one week after planting of the tomato seedlings. Soon after the peak in infestations, thrips numbers dramatically decreased. Estimates of Taylor's power showed that *C. claratris* had an aggregated distribution pattern on the foliage of tomato plants. The importance of these findings for future monitoring programs of *C. claratris* infestations on tomatoes is discussed

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3.2   Introduction

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae) is the most prevalent thrips species attacking field- and greenhouse-grown tomatoes *Lycopersicon esculentum* Mill. (Solanaceae) in Thailand (Murai *et al.,* 2000; Rodmui, 2002; Premachandra *et al.,* 2004). Larvae and adults of *C. claratris* damage tomatoes by voraciously feeding on the foliage, stems and fruits; in addition, oviposition by females on fruits leads to scarification and malformation of tomatoes (Murai *et al.,* 2000; Premachandra *et al.,* 2004). Moreover, *C. claratris* is vectoring a yet to be identified tospovirus of the serogroup IV, serologically and genetically closely resembling the recently described *Capsicum* chlorosis virus (CaCV) (McMichael *et al.,* 2002), which causes severe losses in tomato production in central and northern Thailand (D. Premachandra, unpublished data). Hence, the development of integrated management strategies against *C. claratris* is vital. Information on the spatio-temporal distribution of a pest on a given host plant provides basic information for developing reliable and cost-effective sampling schemes which are a cornerstone in future Integrated Management Programs (IPM) (Ekbom and Xu, 1990) against *C. claratris*. In addition, population density estimates are essential for determining the precise timing of control measures in IPM programs, as well as to assess their effectiveness (Reitz, 2002). To date, little is known about the spatial and temporal distribution of *C. claratris* and its population dynamics on tomatoes. Hence, the objectives of this study were to investigate the intra- and inter-plant distribution of *C. claratris* on tomatoes and to record thrips population development over time under greenhouse conditions in

central Thailand. This research was conducted as part of a project that aims to develop sustainable vegetable production systems under protected cultivation in the humid tropics.

3.3 Materials and Methods

Greenhouses and plants

The trials were conducted in two 200 m^2 big greenhouses, located at the campus of the Asian Institute of Technology (AIT), in Bangkok, Thailand. One was an open side-wall plastic house (polythene plastic, 200 micron UV-stabilized polyfilm, Ludvig Swensson, Netherlands) with an opening of 50-200 cm above the ground level. The second was a closed net-house (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) equipped with two exhaust fans (550 m^3/min, 1.5 HP, 960 rpm, Sriroz Company, India) at the front side of the net-house. The fans were operated by a computerized control system that automatically switched on one fan when temperatures inside the net-house exceeded 25°C, and the second one at temperatures > 30°C. The climate in the two greenhouses was monitored using a data logging system (ITG data logger, University of Hanover, Germany). During the trials mean temperatures and relative humidities were 28-30ºC and 70-80%, and 26-28ºC and 60-80% in the closed and open house, respectively. The total planting area of each greenhouse was 160 m^2. Three weeks-old tomato seedlings (cv. King Kong II, an indeterminate variety) were planted in plastic pots (30 x 25 cm) filled with a commercial growing substrate composed of clay, sand, and silt in proportions of 31, 30 and 39%, respectively, and 29% of organic matter. Pots were

placed on a black ground plastic cover (Chaisiri Nylon Canvas Factory Ltd., Bangkok, Thailand) and arranged in six rows with no inter-pot distances within a row. The distance between rows was 160 cm. Plants were irrigated and fertilized with a drip irrigation system controlled by solar light integral. Tomato plants were irrigated and fertilized 7-9 times per day (2.5 l /day). The fertilizers [Hakaphos® (N-P-K) (2.5 kg/100 l), COMPO Austria, GmbH, and Bai-plus (calcium) (1.8 kg/100l), Bayer Ltd., Thailand] were injected to the irrigation system with mechanical injectors (DI 16, Dosatron®, France). Tomato plants were supported by ropes which were fixed to the structure of the greenhouse.

Intra-plant distribution of C. claratris

In the first trial, the distribution of *C. claratris* on leaves, buds, fruits and flowers of tomatoes were determined in the open and net-house using destructive sampling techniques. The aim of this experiment was to select the most consistent plant parts for subsequent monitoring and sampling. In this experiment tomato plants were maintained in a single-stem system at a density of 360 plants per house. In both houses, plants were naturally colonized by *C. claratris*. Previous investigations showed that *C. claratris* is the predominant thrips species on tomatoes in the greenhouses on the AIT campus (Premachandra *et al.,* 2004). Data was recorded eight weeks after planting (WAP) of the tomato seedlings into the greenhouses. In each house from 30 randomly selected tomato plants, fully expanded non-senescent leaves, fully opened non-senescent individual flowers, terminal buds (length approximately 5 cm) and fruits (both immature and ripen)

were sampled. Plants in the edge rows were excluded for sampling. To minimize the potential diurnal variations in thrips distribution, all data were gathered between 07:00-08:00 h. For flowers, two additional samplings were made at noon and between 16:00-18:00 h to determine any potential diurnal periodicity of thrips occurrence. All samples were separately collected using self-sealing plastic bags by pulling them over leaves, buds or flowers, then breaking the stem and immediately sealing the bag to avoid any losses of thrips. Thereafter, the sealed bags were transported to the laboratory. Tomato leaves, buds and fruits were washed three times for about 10 s in a plastic box (15 × 9 cm) containing 250 ml of 70% ethanol. Subsequently, the thrips-containing solution was poured into a conical flask (200 ml), shook thoroughly, and stored for 30 min for settling. Thereafter, the supernatant was gently decanted to 50 ml and the remaining suspension was poured on to a counting plate and the thrips were then counted under a stereo-microscope. In case of flowers, the petals were carefully dissected and transferred into a Petri dish (8.5 × 1.5 cm) containing 70% ethanol to extract the thrips. Thereafter, they were also counted under a stereo-microscope. Thrips that remained in the plastic bags were also included for the counts.

A second trial was conducted to determine the vertical distribution of thrips on leaves. Data was recorded only on leaves because *C. claratris* densities in the previous experiment were always higher on leaves than on other plant parts (see the results section). For this, the plants were divided into three different strata, i.e., lower (0-50 cm), middle (51-100 cm) and upper (>101 cm), above the soil surface. At least ten plants were randomly selected, and fully expanded non-senescent

leaves were taken from each stratum. Thrips counts were made as previously described.

Inter- and Intra-plant distribution of C. claratris over time

For this, three different trials were carried out. The first trial was conducted to investigate the vertical distribution pattern of *C. claratris* over time using presence-absence sampling techniques. The trial was carried out from June to September 2002 in an open and closed net-house with a plant density of 360 tomatoes (cv. King Kong II) arranged in six rows. Tomato plants were maintained in single- and double-stem systems, arranged in alternating sequence and occupying in total four rows (60 plants / row). Plants in the two edge rows were cultivated in a single-stem manner. Forty plants were randomly selected in each stem system, i.e., 20 plants per row, for weekly monitoring. Monitoring commenced one WAP of tomato seedlings into the greenhouse and continued for ten consecutive weeks. On each monitoring date, the total number of leaves and the number of thrips-infested leaves, i.e., leaves showing thrips feeding damage were recorded with respect to the four different plant strata, i.e., 0-50, 51-100, 101-150, 151-200, and > 200 cm above the soil surface. In the double-stem system, the second stem emerged after the 4th WAP, and thus data collection commenced here from 4th WAP onwards in the 51-100 cm stratum.

A second trial was conducted from January to March 2003 in a closed-net house that was equally partitioned with screens (Econet T, pore size 0.05 mm, Ludvig Swensson, Netherlands) into two partitions. Three weeks old tomato seedlings (n

= 360; cv. King Kong II) were arranged in three rows (60 plants/row) per partition in a single stem system. Monitoring was initiated one WAP of tomato seedlings into the greenhouse and continued until the thrips population declined to low infestation levels, i.e., < 20% attacked leaves per plant. All plants, including the ones in the edge rows, were weekly monitored for thips infestation. Prior to the onset of the monitoring the leaves were numbered in an ascending manner, starting from the cotyledons. On each monitoring date, the total number of leaves and the number of thrips-attacked leaves, together with their position on the stem, were recorded for each plant. In addition, density estimates were made on each sampling date using the above mentioned destructive sampling methods. At least 20 leaf samples were selected from each row in the two sections. Total number of *C. claratris* adults and larvae per leaf and the total leaf area of sampled leaves were recorded using a leaf area meter (LI-3100 area meter, LI-COR. Inc., Nebraska, USA). Moreover, the spatial dispersion pattern of *C. claratris* on tomato leaves was determined by means of Taylor's power law (Taylor, 1961), using combined larval and adult counts in the two partitions over four sampling occasions. Taylor's power law is defined as $s^2 = am^b$, which is the regression of $\log(s^2) = \log(a) + (b) \log(m)$, where s^2 is the variance, m is the mean and *a* and *b* are the intercept and slope of the coefficients, respectively. The intercept (*a*) is a sampling factor and the slope (*b*) is a measure of dispersion of a species with b>1, b=1 and b<1 indicating aggregated, random and regular distribution, respectively.

The third trial was conducted from June to July 2004 also in a sub-divided net-house (for details see previous section). However, only one half of the house was

used for the trial. Three weeks old tomato plants were transplanted into this section in three rows as described previously. Data were recorded for seven consecutive weeks starting from one WAP of the seedlings into the greenhouse. At least ten plants per row were randomly selected, and one fully expanded non-senescent randomly selected leaf per plant was destructively sampled and brought to the laboratory for counting the number of thrips (for details see previous section).

Statistical analysis

Initially, data were subjected to Shapiro-Wilk's test for normality and Brown and Forsythe's test for homogeneity of variance (SAS Institute, 1999); whenever normality and variance homogeneity were violated data was subjected to arcsine transformation. Thrips counts and sex ratios on different plant parts in two types of greenhouses were compared using student's t test. In addition, analysis of variance (ANOVA) was performed for the comparison of thrips counts on different plant parts, diurnal periodicity on flowers and leaf infestation levels among the different plant strata using the GLM procedure in SAS (SAS Institute, 1999). Pearson's correlation analysis was used to determine the relationship between thrips infestation and time, and leaf area of tomatoes and thrips density. The relationship between the percentage infested leaves and the thrips density, and the co-efficients of Taylor's power law (Taylor, 1961) were determined by linear regression. The slopes were compared using dummy variables in the regression analyses (SAS institute, 1999).

3.4 Results

Intra-plant distribution

Compared to the open house in the net-house, adult and larval thrips density was significantly higher on tomato leaves, flowers and fruits (for leaves t = 3.97, P < 0.0002; flowers t = 2.50, P < 0.0149; fruits t = -6.13, P < 0.0001). In both houses, significantly higher thrips numbers were recorded on leaves than on other plant parts (Table 3.1). In both greenhouse types no thrips were detected on the buds. Larvae predominated on the leaves, whereas on fruits in both houses adults outnumbered the larvae. On flowers only adult *C. claratris* were found (Table 3.1). The sex ratio did not differ significantly on flowers and leaves (flowers net-house t = -1.76, P > 0.0823; open house t = -0.51, P > 0.6147; leaves net-house t = -0.46, P > 0.6464; open house t = 0.47, P > 0.6376) whereas on fruits the proportion of male thrips was significantly higher in both houses (net-house t = -6.07, P < 0.0001; open house t = -3.69, P < 0.0004). On flowers no significant diurnal periodicity in adult thrips occurrence was found (net-house F = 2.42; d.f. = 2, 54; P > 0.0989; open house F = 1.99; d.f. = 2, 54; P > 0.1450).

Table 3.1 Mean number (± SE) of *Ceratothripoides claratris* (adults and larvae) recorded on different plant parts of tomatoes in an open and closed greenhouse

Plant structure	Mean (± SE) number of thrips					
	Closed net-house			Open plastic-house		
	Adults	Larvae	Total	Adults	Larvae	Total
Buds	0.00 ± 0.00Ac	0.00 ± 0.00Ab	0.00 ± 0.00b	0.00 ± 0.00Ab	0.00 ± 0.00Ab	0.00 ± 0.00c
Flowers	1.14 ± 0.20Ac	0.00 ± 0.00Bb	1.14 ± 0.20b	0.55 ± 0.12Ab	0.00 ± 0.00Bb	0.55 ± 0.12c
Fruits	5.40 ± 0.50Ab	2.07 ± 0.61Bb	7.47 ± 0.74b	1.78 ± 0.24Ab	0.63 ± 0.19Bb	2.41 ± 0.36b
Leaves	8.83 ± 1.28Ba	27.93 ± 5.67Aa	36.76 ± 6.44a	3.43 ± 0.26Ba	8.30 ± 1.34Aa	10.73 ± 1.70a

For each type of greenhouse means followed by the same lower and upper case letters in columns and rows, respectively, indicate no significant differences (P = 0.05, LSD multiple range test [SAS Institute 1999]).

Combined adult and larval thrips densities varied significantly among the different vertical strata of the tomato foliage irrespective of the house type (net-house F = 70.94, d.f. = 2, 26, P < 0.0001; open house F = 86.81, d.f. = 2, 27, P < 0.0001). The thrips density significantly decreased towards the apical point of the plants, and significantly greater numbers of adults and thrips larvae were recorded on the lower compared to the top stratum in both houses (net-house F = 70.84, d.f. = 2, 26, P < 0.0001; open house F = 86.81, d.f. = 2, 27, P < 0.0001). In general, both adult and larval counts declined towards the top of the tomato plants.

Intra- and inter-plant distribution

In both houses, in the single-stem system, in each plant strata significant positive correlations were found between the extent of leaf infestation by *C. claratris* and time (open and net-houses 0-50 (r = 0.0722 and 0.8035), 51-100 (r = 0.8254 and 0.8657) and 101-150 cm (r = 0.6355 and 0.5635), all P < 0.0001; net-house > 150 cm (r = 0.3239, P < 0.0440); open house > 150 cm (r = 0.3858, P < 0.0007) (Tables 3.2 and 3.3).

In the double-stem system, in the net-house, significant positive correlations were found between leaf infestation by *C. claratris* and time only in the two lower plant strata (1st stem 51-100 cm r = 0. 7732, P > 0.0001; 101-150 cm r = 0.5079, P < 0.0001; > 150 cm 0.2239, P > 0.1829; 2nd stem 51-100 cm r = 0.8305, P < 0.0001; 101-150 cm r = 0.6243, P < 0.0001; > 151 cm r = 0.4913 P > 0.1331). In contrast, in the open houses, positive correlations were found in all the plant strata (1st stem 51-100 cm r = 0.7655, P > 0.0001; 101-150 cm r = 0.6061, P < 0.0001; > 150 cm r

= 0.5385, *P* > 0.0001; 2nd stem 51-100 cm r = 0.8023, *P* < 0.0001; 101-150 cm r = 0.6278, *P* < 0.0001; > 150 cm r = 0.6060 *P* > 0.0001). The development of thrips infestation on the two stems significantly decreased towards the apical point of the tomato plants during the entire observational period in both house types (Tables 3.4 and 3.5). The development of thrips infestation on the two stems significantly decreased towards the apical point of the tomato plants during the entire observational period in both house types (Tables 3.4 and 3.5).

Table 3.2 Mean percentage (± SE) infestation of *Ceratothripoides claratris* on four different strata of tomato plants, maintained in a single-stem system in a net-house over a period of ten weeks.

Week	Mean percentage (± SE) infestation			
	0-50 cm	51-100 cm	101-150 cm	>150 cm
1	48.18 ± 5.49	nl	nl	nl
2	65.67 ± 2.12a	0.00 ± 0.00b	nl	nl
3	80.89 ± 2.42a	11.11 ± 1.82b	nl	nl
4	84.55 ± 2.91a	13.20 ± 2.86b	nl	nl
5	91.11 ± 1.43a	29.18 ± 4.63b	nl	nl
6	97.11 ± 3.08a	48.40 ± 5.30b	11.67 ± 6.89c	nl
7	100.00 ± 0.00a	73.78 ± 6.10b	38.14 ± 8.73c	nl
8	100.00 ± 0.00a	83.30 ± 4.57b	47.58 ± 6.12c	19.05 ± 14.29d
9	100.00 ± 0.00a	85.35 ± 3.70b	58.31 ± 5.74c	30.05 ± 6.97d
10	100.00 ± 0.00a	100.00 ± 0.00a	93.31 ± 2.98b	46.24 ± 8.06c

nl – no leaves. Means followed by the same letters within rows are not significantly different (*P* = 0.05, LSD multiple range test [SAS Institute 1999]).

Table 3.3 Mean percentage (± SE) infestation of *Ceratothripoides claratris* on four different strata of tomato plants, maintained in a single-stem system in an open house over a period of ten weeks.

Week	Mean percentage (± SE) thrips infestation			
	0-50 cm	51-100 cm	101-150 cm	>150 cm
1	43.28 ± 2.02	nl	nl	nl
2	63.45 ± 1.73a	0.00 ± 0.00b	nl	nl
3	81.09 ± 1.46a	0.00 ± 0.00b	nl	nl
4	96.71 ± 1.28a	14.27 ± 3.14b	nl	nl
5	100.00 ± 0.00a	33.41 ± 5.14b	nl	nl
6	100.00 ± 0.00a	69.04 ± 3.02b	19.49 ± 5.10c	nl
7	100.00 ± 0.00a	74.24 ± 2.20b	53.98 ± 7.16b	nl
8	100.00 ± 0.00a	80.50 ± 3.28b	65.91 ± 5.65b	21.85 ± 7.37c
9	100.00 ± 0.00a	86.08 ± 2.21b	67.13 ± 4.86b	39.84 ± 7.79c
10	100.00 ± 0.00a	97.98 ± 1.39b	83.86 ± 4.29b	50.76 ± 5.71c

nl – no leaves. Means followed by the same letters within rows are not significantly different (P = 0.05, LSD multiple range test [SAS Institute 1999]).

In the second trial, in both partitions of the net-house infestation by *C. claratris* started one WAP of tomato seedlings (Figures 3.1 A-D). At four WAP 100% of the plants in all rows of both partitions were thrips infested (Figures 3.1 B and D). At five WAP percentage thrips-infested leaves peaked, and sharply declined thereafter (Figures 3.1 A and C). Cumulative percentage thrips infestation on individual leaves of tomato plants confirmed that infestation commences on the lower leaves and gradually spreads towards the apical point of the plant (Figures 3.2 A and B).

Table 3.4 Mean percentage (± SE) infestation of *Ceratothripoides claratris* on three different strata of tomato plants, maintained in a double-stem system in a net-house over a period of ten weeks.

Week	Mean percentage (±SE) infestation					
	1st stem			2nd stem		
	51-100 cm	101-150 cm	>150 cm	51-100 cm	101-150 cm	>150 cm
1	nl	Nl	nl	nl	nl	nl
2	nl	Nl	nl	nl	nl	nl
3	nl	Nl	nl	nl	nl	nl
4	27.67± 3.54	Nl	nl	1.40 ± 0.65	nl	nl
5	34.06 ± 3.30	Nl	nl	8.76 ± 3.26	nl	nl
6	58.60 ± 4.60a	13.26 ± 5.40b	nl	34.63 ± 5.52a	12.14 ± 6.39b	nl
7	74.19 ± 4.32a	48.36 ± 9.04b	nl	67.90 ± 5.30a	19.65 ± 6.20b	nl
8	84.46 ± 3.04a	61.72 ± 6.74b	30.00 ± 9.74c	69.06 ± 4.88a	35.37 ± 4.36b	nl
9	93.82 ± 1.97a	69.29 ± 5.53b	13.07 ± 4.65c	80.77 ± 4.24a	39.58 ± 4.42b	28.13 ± 11.02c
10	100.00 ± 0.00a	85.81 ± 5.00b	41.10 ± 7.97c	100.00 ± 0.00a	80.54 ± 5.50b	23.24 ± 1.39c

nl – no leaves. Means followed by the same letters within rows with respect to two stems are not significantly different ($P = 0.05$, LSD multiple range test [SAS Institute 1999]).

Table 3.5 Mean percentage (± SE) infestation of *Ceratothripoides claratris* on three different strata of tomato plants, maintained in a double-stem system in an open-house over a period of ten weeks.

Week	Mean percentage (±SE) infestation					
	1st stem			2nd stem		
	51-100 cm	101-150 cm	> 150 cm	51-100 cm	101-150 cm	> 150 cm
1	nl	nl	nl	nl	nl	nl
2	nl	nl	nl	nl	nl	nl
3	nl	nl	nl	nl	nl	nl
4	36.63 ± 4.19	nl	nl	18.73 ± 3.99	nl	nl
5	54.47 ± 2.98	nl	nl	37.96 ± 4.14	nl	nl
6	86.81 ± 3.47a	19.03 ± 3.98b	nl	78.62 ± 4.03	12.55 ± 3.43	nl
7	96.52 ± 1.41a	24.02 ± 4.01b	nl	91.93 ± 2.41	25.16 ± 4.08	nl
8	100.00 ± 0.00a	43.37 ± 5.50b	4.04 ± 3.19c	100.00 ± 0.00a	40.12 ± 5.71b	5.48 ± 2.80c
9	100.00 ± 0.00a	65.49 ± 4.58b	16.05 ± 4.84c	100.00 ± 0.00a	63.74 ± 4.89b	14.44 ± 4.93c
10	100.00 ± 0.00a	71.06 ± 4.11b	30.79 ± 4.28c	100.00 ± 0.00a	70.40 ± 5.03b	32.08 ± 3.91c

nl – no leaves. Means followed by the same letters within rows with respect to two stems are not significantly different (P = 0.05, LSD multiple range test [SAS Institute 1999]).
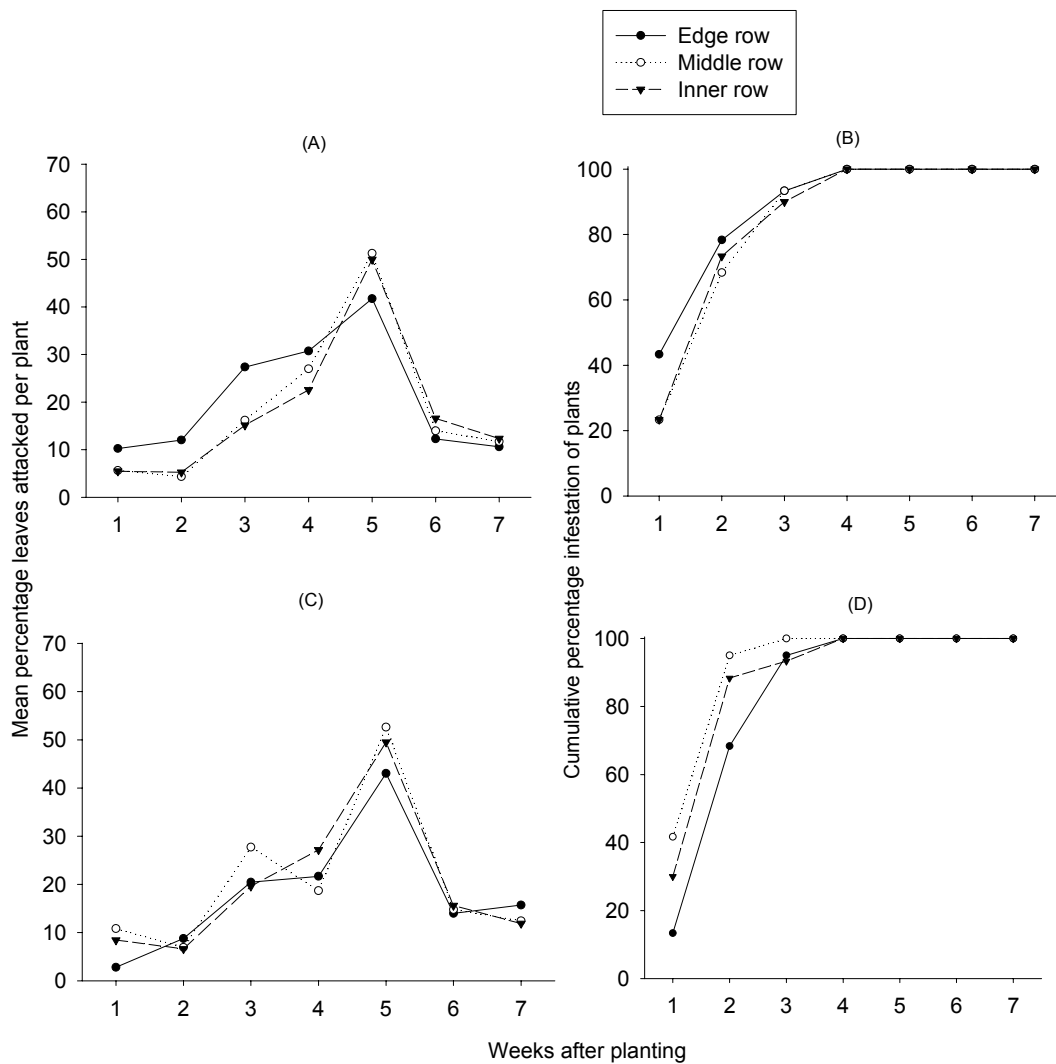
Figure 3.1 Infestation of tomatoes by *Ceratothripoides claratris* in a net-house split in two partitions (partition 1 = (A) and (B), and partition 2 = (C) and (D)) over a period of seven weeks. (A) and (C) show mean percentage of infested leaves per tomato plant and (B) and (D) cumulative percentage of infested plants.
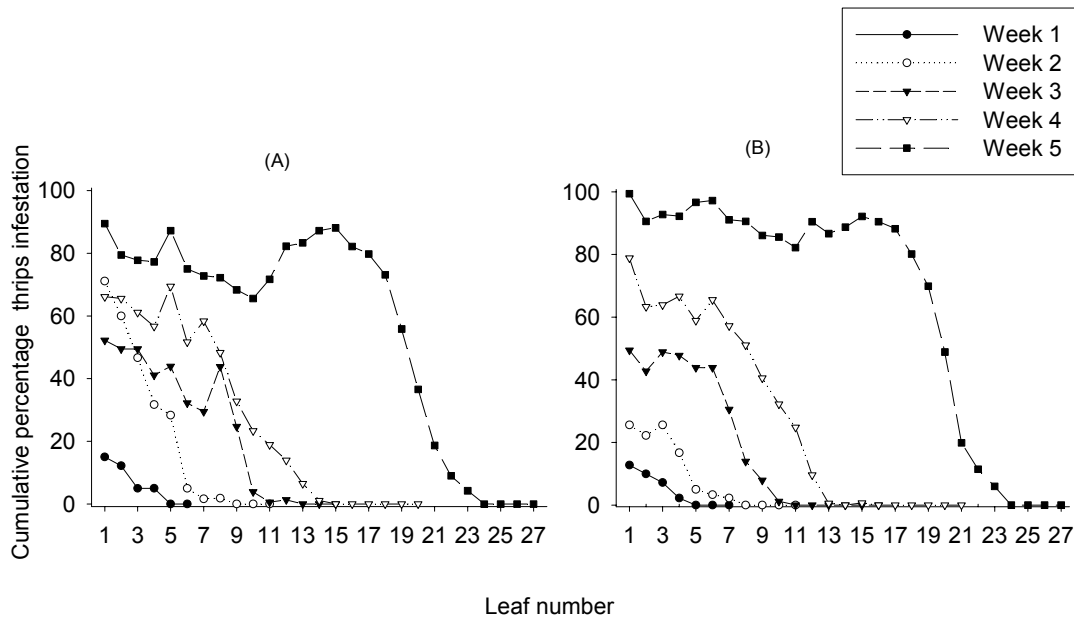
Figure 3.2 Vertical distribution of *Ceratothripoides claratris* on tomato plants in two partitions (A and B) of a net-house over a period of five weeks. Lines show cumulative percentage of infestation of leaves numbered in an ascending manner from bottom to top.

Density estimates showed that *C. claratris* larvae always largely outnumbered thrips adults on tomato leaves (Figures 3.3 A and B). Larval density peaked at five WAP and sharply decreased thereafter while no such population trend was observed in adults (Figures 3.3 A and B). Significant linear relationships were detected between the percentage infested leaves and thrips density per leaf of tomatoes in two partitions (partition 1 R^2 = 0.9345, F = 71.39, P < 0.0004; partition 2 R^2 = 0.9173, F = 55.49, P < 0.0007). No significant correlation between leaf area and thrips density was detected (P > 0.2499).
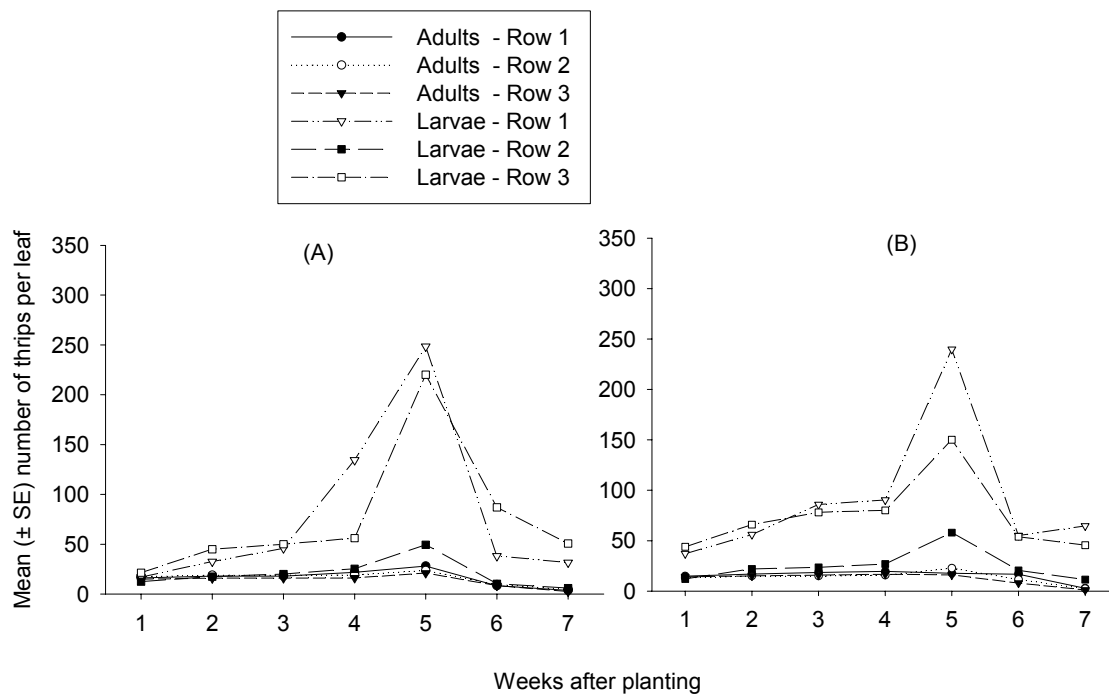
Figure 3.3 Mean (± SE) density of adults and larvae of *Ceratothripoides claratris* on tomato leaves in a net-house split in two partitions (partition 1 = (A) and partition 2 = (B) over a period of seven weeks. Plants were arranged in three rows per partition.

The estimates of Taylor's power law showed that the relationships between log variance and log mean for thrips numbers were linear for all four weeks (Table 3.6). As the slopes of the four regression lines did not differ significantly, data of four weeks were and a common regression line was fitted (Y = −1.08823 + 2.26780X, R^2 = 0.76, F = 304.20, *P* < 0.0001). The value of the slope (b) was significantly >1 (*P* < 0.0001), indicating a clumped distribution of *C. claratris* on tomato leaves.

Table 3.6 Regression statistics for Taylor's power law for combined numbers of adults and larvae of *Ceratothripoides claratris* on the foliage of tomatoes over a period of four weeks.

Week	Regression equation	R^2	F value	*P* >F
1	Y = −1.15776 + 2.31753X	0.67	48.63	0.0001
2	Y = −0.22187 + 1.91317X	0.72	61.08	0.0001
3	Y = −1.29735 + 2.37372X	0.77	75.19	0.0001
4	Y = −0.17025 + 1.66580X	0.30	8.64	0.0074

As in the second trial, in the third trial, larval densities were considerably higher than adult thrips numbers (Figures 3.4 A and B). Yet in contrast to the previous trial larval densities strongly varied among the three rows (Figure 3.4 B) whereas adult densities remained more or less constant (Figure 3.4 A). Peak larval densities were recorded at four, five and six WAP in first, second and third row, respectively, always followed by a sharp decline (Figure 3.4 B).
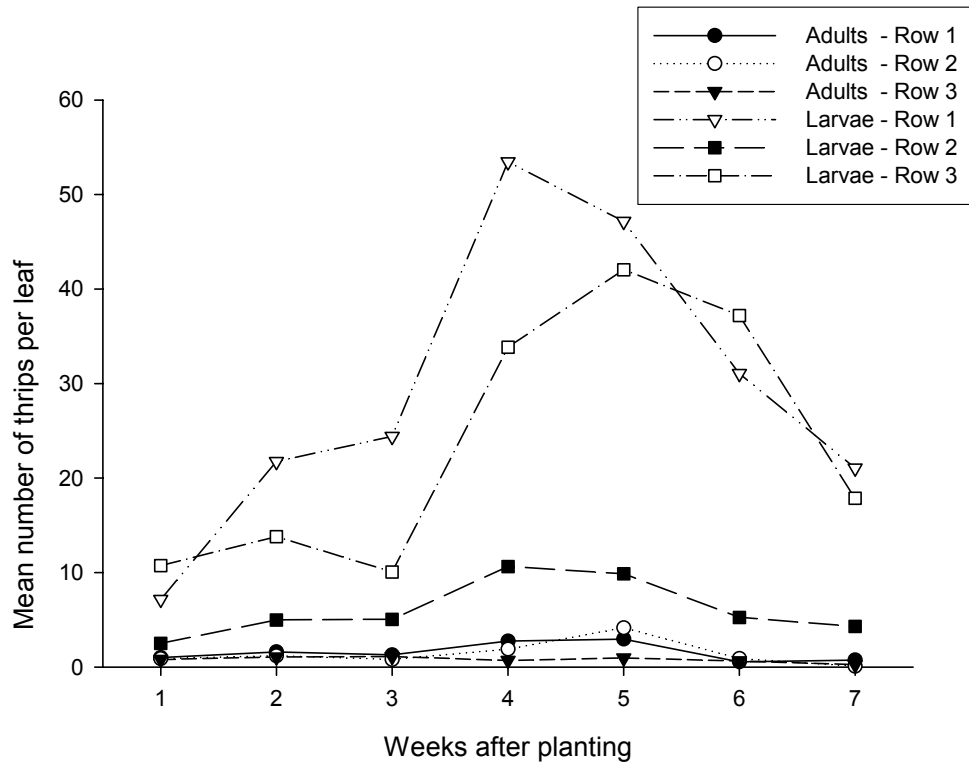
Figure 3.4 Mean (± SE) density of adults and larvae of *Ceratothripoides claratris* on tomato leaves in a net-house over a period of seven weeks. The plants were arranged in three rows.

3.5 Discussion

Among others the objectives of this study were to investigate the intra-plant distribution of *C. claratris* on tomatoes. Thrips densities differed greatly between the closed net-house and the open side-wall house, enabling us to study the effects of different *C. claratris* densities on intra-plant distribution. Evidently, the pore size (0.18 mm) of the nets in the closed house did not completely prevent a thrips invasion from outside. One possible reason for the higher thrips densities in

the closed net-house compared to the open house might have been more favorable microclimatic conditions, particularly in terms of higher temperature and relative humidity. Irrespective of the density, *C. claratris* adults and larvae showed a distinct foliage-biased distribution on tomatoes. Preferential distribution on different plant structures differs among thrips species. Adults of *Frankliniella occidentalis* (Pergande), *F. tritici* (Fitch), *Thrips fuscipennis* Haliday and *T. major* (Uzel) prefer to inhabit flowers compared to the other plant parts (Kirk, 1985a; Atakan *et al.,* 1996; Cho *et al.,* 2000a). In contrast, *T. tabaci* Lindeman (Atakan *et al.,* 1996) and *F. fusca* (Hinds) (Palmer *et al.,* 1989) are mainly foliage feeders. Preference for specific plant parts can also vary among the different life-stages of a given thrips species. For instance in *F. occidentalis*, though a flower feeder, > 85% of the larvae were found on the foliage of bell peppers and cucumbers (Higgins, 1992), indicating niche separation between adults and larvae of *F. occidentalis*. In contrast, for *C. claratris* we did not detect any niche separation between adults and larvae on tomatoes. In addition to the foliage, *C. claratris* occupies flowers and fruits. Lack of noticeable flower damage and the absence of thrips larvae in flowers illustrate that adult thrips inhabit flowers but do not use them as a feeding or reproduction site. Pollen and nectar can be important food sources for thrips (Trichilo and Leigh, 1988; Higgins, 1992). Pollen is rich in protein (Todd and Bretherick, 1942) and in several thrips species positively affects fecundity (Bournier *et al.,* 1979), oviposition rate (Kirk, 1985b), development rate (Trichilo and Leigh, 1988) and larval growth (Murai and Ishii, 1982). Thus, in our study female *C. claratris* most likely aggregated on flowers to feed on pollen and

nectar, while male thrips may visit flowers to copulate with females in addition to feeding on additional food sources. We did not record any diurnal periodicity in occurrence of *C. claratris* on flowers, corroborating earlier findings of Cho *et al.* (2000a) with *F. tritici* and *F. fusca* on tomatoes. However, in *F. occidentalis* the same authors observed significantly higher thrips numbers on tomato flowers in the morning than in the afternoon, indicating species-specific diurnal periodicity in thrips. In our study we detected significant differences in the sex ratio of *C. claratris* only on fruits of tomatoes, with more males than females present. In *F. occidentalis*, Higgins (1992) recorded more females than males on flowers of bell peppers, whereas in *T. major* males outnumbered females on *Calystegia sepium* (L.) (Convolvulaceae) flowers (Kirk, 1985b). The male-biased sex ratio on fruits indicates that tomato fruits are preferred mating and feeding sites for *C. claratris*. Reitz (2002) speculated that male thrips have a tendency to aggregate in certain locations for mating, whereas females tend to leave these sites after mating, supporting our findings on *C. claratris*. The feeding and oviposition of *C. claratris* on tomato fruits cause malformation and scarification, leading to a downgrading of the fruits. Evidently, fruit attack in tomatoes by *C. claratris* commences at a later stage of the infestation cycle, i.e., when the leaves of tomatoes are already hardened and deteriorated as a result of serious feeding by thrips.

We recorded a gradual upward development of *C. claratris* infestations on tomatoes over time irrespective of the stem-system and the house type used. This

was further confirmed by the low abundance of thrips on the top leaves and the absence of *C. claratris* stages on the apical buds. In contrast, Cho *et al*. (2000b) and Salguero-Navas *et al.* (1991) recorded higher densities of *T. palmi* Karny and *F. occidentalis* on the upper canopy of potatoes and tomatoes, respectively. However, *F. fusca* density did not significantly differ among the upper and lower strata in tomatoes (Cho *et al.,* 2000a). Among others vertical distribution pattern in insects is influenced by the species-specific behavior and flying capacity (Ramachandran *et al.,* 2001). The clear preference of *C. claratris* for lower plant strata in tomatoes might be explained by its sluggish movements and/or week flying capacity.

One week after transplanting in one partition of the net-house comparatively higher *C. claratris* numbers were recorded in the edge row. Body width in *C. claratris* ranges from 0.18 - 0.23 mm (Rodmui, 2002). Hence, most likely the pore size (0.18 mm) of the nets did not completely exclude thrips invasion from the outside. Presently little is known on the sources of *C. claratris* infestations in greenhouses. In addition to tomatoes, *C. claratris* was recorded on *Luffa acutangula* L. (Cucurbitaceae) and *Clitoria ternatea* L. (Fabaceae) in field surveys in Thailand (Jangvitaya, 1993). Moreover, it can attack and successfully reproduce on cucumbers, *Cucumis sativus* L. (Cucurbitaceae), eggplants, *Solanum melongena* L. and *S. xanthocarpum* Schard and Wendl (both Solanaceae), pumpkin, *Curcurbita moschata* (Duch.) Poir (Cucurbitaceae), *Vigna sinensis* (L.) Savex- Hass and *V. unguilulata* (L.) Walp (both Fabaceae), and bird pepper,

Capsicum annuum L. (Solanaceae) (D. Premachandra, unpublished data). Finally, preliminary data suggest that *C. claratris* can also accomplish its life cycle on several weed species found in the vicinity of the greenhouse complex at AIT (D. Premachandra, unpublished data). Weeds can serve as reservoirs for many thrips species and their vectored tospoviruses (Duffus, 1971; Tresh, 1981; Chatzivassiliou and Boubourakas, 2001). Possibly a *C. claratris* invasion from yet to be identified sources outside the greenhouses, coupled with its high reproductive potential at high temperatures, frequently prevailing in greenhouses in the humid tropics (Premachnadra *et al.,* 2004), as well as the arrangement of the pots within the rows with no inter-pot distance, were responsible factors for the rapid distribution of *C. claratris* in the net-houses. Other factors for the relatively higher *C. claratris* infestation in the edge row of the first and the middle row of the second partition might have been the prevailing wind direction and the wind stream caused by the exhaust fan of the opposite greenhouse, respectively.

In the first inter-plant distribution trial, four weeks after transplanting the tomato seedlings into the net-house, 100% of the plants were infested by *C. claratris*. One week later the highest thrips density was recorded, probably a result of a previous oviposition and subsequent emergence of the larvae. The following sharp drop in infestation was most likely due to a deterioration of the host plant quality of the tomatoes as a result of severe damage.

An important reason for the differing *C. claratris* densities between the two inter-plant distribution experiments might have seasonal effects. Thrips densities were

relatively low during the rainy season (April to September) compared to the dry season (from October to March). Similar findings have been observed in a greenhouse study in central Thailand from November 2000 to January 2002 using a different tomato variety (Rodmui, 2002).

Estimates of Taylor's power law clearly indicated a highly aggregated distribution of *C. claratris* on tomatoes. This information will facilitate the future development of sampling plans for *C. claratris*. An aggregated distribution has been observed in several other thrips species, e.g., *T. tabaci*, *T. angusticeps* Uzel, *F. occidentalis*, *F. intonsa* (Trybom) and *Aeolothrips intermedius* Bagnall (Deligeorgidis *et al.,* 2002; Steiner, 1990).

In conclusion, our results provide basic information for the future development of monitoring programs for *C. claratris*. Though presence-absence sampling is generally believed to be not a very reliable sampling method, the large number of observations made over an extended period of time in our study largely increased the accuracy of the results. In addition, the linear relationship between thrips infested leaves and thrips density indicates that in future the proportion of infested leaves can be used for density estimations. Moreover, at extremely high densities, as observed in this study, density estimates are very time-consuming to obtain and thus less practical. Our results clearly indicate that for *C. claratris* the most consistent sampling unit in tomatoes is the leaf. As larvae are the predominant developmental stage on the leaves, in future monitoring programs larval counts on tomato leaves will be appropriate to assess the population densities of *C. claratris*.

Monitoring should start during an early phase of the crop cycle, and first observations should be made in the lower parts of the tomato plants and continue upwards over time. As *C. claratris* is strongly attracted to white and blue colors (Wickramarachchi, 2004), white and blue sticky traps could be used in future for monitoring purposes. In ongoing experiments we are studying the relationship between thrips densities and damage in tomatoes, and the sources of *C. claratris* infestations outside the greenhouses, and the role of alternative host plants for the population dynamics of the thrips and the epidemiology of its vectored tospovirus.

4   *Ceratothripoides claratris*, a new vector of a tospovirus infecting tomatoes in Thailand[3]

4.1   Abstract

Ceratothripoides claratris, a predominant thrips species on tomatoes in Thailand, was tested for its vector competence and efficiency to transmit Capsicum chlorosis virus (CaCV) (isolate AIT) to tomatoes. The results showed that the efficiency of adult-stage transmission is influenced by the larval stage at which the virus was acquired. Adult *C. claratris* showed 69% transmission efficiency after obtaining the virus as first instar larvae. However, when second instar larvae fed on virus-infected plants the percentage of adult transmitters significantly decreased (48%). Considerable transmission efficiency of up to 47% was detected with second instar larvae of *C. claratris* which had acquired the virus as first instar larvae. Transmission efficiency did not significantly differ between adult males and females, irrespective of the larval stage at which the virus was acquired. Highest transmission efficiency for CaCV was recorded in adult *C. claratris* that derived from second instar larvae which had been previously collected from infected tomato plants in a greenhouse. The lowest transmission was observed in adults directly collected from infected tomato plants in the greenhouse. Fluctuations in thrips infestations and spread of the virus in the greenhouse were investigated and discussed.

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4.2 Introduction

Worldwide tospoviruses (Genus: *Tospovirus*, Family: *Bunyaviridae*) cause severe losses in various economically important crops (Brittlebank, 1919, Francki and Hatta, 1981; Ullman, 1996). They are exclusively transmitted by several thrips species in a persistent propagative manner (Wijkamp *et al.*, 1993; Ullman *et al.*, 1993). The first instar larvae acquire the virus from viruliferous plants (Ullman *et al.*, 1991, 1992, 1995; German *et al.,* 1992) and after a latent period it can be transmitted by second instar larvae and adults (Wijkamp and Peters, 1993; Sakimura, 1962). To date of the approximately 5,500 known species of Thysanoptera, only 10 have been recorded as vectors of tospoviruses (Ullman *et al.,* 2002).

Since 2001, tomato plants in fields and greenhouses at the Asian Institute of Technology (AIT) in Bangkok, Thailand, showed symptoms of a severe tospovirus infection. Serological studies confirmed that the virus belongs to the serogroup IV of tospoviruses. Sequence analysis of the N-gene (D. Knierim and E. Maiss, unpublished data) revealed a close relationship to a tospovirus recently described in Queensland, Australia, by McMichael *et al.* (2002) which was tentatively named Capsicum chlorosis virus (CaCV). In addition, similar tospovirus infections have been recorded in north-eastern parts of Thailand (Pongsapich and Chiemsombat, 2002). The predominant thrips species on field- and greenhouse-grown tomatoes in central Thailand in general, and in the greater Bangkok area in particular is *Ceratothripoides claratris* (Shumsher) (Thysanoptera: Thripidae) (Murai *et al.,*

2000; Rodmui, 2002; Premachandra *et al.,* 2004). Moreover, symptoms of tospovirus infections on tomatoes were always associated with the presence of this thrips species, and severe *C. claratris* infestations coupled with a virus infection resulted in the death of the plants prior to fruit setting (D. Premachandra, unpublished data). Thus, we hypothesized that the tospovirus is vectored by *C. claratris.* The principal objective of this research was therefore to verify the potential vector competence of *C. claratris* in transmitting this tospovirus species. In addition, acquisition of the virus by first and second instar larvae and transmission efficiencies of second instar larvae and adults were studied under laboratory conditions. Finally, the spread of the virus and infestation pattern of *C. claratris* were investigated under greenhouse conditions at AIT, and the transmission efficiency of thrips larvae and adults collected from infected tomato plants in the greenhouse were determined.

4.3   Materials and Methods

Thrips

Virus-free *C. claratris* colonies were reared on potted tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) in thrips-proof cages at 25 ± 2°C and a L12:D12 h photoperiod. The culture was originally started from adult *C. claratris* collected from tomato plants at AIT. For the acquisition tests synchronized-aged first instar larvae (L1) were obtained by allowing female *C. claratris* (approximately 500) to oviposit on excised healthy tomato leaflets for 4 h in a sealed Petri dish (8.5 × 1.5 cm) containing on the bottom a thin layer

(approximately 1 cm thickness) of a mixture of Plaster of Paris and charcoal (ratio 9:1). In order to keep the leaflets viable, the petioles were wrapped with water-soaked cotton. After 4 h, the adults were removed from the leaflets with a fine camel hair brush, and the eggs were allowed to develop into L1 or L2. The L2 stage was determined by the occurrence of an exuvia.

Tospovirus isolate and test plants

Capsicum chlorosis virus (Isolate AIT; CaCV-AIT) was originally detected in tomato plants (cv. King Kong II) in greenhouses at AIT. The virus was propagated and maintained after mechanical inoculation in *Nicotiana benthamiana* Domin. (Solanaceae) plants. Inoculations were made on plants at the 5-leaf stage with sap of infected tomato or *N. benthamiana* leaflets grinded in 0.01 M phosphate buffer (ratio 5:1), pH 7.0, containing 0.1% sodium sulfite and a small amount of Celite as an abrasive. In the acquisition experiments, two weeks-old tomato seedlings (cv. King Kong II) were mechanically inoculated with extracts of systemically infected leaves of *N. benthamiana* plants. Both tomato and *N. benthamiana* plants were grown at 25 ± 2°C and a L12:D12 h photoperiod. Tomato leaflets showing systemic symptoms (Figure 4.1), generally appearing 14-21 days after mechanical inoculation, were selected for the acquisition tests.

Figure 4.1 Tomato seedling showing symptoms of CaCV-AIT after mechanical inoculation

Virus detection by enzyme-linked immunosorbent assay (ELISA)

Standard enzyme-linked immunosorbent assay (ELISA) (Clark and Adams, 1977; Resende *et al.,* 1991) was conducted for the detection of CaCV-AIT in leaf extracts of infected *N. benthamiana* and tomato plants and to confirm the infection in tomato leaf discs used for the transmission tests. Polyclonal and monoclonal antibodies raised against the N-protein of *Watermelon silver mottle virus* (WSMV) and *Groundnut bud necrosis virus* (GBNV) (Agdia, Inc., Elkhart, ID, USA) were used. In the ELISAs antibodies and conjugated antibodies were applied in a concentration of 1: 200 (wt/vol). Plant extracts were prepared by homogenizing

leaf material at a ratio of approximately 200 mg per ml of PBS-T (2.5 mM KCl, 1 mM KH_2PO_4, 8 mM Na_2HPO_4, 0.14 M NaCl, and 0.5 ml/l Tween 20) containing 20 g / l polyvinylpyrollidone K25 (PVP). Leaf discs from healthy *N. benthamiana* and tomato plants were used as controls. Absorbance values were determined with an EL 312 ELISA-reader (BIO-Tek Instruments, Inc, Vermont, USA) at 405 nm (A_{405}). Wells containing only PBS-T in the sample incubation step were used as blanks. The A_{405} were corrected by subtracting the mean (average of three wells) of the buffer control absorbance values from sample values. Samples having absorbance values at least three times than that of mean healthy controls were considered as positive.

Virus acquisition by first and second instar larvae and transmission by adults

The ability to acquire the virus by L1 and L2 of *C. claratris* was determined by the subsequent capability of the emerging adults to transmit the virus. Newly emerged (0 h) L1 and just molted L2 from the same cohort were given an acquisition access period (AAP) until pupation on systemically infected tomato leaflets with equally high virus titers. Prior to the tests the virus titer in tomato leaflets was checked by DAS-ELISA. Groups of L1 and L2 (at least 40 in each category) were separately placed on infected tomato leaflets. Prior to the AAP, L2 were reared on virus-free tomato leaves (cv. King Kong II). Previously it was observed that in *C. claratris* the late L2 drop from the foliage to pupate in the soil or in the leaf litter (D. Premachandra, unpublished data). Thus, a water-filled Petri dish (8.5 × 1.5 cm)

was placed beneath leaflets to collect the dropping larvae and was controlled every 6 h for the presence of thrips larvae. In preliminary experiments, no detrimental effects for thrips floating on the water surface for up to 6 h were recorded (D. Premachandra, unpublished data). L1 and L2 reared on virus-free tomato leaflets were used as controls. The collected larvae were reared until adulthood in small plastic containers (5 × 4 × 3 cm) containing on the bottom a thin layer (approximately 1.5 cm thickness) of a mixture of Plaster of Paris and charcoal (9:1) at 25 ± 2ºC, 60-70% relative humidity (RH) and a 12L:12D photoperiod. The sex of the adult thrips was determined upon emergence. The transmission efficiency of individual adult thrips, i.e., both males and females, was determined using leaf discs (approximately 10 mm diameter) of healthy tomato plants. Newly emerged adults were individually placed on a tomato leaf disc in 2 ml Eppendorf tubes. The transmission ability of the adult thrips was determined in three successive inoculation access periods (IAP) of 48 h each. After each IAP, the leaf discs were incubated on tap water for six days in 24 well plates (Costar Europe Ltd., Badhoevedorp, The Netherlands) at 25 ± 2ºC. Infection of the leaf discs was estimated by development of local lesions (Figure 4.2) and subsequently confirmed by DAS-ELISA at the end of the incubation period. At least 30 adult thrips deriving each from the L1 and L2, respectively, were tested individually on tomato leaf discs and the experiment was repeated three times. The rate of the virus transmission was calculated as the percentage of leaf discs infected by the virus.
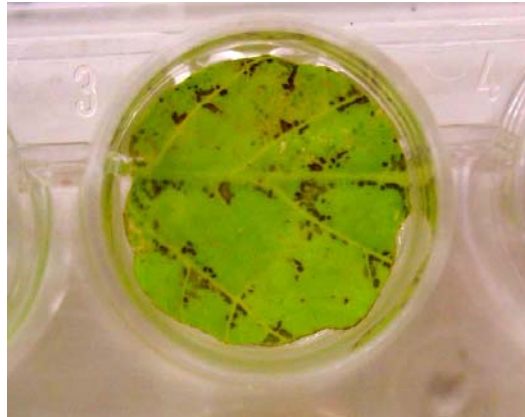
Figure 4.2 Tomato (cv. King Kong II) leaf disc showing local lesions caused by CaCV-AIT infection 5 days after the start of the inoculation access period (IAP). Infected adult *Ceratothripoides claratris* were given an IAP of 48 h on the disc.

In addition to tomato leaf discs, the potential virus transmissibility of adult *C. claratris* was determined on tomato seedlings in microcosm experiments providing the thrips with an IAP of 5 days at 25 ± 2ºC. Previously L1 were given an AAP on infected tomato leaflets and reared until adult emergence following the above mentioned protocols. Adults emerging from larval instars reared on healthy tomato leaves were used as controls. Two weeks-old potted tomato plants were covered with Plexy glass cylinders (10 cm diameter, 30 cm height). The upper opening of the cylinder was closed with thrips-proof nylon gauze (64 µm mesh size) to provide additional ventilation. In addition, four holes were drilled at the upper and lower edges of the cylinder and except for one hole at the lower edge all other holes were covered with thrips-proof nylon gauze to aid ventilation. The

lower uncovered hole was used for releasing adult thrips into the cylinders and was subsequently covered by a piece of glue tape. After the IAP, the cylinder was removed and the seedlings were sprayed with spinosad (12% w/v SC, Dow Agrosciences, Indianapolis, USA) and kept at 25 ± 2°C, 60-70% RH and a 12L:12D photoperiod for symptom development. Percentages infected tomato plants were recorded on the basis of visual symptoms, i.e., brown necrotic spots, and later confirmed by DAS-ELISA. The trial was repeated three times with a minimum of 30 thrips per repetition.

Virus acquisition by first instar larvae and transmission by second instar larvae

In this experiment the ability of L2 to transmit the virus, obtained during the L1, was determined on tomato leaf discs. In addition, after adult emergence the transmission efficiency of unsexed adult thrips was also tested. Cohorts (at least 15) of newly emerged L1 were given an AAP on virus-infected tomato leaflets until the molting to the L2 stage, as confirmed by the presence of exuviae. Immediately after molting, L2 were individually transferred on healthy tomato leaf discs floating on tap water in 24-well plates and allowed to feed there until pupation. Thereafter, the prepupae were removed to the above mentioned small plastic containers and reared until adulthood. The leaf discs were incubated for six days and a possible virus infection was confirmed by DAS-ELISA. The adult-stage transmission efficiency was determined following the previously described protocol. Thrips

larvae reared on healthy tomato leaves were used in the control treatments. The experiment was repeated three times with a minimum of 10 thrips per repetition.

Greenhouse trials

Two trials were conducted in a 200 m^2, closed net-house (Econet M, pore size 0.18 mm, Ludvig Svensson Inc., Kinna, Sweden), sub-divided into two partitions, located at AIT. The experiments were carried out between January to March 2003 and June to August 2004, respectively. Only one partition of the net-house was used for the trials. For this 180 3-weeks-old potted (30 x 25 cm) tomato seedlings (cv. King Kong II) were arranged in three rows with no inter-pot distances within a row. The distance between rows was 160 cm. During the trials mean temperature and RH were 28-30ºC and 70-80%, respectively. Plants were irrigated and fertilized 7-9 times per day with an automised drip irrigation system. One week post-planting (WPP), thrips density (larvae and adults) on tomato leaves was estimated using destructive sampling methods. For this ten leaf samples (10/plant) from ten randomly selected plants were taken from each row. Sampling continued for nine consecutive weeks. In addition, number of virus-infected plants was recorded based on the presence of visual symptoms, i.e., brown lesions on the leaves and ring spots on fruits, in each row at all sampling dates. At least five leaf samples per plant were tested by means of DAS-ELISA for confirmation of the virus infection. In addition, during the second trial, five WPP, adult thrips were randomly collected from tomato plants showing signs of a tospovirus infection and their vector competence was investigated using the tomato leaf disc assay as

previously described. Moreover, late L2 were collected from supposedly infected leaves in the same week, reared in the laboratory until adulthood and their transmission efficacy was then studied using the tomato leaf disc assay. These tests were repeated at least three times with 35 thrips larvae and adults per repetition, respectively.

Statistical analysis

At first, percentage transmission data were subjected to Shapiro-Wilk's test for normality and Brown and Forsythe's test for homogeneity of variance, and whenever normality and variance homogeneity were violated data was subjected to arcsine transformation. Subsequently transmission efficacies between the sexes, and life-stages were compared using student's t-test and analysis of variance (ANOVA) (SAS Institute, 1999).

4.4 Results

Virus acquisition by first and second instar larvae and transmission by adults

Adult *C. claratris* that had been kept as L1 on virus-infected tomato leaflets transmitted the tospovirus to tomato leaf discs and plants at rates of 69% and 64%, respectively (Table 4.1). Transmission efficiencies did not differ significantly between the two assay methods (t = 0.86, *P* > 0.4374). When using the leaf disc method uptake of the virus by L2 resulted in a transmission efficiency of 48% by adults, which was significantly lower than in L1 (t = 3.36, *P* < 0.0283) (Table 4.1).

The highest transmission efficiency was observed in adult male *C. claratris* that had acquired the virus as L1 on tomato leaf discs (Table 4.1). Transmission efficacy did not differ significantly between the sexes, though when acquired as L1 transmission was higher in males than in females, whereas the reverse was true for L2 (Table 4.1). The majority of the males (i.e., 83%) which had acquired the virus as L1 transmitted the virus for the first time during the first IAP, i.e., immediately after emergence (Figure 4.3). In contrast, in females similar transmission frequencies were observed during the first and second IAP; however, in the third IAP transmission frequency was significantly lower compared to the first and second IAPs. All thrips that had transmitted the virus in an IAP also transmitted it in the subsequent IAP.

Virus acquisition by first instar larvae and transmission by second instar larvae

In tomato leaf disc assays L2 *C. claratris* transmitted the virus with an efficiency of 47% (± 5.68) compared to 66% (± 2.76) in adults, the latter being significantly higher than the former (t = 2.95, *P* < 0.0419). Thirty-nine percent of the thrips could not transmit the virus, neither as L2 nor adults. All thrips that had already transmitted the virus as L2 also transmitted it as adults.

Table 4.1 Transmission efficiency of a tospovirus isolate on tomato leaf discs and whole plants by adult *Ceratothripoides claratris*. Both first and second instar larvae were given an acquisition period until pupation on virus-infected tomato leaves.

Acquisition life-stage	Assay method	Repetitions	Mean (± SE) transmission by adult thrips (%)		
			Female	Male	Total
First instar larvae	Leaf disc assay	3	62.85 ± 1.98 (87)a	77.69 ± 9.30 (38)a	68.67 ± 4.48 (125)
First instar larvae	Whole plant assay	3	63.58 ± 2.29 (57)a	73.00.± 4.77 (36)a	64.46 ± 2.23 (93)
Second instar larvae	Leaf disc assay	3	51.52 ± 3.33 (54)a	40.28 ± 5.00 (20)a	48.43 ± 3.92 (74)

Means followed by the same lower case letters in rows indicate no significant differences ($P = 0.05$, LSD multiple range test [SAS Institute 1999]). Numbers in parentheses represent sample sizes.
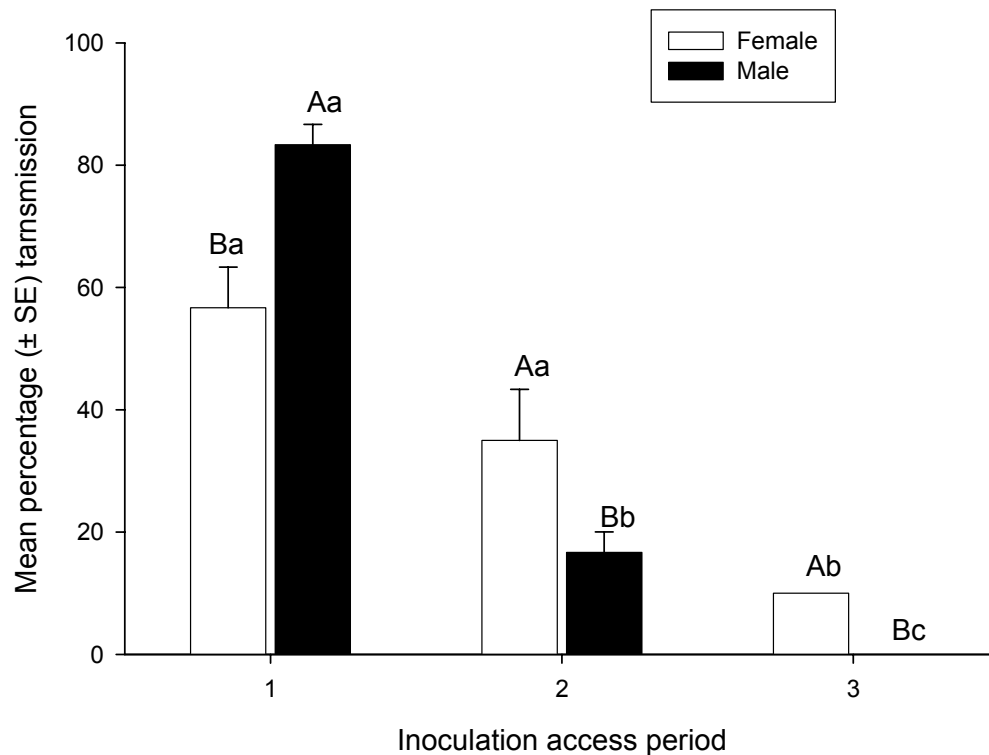
Figure 4.3 Virus transmission of adult *Ceratothripoides claratris* over three inoculation access periods (IAPs). Thrips had acquired the virus in the first instar larval stage and had been provided with an acquisition period until the pupation. Means followed by the lower case and upper case letters for a given sex over three IAPs and for a given IAP between the two sexes, respectively, are not significantly different (*P* = 0.05, LSD multiple range test and t test [SAS Institute 1999]).

Thrips density and spread of the virus in the greenhouse

In both trials *C. claratris* infestations commenced one WPP of the tomato seedlings (Figure 4.4). The thrips density in the first trial was considerably higher than in the second one. In the first trial, the peak thrips density was recorded at five WPP, and was followed by a sharp decline. In contrast in the second trial

thrips densities remained more or less constant over time. In both trials tomato plants started to show symptoms of a tospovirus infection four WPP. In the first trial, the proportion of virus-infected plants exceeded 50% at five WPP, whereas in the second trial, it took eight weeks to infect at least 50% of the tomato plants. In both trials at the end of the observation period more than 75% of the plants showed signs of a tospovirus infection (Figure 4.4).

Transmission tests with greenhouse collected thrips

Adult *C. claratris* which were reared from L2 collected from infected tomato plants in the net-house transmitted the virus at a rate of 87% to tomato leaf discs. In contrast, adults directly collected from infected tomato plants transmitted the virus at a significantly lower rate (Table 4.2). As in the previous experiment, transmission efficiency of the virus did not differ significantly between the sexes (Table 4.2).
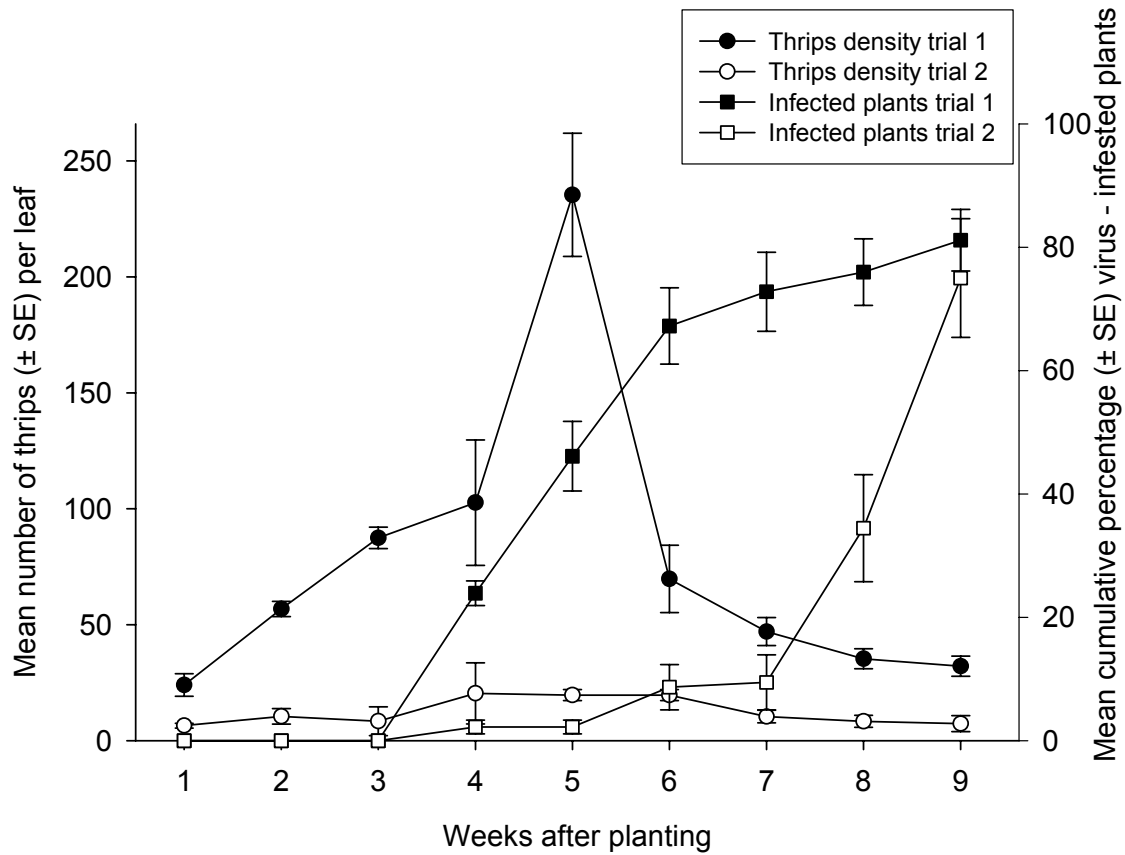
Figure 4.4 Mean density (± SE) of *Ceratothripoides claratris* on tomato leaves and mean cumulative percentage (± SE) infested tomato plants recorded in two trials in a net-house over a period of nine weeks. The plants were arranged in three rows.

Table 4.2 Transmission efficiency of a tospovirus isolate on tomato leaf discs by adult *Ceratothripoides claratris* reared from second instar larvae previously collected from infected tomato plants in a net-house and adult thrips directly collected from infected tomato plants in a net-house.

Source of adult thrips	Repetitions	Mean (± SE) transmission by adult thrips (%)		
		Female	Male	Total
Reared from second instar larvae	3	86.23 ± 7.53Ba (77)	87.18 ± 6.78Ba (36)	86.54 ± 7.28B (113)
Collected as adults from infected plants	3	11.18 ± 8.02Aa (137)	22.50 ± 11.55Aa (70)	15.05 ± 7.39A (207)

Means followed by the same lower and upper case letters in rows and columns, respectively, indicate no significant differences (P = 0.05, t test [SAS Institute 1999]). Numbers in parentheses represent sample sizes.

4.5 Discussion

Our results clearly demonstrate that *C. claratris* is highly competent to transmit CaCV-AIT to tomatoes. The tomato cultivar used in our experiments (i.e., King Kong II) proved to be a very suitable host plant for *C. claratris* and at the same time highly susceptible to an infection by CaCV-AIT. In preliminary experiments we found that the two hypersensitive tobacco species *N. benthamiana* and *N. glutinosa* L. greatly supported the propagation of the virus, but with 100 % larval mortality no thrips survival and development, suggesting that both are toxic to *C. claratris*. Moreover, we recorded reduced feeding preference of *C. claratris* and propagation of CaCV-AIT on *Petunia* hybrids compared to tomatoes (data not shown). Thus, the compatibility of the virus isolate, thrips and host plant species used for the acquisition and inoculation experiments was most likely the reason for the very high transmission efficiency recorded in this study. Moreover, we observed almost identical virus transmission efficiencies using the leaf disc and the whole plant assay methods. However, approximately 10-14 and 5-6 days were needed to detect the virus by means of DAS-ELISA in the whole plant and the leaf disc assay methods, respectively, indicating that the latter is the more appropriate protocol for studying transmission efficiencies in *C. claratris*.

The transmission efficiencies of adult *C. claratris* were influenced by the larval stage at which the virus was acquired. The percentage of adult transmitters decreased when the virus was acquired during L2, indicating a lower virus acquisition rate of L2 compared to L1. The transmission efficiencies of *Tomato*

spotted wilt virus (TSWV) and *Impatiens necrotic spot virus* (INSV) by *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) decreased with the age at which the larvae had acquired the virus (van de Wetering *et al.,* 1999), corroborating our findings. However, the ability of the larval instars to acquire the virus greatly depends on the thrips species/population as well as on the virus isolates (German *et al.,* 1992; Ullman *et al.,* 1992; Wijkamp *et al.,* 1995; van de Wetering *et al.,* 1999). The lower acquisition rate of *C. claratris* L2 might be associated with the presence of midgut barriers in this larval stage which prevent acquisition and subsequent virus replication required for the transmission (van de Wetering *et al.,* 1996).

Our results show that the L2 of *C. claratris* can transmit CaCV-AIT after acquisition during L1. Moreover, 19% of the thrips that failed to transmit the virus as L2 were subsequently able to as adults, indicating that these adults had become infectious as prepupa, pupa or immediately after adult emergence (Wijkamp and Peters, 1993). Virus transmission by L2 *C. claratris* is particularly important as they can migrate between plants if they touch each other (Wijkamp and Peters, 1993). *Ceratothripoides claratris* has a high reproduction capacity at the high temperatures frequently prevailing under tropical greenhouse conditions (Premachandra *et al.*, 2004). Hence, transmission ability of L2 of *C. claratris* could contribute for a secondary spread of the virus.

Adult male and female *C. claratris* did not differ in their competency to transmit CaCV-AIT, corroborating findings of previous studies with *F. occidentalis* and

TSWV (Sakurai *et al.,* 1998; Tavella *et al.,* 2002). Conversely, van de Wetering *et al.* (1998, 1999) detected higher transmission efficiency of TSWV by male thrips of *F. occidentalis*. Differences in tospovirus transmission between male and female adult thrips can depend on the feeding behavior (van de Wetering *et al.,* 1998), and the degree of accumulation of virus particles and physiological conditions in the body of the vectors (Sakurai *et al.,* 1998).

The higher transmission efficacy of male *C. claratris* in the first IAP might have been the result of a shorter latent period than in females. In *F. occidentalis* concentration of TSWV particles in adults reached maximum levels four days after adult emergence and the transmission efficiency decreased with age (van de Wetering *et al.,* 1999). In our studies adult *C. claratris* retained equivalent transmission abilities at least for six days after adult emergence.

In both net-house trials natural infestation by *C. claratris* commenced one WPP, indicating that the net could not exclude a thrips invasion from the outside. Meanwhile, symptoms of a tospovirus infection first appeared on tomato plants four WPP, strongly suggesting that the tosopovirus infection was initiated by viruliferous *C. claratris* that had invaded the net-houses from outside. Seed transmission of tospovirus is not possible (Kritzman *et al.,* 2002). The differences in thrips densities between the two trials were most likely associated with seasonal effects, with relatively low *C. claratris* densities during the rainy (April to September) compared to the dry season (from October to March), which is in agreement with the previous findings of Rodmui (2002). The level of thrips

densities influenced the initiation and subsequent spread of the virus in the net-houses. Thus, after the invasion of viruliferous thrips into the net-houses, the prevailing microclimatic conditions (i.e., high temperatures and relative humidity) inside the net-house provided highly favorable conditions for growth and reproduction of *C. claratris* (Premachandra *et al.,* 2004). Consequently the few initially infected tomato plants might have served as potential sources for the newly emerged thrips larvae which in turn caused the spread of the virus once they developed into adults. In addition to adult thrips, L2 *C. claratris* also could have contributed to the spread of the virus infection because the arrangement of the pots in the net-house allowed tomato plants to have leaf contacts.

We recorded high efficiency in virus transmission (up to 87%) by adults emerging from L2 that had been previously collected from infected tomato plants in the net-house, possibly because of the high virus replication rate in second instar larvae. Wijkamp and Peters (1993) reported that in *F. occidentalis* TSWV particles replicated from first to second instar larvae. In addition, virus replication and movement of the virus are higher inside the vectors at higher temperatures (Tamada and Harrison, 1981). The lowest virus transmission rate was recorded in *C. claratris* adults collected from infected tomato plants in the net-house. At the time of collection, very low numbers of adults were present on leaves, and most of the thrips were collected from fruits of infected plants. Moreover, many of the collected thrips showed abnormalities, especially on their legs and wings, indicating that they were either at the end of their life span or they had suffered

because of the deterioration of the host plant quality, possibly explaining the low virus transmission rate in these adults. Viruliferous thrips can transmit the virus until their death (Wijkamp *et al.,* 1996), though young adults of *F. occidentalis* are more efficient in transmitting TSWV than older ones (van de Wetering *et al.,* 1999).

In conclusion, *C. claratris* is an efficient vector for transmitting CaCV-AIT. Both first and second instar larvae can acquire the virus, and second instar larvae and adults can transmit the virus. The short life cycle of *C. claratris*, coupled with its high reproductive capacity at high temperatures (Premachandra *et al.,* 2004) can lead to serious virus epidemics under tropical conditions. Investigations are underway to study the median latent period, acquisition access period and inoculation access period, as well experiments to detect the amount of virus titers inside the thrips body.

5 Effects of neem and spinosad on *Ceratothripoides claratris* (Shumsher) (Thysanoptera: Thripidae), an important vegetable pest in Thailand, under laboratory and greenhouse conditions[4]

5.1 Abstract

Toxicity of three bio-pesticides, i.e., two neem products and spinosad, was determined on foliage-dwelling life-stages of *Ceratothripoides claratris* (Thysanoptera: Thripidae), a major thrips pest on tomatoes in central Thailand. Direct and residual contact toxicities of NeemAzal-TS® (1% azadirachtin) and systemic activity of NeemAzal-MD 5 (5% azadirachtin) affected the survival of first instar larvae (L1) in a dose-dependent manner. However, neither second instar larva (L2) nor adult survival was influenced by both neem products. On the contrary, spinosad caused 100% mortality in both larval stages and adults of *C. claratris* regardless of the dose rates tested. No strong ovicidal effects were detected in three different age groups of eggs (i.e., one-, two- and three-days-old) topically treated with both NeemAzal-TS® and spinosad. Residual toxicity was highest with fresh residues of NeemAzal-TS® compared to one-, three-, five- and seven-days-old residues, and in general was higher under laboratory than greenhouse conditions. Irrespective of the age of the spray residues spinosad always caused 100% mortality in larvae and adults. Strongest systemic effects

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were observed in L1 one day after soil drenching with NeemAzal-MD 5 at the highest dose rate tested. Foliar and soil applications of NeemAzal-TS$^{®}$ and NeemAzal-MD 5, respectively, did not cause any oviposition deterrent effects.

5.2   Introduction

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae) is a serious pest attacking tomatoes in Thailand both in the field and in greenhouses (Murai *et al.,* 2000, Rodmui, 2002, Premachandra *et al.,* 2004). First records of *C. claratris* date back to 1987 on melons, *Cucumis melo* L. (Cucurbitaceae), in northern Thailand (Okajima *et al.,* 1992). In 1991, *C. claratris* was first detected on tomatoes in central Thailand (Murai *et al.,* 2000). Apart from Thailand, *C. claratris* has been recorded in India (Jangvitaya, 1993) and Malaysia (S. Okajima cited in Murai *et al.,* 2000). The life cycle of *C. claratris* comprises of six developmental stages, i.e., eggs, first (L1) and second instar larvae (L2), prepupae, pupae and the adult. Eggs hatch into small white first instar larvae (L1) which start to feed on the foliage immediately after emergence (Rodmui, 2002). Late L2 drop from the foliage to the soil or leaf litter (D. Premachandra, unpublished data) where they develop into the non-moving and non-feeding prepupae and pupae. Adults are about 1 mm long and dark brown in color (Jangvitaya, 1993; Mound and Kibby, 1998; Rodmui, 2002).

Thrips directly damage tomatoes by voracious feeding of adults and larvae and by oviposition of females on leaves, stems and on fruits (Murai *et al.,* 2000; Rodmui, 2002). Moreover, *C. claratris* is most likely vectoring a yet to be identified

tospovirus, serologically closely resembling the recently described Capsicum chlorosis virus (CaCV) (McMichael *et al.,* 2002), an important disease of tomatoes in central Thailand (D. Premachandra, unpublished data). Previous studies have shown that *C. claratris* is particularly well adapted to the high temperatures frequently prevailing in the tropics (Premachandra *et al.,* 2004). For instance it takes *C. claratris* only nine days to complete its life cycle at 30°C. In addition to tomatoes, *C. claratris* attacks other economically important vegetables like cucumbers, *Cucumis sativus* L. (Cucurbitaceae), eggplants, *Solanum melongena* L. and *S. xanthocarpum* Schard and Wendl (Solanaceae), pumpkin, *Curcurbita moschata* (Duch.) Poir (Cucurbitaceae), cowpea, *Vigna sinensis* (L) Savex- Hass (Fabaceae), bird pepper, *Capsicum annuum* L. (Solanaceae), and yard long bean, *Vigna unguilulata* (L.) Walp (Fabaceae) (Jangvitaya, 1993; D. Premachandra, unpublished data).

To date, no integrated control strategies against *C. claratris* have been developed. According to Murai *et al.* (2000) and Rodmui (2002) the two eulophid parasitoids *Ceranisus menes* (Walker) and *Goethena shakespearei* Girault are key natural enemies of *C. claratris* though little is known on their efficacy in controlling thrips. Moreover, so far no attempts for mass releases in greenhouses and/or fields have been made. In recent years, bio-pesticides have been increasingly used for integrated control of important crop pests. Products derived from the Neem tree, *Azadirachta indica* Juss. (Meliaceae) (Larew *et al.,* 1985; Labanowski and Soika, 1999; Ascher *et al.,* 2000; Thoeming *et al.,* 2003) and

spinosad, a fermentation product of the soil actinomycete *Saccharopolyspora spinosa* Mertz and Yao (Drinkall and Boogaard, 2001; Ishaaya *et al.,* 2001; Schoonejans and Van Der Staaij, 2001; Nawrocka, 2002) can efficiently control important pests with considerably less impact on non-target organisms than synthetic insecticides. Hence, the objectives of this study were to determine the effects of two neem-based products and spinosad on survival and reproduction of *C. claratris*. This research is part of a larger study that seeks to develop sustainable vegetable production systems under protected cultivation in the humid tropics.

5.3 Materials and Methods

Host plants and thrips

All trials were conducted with tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II). Adult *C. claratris* were initially collected from tomato plots at the Asian Institute of Technology (AIT), Bangkok, Thailand. Thereafter, thrips were reared on potted tomato plants in a small outdoor net-house (10 × 20 m, mesh size of the net approximately 400 µm). Synchronized-aged developmental stages of thrips, i.e., L1, L2 and adults, were obtained by allowing female *C. claratris* (approximately 500) to oviposit on excised tomato leaflets for 4 h in a sealed Petri dish (8.5 × 1.5 cm) containing on the bottom a thin layer (approximately 1 cm thickness) of a mixture of Plaster of Paris and charcoal (ratio 9:1), at 25 ± 2ºC, 60-70% relative humidity (RH) and a 12L:12D photoperiod. Leaflets were kept in a Petri dish. After 4 h, the adults were removed from the

leaflet with a fine camel hair brush, and the eggs were allowed to develop into L1 or L2. The L2 was determined by the occurrence of an exuvia. Second larval instars were further reared until adulthood to obtain similar-aged adults. Twelve h after molting, the L2 were transferred to small sealed plastic containers (5 × 4 × 3 cm) with a bottom layer (approximately 1.5 cm thickness) of a mixture of Plaster of Paris and charcoal (9:1) and were kept at 25 ± 2ºC, 60-70% RH and a 12L:12D photoperiod until adult emergence.

Bio-pesticides

NeemAzal-TS® (1% Azadirachtin A) NeemAzal-MD 5 (5% Azadirachtin A) (Trifolio-M GmbH, Lahnau, Germany) and spinosad (12% w/v SC, Dow Agrosciences, Indianapolis, USA) were used in bioassays against different life-stages of thrips. For both Neem products the blank formulations (i.e., the formulation products without the active ingredients) T/S Forte and NeemAzal-MD blank (Trifolio-M, GmbH, Lahnau, Germany) were used in the untreated controls.

Experiment 1 - Direct contact toxicity

Effect of direct contact toxicity of NeemAzal-TS® and spinosad was tested against eggs, larvae (L1 and L2) and adults of *C. claratris* at three dose rates, i.e., 3, 5 and 7 ml / liter water (ml / lw), and 5, 10 and 20 ml / 20 lw, respectively. In the adult bioassay, 1-day-old unsexed adult *C. claratris* (approximately 300) were exposed to NeemAzal-TS® and spinosad on tomato leaflets by dipping the leaflets individually in the bio-pesticide solutions for 10 s. One minute after exposure, treated adults were transferred to fresh, i.e., untreated, leaflets of 3-weeks-old

potted tomato plants using a camel hair brush. These tomato leaflets containing thrips were individually confined into small plastic containers (5.5 × 6.5 cm) which were set upright with the aid of thin wooden sticks (0.5 cm diameter) embedded in the soil using a rubber band. Subsequently, the containers were tightly closed with corresponding lids. Two holes (2 cm diameter) were punched in the lid and sealed with thrips-proof nylon gauze (64 µm mesh size) to allow ventilation. A group of 30 thrips per leaflet formed a replicate and at least five groups were assigned to each life stage-dose rate combination of both bio-pesticides. Thrips were kept on the tomato leaflets at 25 ± 2ºC, 60-70% RH and a 12L:12D photoperiod for 6 days. After six days the leaflets were taken out of the containers and the survival of adults was recorded. The adults were considered dead if they did not move when prodded with a fine camel hair brush. Thrips of control groups were treated with T/S Forte (10 ml / lw) and distilled water for NeemAzal-TS® and spinosad, respectively, following the same protocols.

Larval bioassays were performed with L1 and L2 of *C. claratris* using the same dose rates as in the adult bioassay. Twelve-h–old L1 and L2 (approximately 300) were exposed to NeemAzal-TS® and spinosad in separate groups as previously described. Treated larvae were then transferred to fresh, i.e., untreated, leaflets of 3-weeks-old potted tomato plants. The bases of the petioles were treated with insect glue to confine the larvae to the leaflets on which they were placed. In the L1 assay, dead larvae on the leaflets were recorded daily for nine consecutive days, commencing from 24 h after the insecticide treatment. As late L2 in

C. claratris drop from the foliage to pupate in the soil/leaf litter (D. Premachandra, unpublished results), the number of dropped larvae was also recorded. For this, a Petri dish (8.5 × 1.5 cm) containing water was placed beneath a leaflet. Water was checked every 6 h for any dropped larvae. In preliminary experiments, for up to 6 h no detrimental effects for thrips floating on the water surface were recorded. Larvae were scored as survived if they had dropped from the leaflets for pupation. In the L2 bioassays, numbers of dead and dropped larvae were recorded for 1-5 days after exposure to the bio-pesticides. In addition, the dropped larvae were reared until they molted to prepupae in small sealed plastic containers. The number of L2 that successfully molted into prepupae was recorded. The dropped L2 that successfully molted into prepupae were scored as survivors. Thrips of control groups were treated with T/S Forte and distilled water for NeemAzal-TS$^®$ and spinosad, respectively, following the same protocols.

Potential ovicidal effects of NeemAzal-TS$^®$ and spinosad were evaluated using *C. claratris* eggs of three different age groups, i.e., 1-, 2- and 3-days-old eggs. Synchronized-aged eggs were obtained by allowing female thrips (approximately 300) to oviposit on 10 excised tomato leaflets (i.e., 30/leaflet) for 4 h. After 4 h the females were removed and the petioles of the leaflets were inserted into a small glass vial (1.4 × 1.5 × 4.4 cm) filled with water agar (0.9%). The vial was placed in a plastic container (7.5 × 5.5 × 7 cm) and the base of the vial (approximately 1 cm in depth) was embedded in a layer of Plaster of Paris and charcoal (9:1 ratio, 3 cm thickness). The containers were closed and held at 25 ± 2ºC, 60-70% RH. A hole

(1 cm diameter) was cut into the center of the lid and covered with thrips-proof nylon gauze (64 µm mesh) to allow ventilation. One, 2 and 3 day/s after removal of the females, the number of eggs per leaflet was counted and the leaflets were then individually submerged in different bio-pesticide solutions for 30 s, and then allowed to air-dry for 30 min. Thereafter, the leaflets with treated eggs were transferred back to the plastic containers and held at the same conditions until emergence of larvae. Eggs of control groups were treated with T/S Forte and distilled water for NeemAzal-TS® and spinosad, respectively. The number of emerged larvae was recorded and observations were continued until no further eggs hatched. The duration of the entire pre-larval development time, i.e., the time from oviposition to emergence of larvae, was recorded. Moreover, emerged larvae were reared on fresh leaflets of tomato plants until L2 to evaluate their survival. The remaining experimental protocol and data collection were identical to the above described L1 assay.

Experiment 2 - Residual contact toxicity

Residual effects of NeemAzal-TS® and spinosad were evaluated using fresh and 1-, 3-, 5- and 7-days-old residues at the before mentioned three dose rates against larvae and adults of *C. claratris.* Two experiments were conducted. In the first experiment, tomato leaflets on a 3-weeks-old tomato plant were sprayed with the different dose rates on both sides until run-off, using a hand-held sprayer, and allowed to air-dry under laboratory conditions (25 ± 2ºC, 60-70% RH and a 12L:12D photoperiod). Thereafter, 12-h-old L1 and L2 were separately placed on

the treated leaflets (30 thrips/leaflet) immediately after drying (approximately 1 h after spraying) and 1, 3, 5 and 7 days. Treated plants were kept at the same laboratory conditions. At least five groups of thrips were tested for each bio-pesticide / dose rate combination. T/S Forte and distilled water were applied in untreated controls for NeemAzal-TS® and spinosad, respectively. Data recording was identical to the before mentioned L1 and L2 bioassays of direct contact toxicity. In the second experiment effects of the same bio-pesticide / dose rate combinations and residue age classes on the survival of adult thrips were studied. Test plants and treatments followed the previously described protocol. Thereafter, 30, 1-day-old thrips adults were separately placed on at least five treated leaflets immediately after drying (approximately 1 h after spraying) and 1, 3, 5 and 7 days. The remaining experimental set-up and data collection were identical to the adult assay on direct contact toxicity. In addition, the potential impact of fresh residues of NeemAzal-TS® (same dose rates as above) on reproduction of *C. claratris* was determined. Effects of spinosad on thrips reproduction could not be evaluated due to the high and rapid adult mortality (i.e., 100% mortality after 24 h exposure) at all three dose rates tested (for details see results). For NeemAzal-TS® the leaflets with fresh residues were inserted in a plastic container and one adult female was introduced and allowed to oviposit for 6 days. Thereafter, the female and the container were removed and survival of the adults was recorded. The adults were considered dead if they did not move when prodded with a fine camel hair brush. The leaf bearing eggs was incubated and the number of emerging L1 was counted using a stereo microscope. Observations were made until no further larval

emergence. Effects of NeemAzal-TS® residues on reproduction were evaluated based on the progeny production during the 6 days exposure period. Controls were treated with T/S Forte. Five replications were used in the trial.

Since under laboratory conditions spinosad (at all three dose rates) and NeemAzal-TS® (at 7 ml / lw) caused high residual toxicity in L1 of *C. claratris* (for details see results) a second experiment was conducted to evaluate the effects under greenhouse conditions. The aim of this trial was to simulate the residual levels of NeemAzal-TS® and spinosad on tomato plants in the greenhouse after different intervals following a treatment. Three weeks old tomato plants were sprayed with NeemAzal-TS® (7 ml / lw) and spinosad (5, 10 and 20 ml / 20 lw) and the respective blank formulation and distilled water in a greenhouse (mean temperature and relative humidity ranged from 25-36ºC and RH 53-98%, respectively). After spraying plants were allowed to air-dry. Residual effects were evaluated on fresh, 1, 3, 5 and 7-days-old residues. The experimental protocol and the data collection were identical to the previously described laboratory assay except that bigger plastic containers (10 × 8.5 cm) were used to collect the dropping larvae.

Experiment 3 - Systemic effects of NeemAzal-MD 5

In the laboratory, the effects of soil applications of NeemAzal-MD 5 on the survival of larvae and adult *C. claratris* were assessed at three dose rates, i.e., 10, 12.5 and 15 gram / liter water (g / lw). Three weeks old tomato seedlings (cv. King Kong II) transplanted in plastic pots (11 × 7.5 × 8.5 cm) containing a commercial growing

substrate composed of clay, sand, and silt in proportions of 31, 30 and 39%, respectively, and 29% of organic matter were used for the trial. Tomato plants were not watered for 2 days before drenching the soil with the NeemAzal-MD 5 solutions. Per pot plants were treated with a soil drench of 170 ml of NeemAzal-MD 5, the respective blank formulation, i.e., NeemAzal-MD blank (10 g/1 l water) and the distilled water in the controls. This volume of solution was sufficient to cause little to no drainage. In the larval assay, 20 L1 and L2 of *C. claratris* were separately placed on tomato leaflets 6 h, and 1, 3, 5 and 7 days post-application. At least 5 groups of larvae were allocated for each treatment combination. The remaining experimental set-up and data collection were identical with that described to the larval assays in experiments 1 and 2.

The assays with adult thrips were conducted in microcosms. Plants were treated in the same manner and with the same dose rates as in the larval assay. Six h and 1, 3, 5 and 7 d post-application the pots were covered with a Plexy glass cylinder (10 cm diameter, 30 cm height). The upper opening of the cylinder was closed with thrips-proof nylon gauze (64 µm mesh size) to provide additional ventilation. Additional four holes were drilled at the upper and lower edges of the cylinder and except for one hole at the lower end all the other holes were covered with thrips-proof nylon gauze to aid ventilation. The lower uncovered hole was used for releasing adult thrips into the cylinder and was subsequently covered by piece of glue tape. Twenty 1-day-old unsexed adult thrips were released onto plants in

each cylinder. The plants were watered whenever needed. After 6 days the cylinder was carefully removed and the survival of the thrips recorded.

An additional trial was carried out to evaluate the influence of NeemAzal-MD 5 on thrips reproduction at a single dose rate of 15 g / lw. The experiment was conducted using the same Plexy glass cylinders and set-up as to the one described above. The soil was drenched with 170 ml of NeemAzal-MD 5 and the respective blank formulation (10 g/1 l water) in the control. Seven replications were used in the trial. Six h after the soil treatment a 1-day-old virgin female was placed on the tomato plant in the cylinder and allowed to reproduce for 6 days. Thereafter, the cylinder and the female were removed and the eggs on the plants were allowed to hatch. The number of emerging larvae was recorded daily on the leaflets and the data collection continued until no more larvae emerged.

Statistical analysis

All bio-pesticide treatments were arranged in a complete randomized design. Initially, data were subjected to Shapiro-Wilk's test for normality and Brown and Forsythe's test for homogeneity of variance. Percentage mortality data of the different life stages of *C. claratris* were subjected to arcsine transformation whenever normality and variance homogeneity were violated. Data on duration of egg development, fecundity and mortality were analyzed using the GLM procedure in SAS to determine single or interaction effects of factors. When the ANOVA yielded significant F-values, means were compared using the Least Significant Difference (LSD) multiple-range test. Whenever significant interactions were

observed between factors, treatment means of one factor were compared at each level of the other factor. In contrast, when no significant interactions were detected means of the levels of one factor were compared regardless of the levels of the other factor (Sokal and Rohlf, 1995). All analyses were performed using SAS (SAS Institute, 1999) and a significance level of $\alpha = 0.05$ was used in all analyses.

5.4   Results

Experiment 1 - Direct contact toxicity

In the two untreated controls, i.e., T/S Forte (blank formulation) and distilled water, 100% survival was recorded in L1, L2 and adults of *C. claratris*. No mortality was detected in L2 and adults after direct exposure to NeemAzal -TS$^®$ at any of the dose rates tested. In contrast, survival of L1 was significantly reduced ($P < 0.0001$) by NeemAzal-TS$^®$ at all the dose rates when compared to the untreated controls. Mortalities caused by NeemAzal-TS$^®$ gradually increased with time and reached the highest values 9 days post-application; at this time cumulative percentage mortalities of L1 significantly differed (F = 154.52; d.f. = 3, 17; $P < 0.0001$) among the dose rates with 32.36 ($\pm$ SE 4.24), 62.69 ($\pm$ 3.79) and 75.71 ($\pm$ 3.46) for concentrations of 3, 5 and 7 ml, respectively (Figure 5.1). Spinosad induced 100% mortality in all tested life-stages of *C. claratris* irrespective of the dose rates tested. At all dose rates, spinosad-treated thrips died within 1 day after exposure.
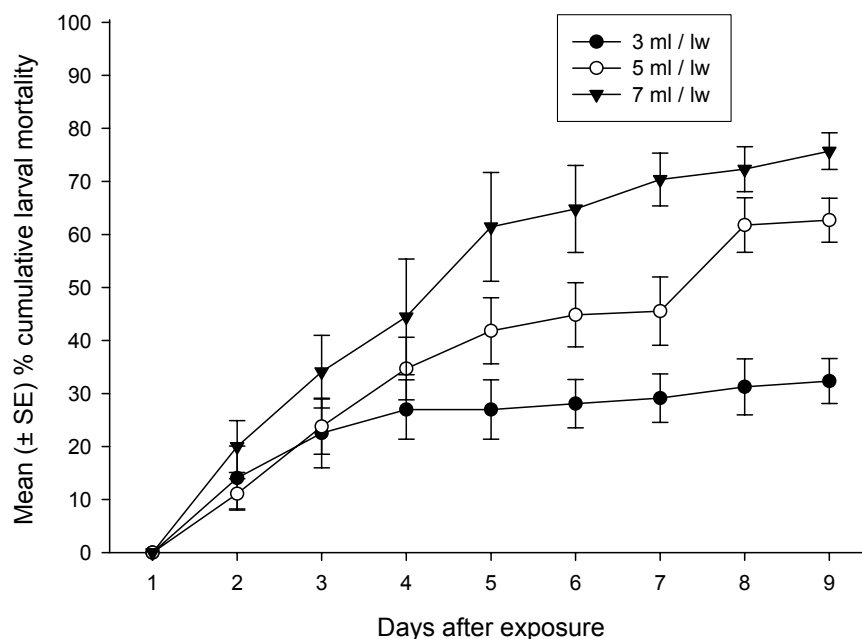
Figure 5.1 Mean (± SE) percentage cumulative mortality in first instar larvae of *Ceratothripoides claratris* exposed to three dose rates (3, 5 and 7 ml / lw) of NeemAzal-TS® during nine consecutive days.

Egg hatchability, expressed in proportion of larval emergence, did not significantly differ among the eggs treated at different times in their pre-larval development, i.e., age classes, in the two untreated controls (F = 0.5; d.f. = 2, 12; *P* = 0.6186 for the blank neem formulation; F = 1.00; d.f. = 2, 12; *P* = 0.3966 for distilled water). Hence, data were pooled over the age classes in the two untreated controls and then compared. No significant differences in development time of eggs were found between the blank formulation of NeemAzal-TS® and distilled water (t = -0.68; *P* > 0.5039). Thus, data on percentage larval emergence in the blank formulation and distilled water were used for comparisons with NeemAzal-TS® and spinosad

treatments, respectively. Larval emergence was affected by the interaction of the age classes of eggs and the tested NeemAzal-TS® dose rates (dose rate * age class F = 9.71; d.f. = 4, 45; *P* < 0.0001). Hence, percentage larval emergence for each age class was compared at each level of the tested NeemAzal-TS® dose rates. Larval emergence was reduced, though not significantly, by NeemAzal-TS® only in three days-old eggs at dose rates of 5 and 7 ml / lw (i.e., 1 and 18%, respectively); however, compared to the blank formulation, in which 100% of the larvae emerged, significant reductions (i.e., 18%) were detected at the highest dose rate of 7 ml / lw (*P* < 0.0001). In contrast, no significant interactions were found in larval emergence between the age classes of eggs and the tested dose rates of spinosad (F = 1.19; d.f. = 4, 36; *P* = 0.3325). Thus, dose rates were compared irrespective of the levels of the age classes and vice versa. Significantly lower larval emergence was recorded in spinosad treated eggs at all dose rates- age classes combinations compared to the distilled water control (*P* < 0.0001). The proportion of larval emergence in the distilled water control was between 98- 100% (Figure 5.2). Larval emergence did not differ significantly among the spinosad dose rates tested (F = 1.98; d.f. = 2, 42; *P* = 0.1503). In contrast, significant differences were found in larval emergence among the three age classes of eggs (F = 13.22; d.f. = 2, 42; *P* < 0.0001) (Figure 5.3). Larval emergence was significantly lower in 3–days-old eggs compared to 1- and 2– days-old eggs, with no significant differences among the latter two age classes (Figure 5.3). Despite larval emergence in spinosad treated eggs, 100% of the L1

died immediately after hatching, irrespective of the age classes of eggs and dose

rates tested.
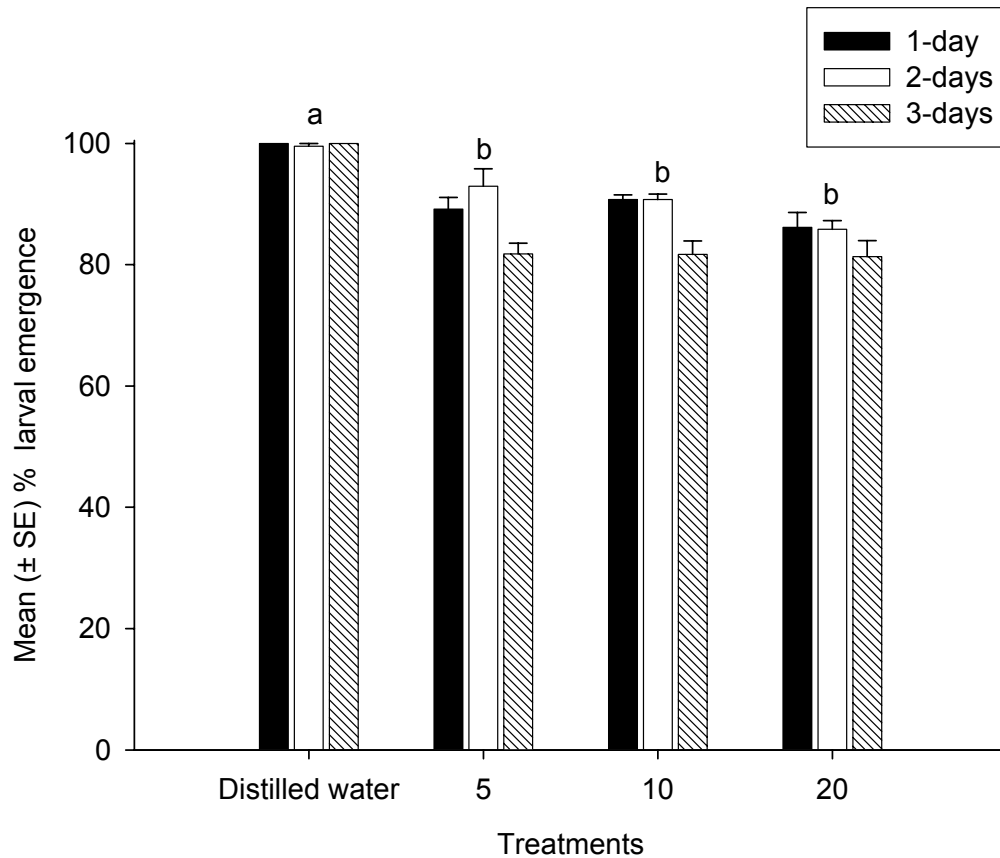
Figure 5.2 Mean (± SE) percentage larval emergence in three age classes of eggs (1-, 2- and 3-days–old) of *Ceratothripoides claratris* treated with three dose rates of spinosad (5, 10 and 20 ml / 20 lw). No significant interactions were found between the dose rates and the age classes of eggs. Therefore, comparisons of the dose rates were performed irrespective of the age classes. Same letters above a group of three bars (i.e., means of different age classes of eggs) of a given spinosad dose rate and distilled water, i.e., untreated control, indicate no significant differences (*P* < 0.05). LSD multiple range test [SAS Institute 1999]).
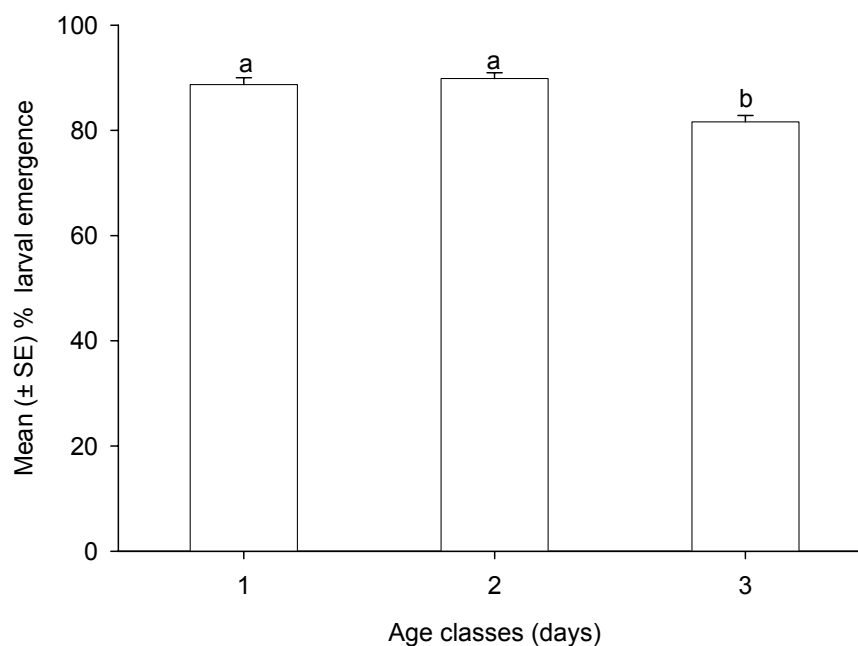
Figure 5.3 Mean (± SE) percentage larval emergence in three age classes of eggs (1-, 2- and 3-days–old) of *Ceratothripoides claratris* treated with three dose rates of spinosad (5, 10 and 20 ml / 20 lw). No significant interactions were found between the dose rates and the age classes of eggs. Therefore, comparisons of the age classes were performed irrespective of the spinosad dose rates. Same letters above bars indicate no significant differences between age classes of *C. claratris* eggs (*P* < 0.05). LSD multiple range test [SAS Institute 1999]).

Both distilled water or blank formulation of NeemAzal-TS® had no significant effects on the development time of eggs from the three age classes (F = 0.79; d.f. = 2, 15; *P* = 0.4729 for the blank formulation, mean development time 4.11 ± 0.03 days; F = 1.22; d.f. = 2, 15; *P* > 0.3229 for distilled water, mean development time 4.15 ± 0.01 days). Hence, data were pooled over the age classes in the two untreated controls and then compared. No significant differences in development

time of eggs were found between the blank formulation of NeemAzal-TS® and distilled water (t = 1.22; *P* > 0.2344). Thus, data from the blank formulation was used for comparisons in the NeemAzal-TS® experiments whereas data from the distilled water treatment was used for comparisons in the spinosad trials. No significant interactions were detected between the dose rates of both bio-pesticides and the age classes of the thrips eggs (dose rate * age class F = 2.23; d.f. = 4, 42; *P* = 0.0825 for NeemAzal-TS®; F =1.44, d.f. = 4, 42; *P* = 0.3503 for spinosad). Thus, data on development time in the different age classes of eggs were compared irrespective of the dose rates of the bio-pesticides and similarly, dose rates were compared irrespective of the age classes of eggs. No significant differences in development time were found among the dose rates of both bio-pesticides (F = 0.62; d.f. = 2, 48; *P* = 0.5397 for NeemAzal-TS®; F = 1.49; d.f. = 2, 48; *P* = 0.2350 for spinosad) and age classes of eggs (F = 1.48; d.f. = 2, 48; *P* = 0.2371 for NeemAzal - TS®; F = 2.29; d.f. = 2, 48; *P* = 0.1124 for spinosad).

Treating eggs with the blank neem formulation and/or distilled water resulted in no mortality in the emerging L1. In contrast, 100% of the L1 that emerged from spinosad-treated eggs immediately died after emergence. Hence, survival was only evaluated for first instar larvae emerging from NeemAzal-TS®-treated eggs until the next ecdysis, i.e., until they reached the L2 stage. Compared to the blank neem formulation the survival of L1 was significantly reduced (*P* < 0.0001) by NeemAzal-TS® at all tested dose rates in the three age classes of thrips eggs. However, mortality caused by the different NeemAzal-TS® concentrations was independent of the egg age classes (dose rate * age class F = 0.15; d.f. = 4, 42; *P*

= 0.9617). Hence, percentage survival of L1 among the different dose rates was compared irrespective of the egg age classes and vice versa. Mortalities of L1 differed significantly among the dose rates (F = 154.89; d.f. = 2, 48; *P* < 0.0001) with the highest mortality (65%) recorded at the highest dose rate of NeemAzal-TS[®] (Figure 5.4).
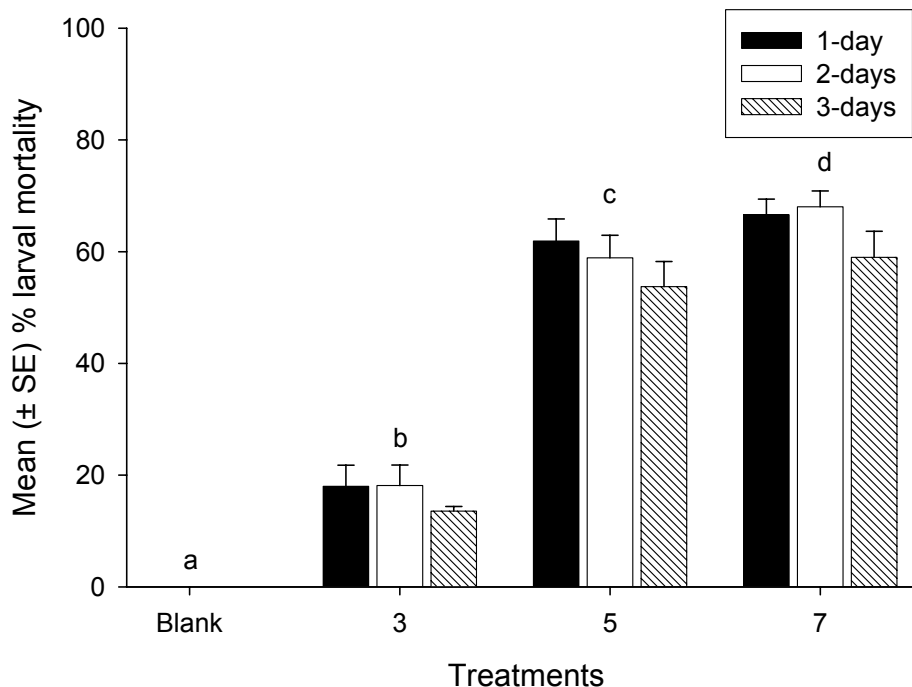
Figure 5.4 Mean (± SE) percentage of mortality in first instar larvae of *Ceratothripoides claratris* emerging from eggs of three different age classes (1-, 2- and 3–days old) that had been treated with three dose rates of NeemAzal-TS[®] (3, 5 and 7 ml / lw) and the blank formulation. No significant interactions were found between the dose rates and the age classes of eggs. Therefore, comparisons of the dose rates were performed irrespective of the age classes of eggs. Same letters above the group of bars (i.e., means of the different age classes of thrips eggs) of a given dose rate indicate no significant differences (*P* < 0.05). LSD multiple range test [SAS Institute 1999]).

No significant differences in L1 mortality were detected among the three age classes of eggs (F = 0.39; d.f. = 2, 48; *P* = 0.6775).

Experiment 2 - Residual contact toxicity

In both untreated controls, 100% of the L2 and adult thrips survived. In contrast, for the L1 0.1 – 1% mortality was recorded in the two untreated controls. However, no significant differences in L1 mortality were found among the different residual levels in the two controls (F = 0.90; d.f. = 4, 20; *P* > 0.4881 for blank formulation; F = 0.75; d.f. = 4, 20; *P* > 0.5696 for distilled water). Hence, data from the untreated controls were pooled over the residual levels and then compared. No significant differences between the blank formulation of NeemAzal-TS® and distilled water were found (t = -1.44; *P* > 0.1551). Thus, percentage larval mortality of the blank formulation was used for comparisons with NeemAzal-TS® treatments and data from the distilled water control was used in the spinosad experiments. Like in the previous experiment, 100% survival of L2 and thrips adults was recorded in all NeemAzal-TS® treatments. Yet, compared to the blank formulation NeemAzal-TS® significantly reduced survival of L1 at all dose rates and residual levels tested. In addition, significant interaction was found between the dose rates and the age of the residues (residue * dose rate F = 6.20; d.f. = 8, 65; *P* < 0.0001). Hence, % mortalities of L1 at each residual level were compared with each level of the dose rates tested. Significant differences were detected in mortality of L1 across the residual levels at all three dose rates of NeemAzal-TS® (Table 5.1). In general, mortalities of L1 declined with increasing age of residues and increased with

increasing dose rates. At the lower and intermediate dose rates mortality of L1 on fresh residues was significantly higher than on all other residual levels. At the highest dose rate no significant differences in larval mortality were found between fresh and 1-, and 3-days-old residues. In general, lowest mortality levels were recorded on the oldest residues, though at the highest dose rate mortality was approximately two and three times higher than at the intermediate and lower concentrations, respectively. Except for fresh residues, where no significant differences among the tested dose rates were found, and for five-days-old residues mortality levels caused by the highest neem dose rate were always significantly higher than those of the two lower concentrations (Table 5.1).

Table 5.1 Mean (± SE) percentage mortality in first instar larvae (L1) of *Ceratothripoides claratris* exposed to different residual levels of NeemAzal - TS® under laboratory conditions.

Residual levels	Mean (±SE) percentage larval mortality		
	3 ml / lw	5 ml / lw	7 ml / lw
Fresh	96.85 ± 2.08aA	100.00 ± 0.00aA	100.00 ± 0.00aA
1-day-old	79.73 ± 2.01bA	92.99 ± 3.60bB	99.31 ± 0.69aC
3-days-old	58.46 ± 2.35cA	80.39 ± 4.31cB	98.67 ± 1.33aC
5-days-old	17.98 ± 3.70dA	61.97 ± 4.82dB	67.58 ± 5.90bB
7-days-old	17.07 ± 2.67dA	33.11 ± 2.69eB	66.30 ± 3.43bC

Means followed by the same lower letters within columns and upper case letters within the rows are not significantly different (*P* = 0.05, LSD multiple range test [SAS Institute 1999]). Control mortality was 0% (see text for details). Data were subjected to arcsine square root transformation before the analysis; non-transformed percentage mortalities are presented in the table.

In general mortality increased faster at the two higher dose rates, particularly on fresh residues. Here, 100% was recorded already two days after of exposure of the thrips to the bio-pesticide (Figure 5.5).
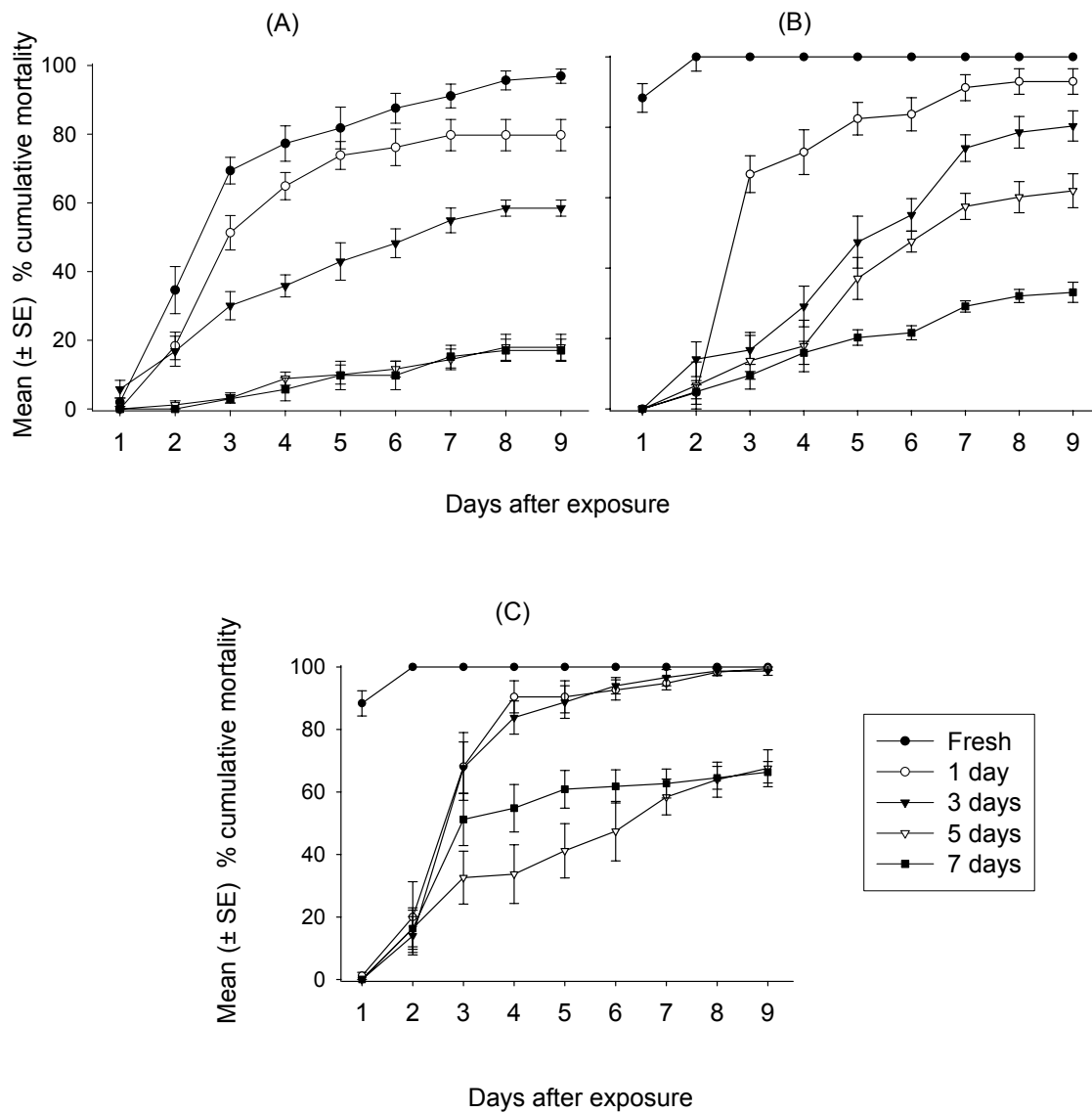
Figure 5.5 Mean (± SE) percentage cumulative mortality in first instar larvae of *Ceratothripoides claratris* exposed to different aged residues of NeemAzal -TS® at three dose rates, i.e., 3 (A), 5 (B) and 7 ml / lw (C)) during 9 consecutive days.

As in the previous experiment on contact toxicity, spinosad caused 100% mortality in all life-stages of *C. claratris* within a day after exposure to the bio-pesticide, irrespective of the residual levels or dose rates tested.

Under greenhouse conditions, 100% survival was recorded in L2 and adults of *C. claratris* in the blank formulation as well as in the NeemAzal-TS® treatments, irrespective of the age of the residues. However, in L1 0-1% mortality was observed in both untreated controls (Table 5.2).

Table 5.2 Mean (± SE) percentage mortality of first instar larvae of *Ceratothripoides claratris* exposed to different aged residues of NeemAzal-TS® , spinosad and in two untreated controls under greenhouse conditions.

Residual levels	Mean (± SE) percentage larval mortality			
	Controls		NeemAzal-TS®	Spinosad
	Blank	Dis. water	7 ml / lw	5, 10 , 20 ml / 20lw
Fresh	0.67 ± 0.67a	0.50 ± 0.52a	100.00 ± 0.00d*	100.00 ± 0.00a*
1-day-old	0.00 ± 0.00a	0.00 ± 0.00a	70.87 ± 4.37c*	100.00 ± 0.00a*
3-days-old	0.69 ± 0.69a	0.50 ± 0.52a	3.39 ± 1.44b	100.00 ± 0.00a*
5-days-old	0.50 ± 0.52a	0.00 ± 0.00a	3.28 ± 2.14ab	100.00 ± 0.00a*
7-days-old	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	100.00 ± 0.00a*

Means followed by the same letters within columns are not significantly different (P = 0.05, LSD multiple range test [SAS Institute 1999). Mortality levels that differ from the untreated controls are indicated by * (LSD multiple range test). Data were subjected to arcsine square root transformation before the analysis; non-transformed percentage mortalities are presented in the table.

Oviposition of virgin females on fresh NeemAzal-TS®-treated residues did not significantly differ from those treated with the blank formulation only (mean fecundity 28.0 ± 3.05). Moreover, no significant differences were detected among the three dose rates tested (mean fecundity (± SE) of 22.0 ± 1.71, 29.0 ± 3.95 and 24.0 ± 3.60 for 3, 5 and 7 ml / lw, respectively) within the 6-days time period (F = 0.89; d.f. = 3, 27; *P* > 0.4578).

Experiment 3 - Systemic effects of NeemAzal-MD 5

In the untreated controls, i.e., the blank formulation and distilled water, 100% survival of all life-stages of *C. claratris* was recorded. Like in the previous experiments, NeemAzal-MD 5 did not affect survival in L2 and adults, i.e., 100%, survival was recorded, irrespective of the dose rates and the days after the bio-pesticide treatment. However, compared to the untreated controls except for 6 h after the treatment, neem caused significantly higher mortality in L1 at the intermediate and higher dose rates, (*P* < 0.0001). In addition, significant interactions were found between the dose rates and days after treatments (F= 26.53; d.f. = 8, 60; *P* < 0.0001). Hence, percentage mortalities at each level of the dose rates were compared with each level of days after treatments. The lowest dose rate did not cause any mortality in first instar larvae of *C. claratris* (Table 5.3)*.* In the intermediate and higher dose rates, from one day after the treatments onwards, neem-induced mortality in thrips larvae was found. Significantly higher mortality was recorded one day after the treatment compared to the later evaluation dates in the highest neem dose rate, whereas in the intermediate dose

rate mortality levels on evaluation date one and two did not differ significantly. The lowest mortality in both dose rates was recorded seven days after the treatments. Larval mortalities significantly increased with increasing dose rates except at 6 h and 7 days after soil application of NeemAzal-MD 5 (Table 5.3).

Table 5.3 Mean (± SE) percentage mortality in first instar larvae of *Ceratothripoides claratris* at different time intervals after soil application of NeemAzal-MD 5 at three dose rates.

Time after application	Mean (± SE) percentage larval mortality		
	10 g / lw	12.5 g / lw	15 g / lw
6 hours	0.00 ± 0.00 aA	0.00 ± 0.00 aA	0.00 ± 0.00 aA
1-day	0.00 ± 0.00 aA	16.49 ± 4.52 bB	80.96 ± 2.08 bC
3-days	0.00 ± 0.00 aA	22.41 ± 4.81 bB	62.04 ± 5.06 cC
5-days	0.00 ± 0.00 aA	13.22 ± 5.70 cB	54.79 ± 4.88 cC
7-days	0.00 ± 0.00 aA	12.42 ± 3.39 cB	19.55 ± 5.11 dB

Means followed by the same lower case letters within columns and upper case letters within the rows are not significantly different (P = 0.05, LSD multiple range test [SAS Institute 1999]). Control mortality was 0% (see text for details). Data were subjected to arcsine square root transformation before the analysis; non-transformed percentage mortalities are presented in the table.

Oviposition of virgin females on plants systemically treated with 15 g / lw NeemAzal-MD 5 did not significantly differ from those treated with the blank formulation only (mean fecundities of 30.0 ± 3.77 and 28.0 ± 4.06 for the blank formulation and NeemAzal-MD 5, respectively) within the 6-days time period (t = 0.30; d.f. = 10; P > 0.7695).

5.5   Discussion

This is first report on the effects of neem and spinosad on *C. claratris*, an important thrips pest of vegetables in Southeast Asia. The blank formulations of neem and distilled water used as untreated controls acted in a similar manner, implying absence of any deleterious effects of the neem solvents on *C. claratris*. In addition, thrips mortalities were negligible to zero in the untreated controls, confirming that our bioassay protocols were well suited for the investigations. Direct and residual contact toxicity of NeemAzal-TS® as well as systemic activity of NeemAzal-MD 5 greatly affected the L1 of *C. claratris*. However, the neem products had no deleterious effects on L2 and adult thrips. In all three bioassays the first instar larvae could not accomplish their development and died during molting. Ascher *et al.* (2000) showed that two neem-based formulations, i.e., dried neem seed kernel extracts (NSKE) and Azatin, containing 3% azadirachtin, prevented the development from L1 to L2 in western flower thrips *Frankliniella occidentalis* (Pergande) and onion thrips *Thrips tabaci* Lindeman (both Thysanoptera: Thripidae). Moreover, they found no direct contact, residual and systemic effects on the survivorship of L2 and adults in both thrips species, corroborating findings of this study. Harris (1972) suggested that the insensitivity of older insect instars to insecticides might be attributable to an increase in body weight, a decrease in penetration of the active ingredients, as well as biochemical and physiological changes of the insect itself. High susceptibility of first instar larvae to neem products, as found in this and previous studies (e.g., Ascher *et al.,*

2000), is most likely due to the thinner integument of L1 compared to older instars adults, facilitating the penetration of the active ingredients.

In our study NeemAzal-TS® induced in a dose-dependent manner high contact toxicity in first instar larvae of *C. claratris*, corroborating results of Banken and Stark (1977) who reported dose-dependent effects of Neemix 4.5 EC against larvae of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) and Schroer *et al.* (2001) who recorded 97% corrected mortality in *F. occidentalis* larvae caused by direct contact effects of 0.2% NeemAzal-TS®.

A topical application with NeemAzal-TS® caused no strong ovicidal effects in eggs of *C. claratris*. In all treated eggs, we observed larval development and sometimes even partial larval emergence. Thus, NeemAzal-TS® had no detrimental effects on the embryonic development of *C. claratris*, as also shown by the unaffected duration of egg development following a neem treatment. However, the survival of the L1 emerging from neem-treated eggs was greatly reduced. Most likely, the neem residues on the egg surfaces were still toxic for the emerging larvae when they contacted the active ingredients while hatching. Even though the L1 were transferred to fresh tomato leaflets within half an hour upon emergence, apparently the larvae ingested the active ingredients from the still active neem residues through feeding or probing on the treated leaflets immediately after emergence, resulting in the observed high mortality. Ascher *et al.* (2000) reported that egg hatching in *F. occidentalis* and *T. tabaci* was not influenced on NSKE- and Azatin-treated cotton. Similar observations were made by von Elling *et al.*

(2000) with *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) on tomatoes. On the contrary, ovicidal activity of neem has been found in *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Loke *et al.,* 1992) and *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) (Jeyakumar and Gupta, 1999). These discrepancies are probably associated with insect species tested, and type of formulations and experimental protocols used (Ermel and Kleeberg, 1995; Singh and Raheja, 1996; Adán *et al.,* 1998; Walter, 1999). As in our study, Li *et al.* (2003) observed a direct kill of emerging larvae of the Balsam fir fly *Neodiprion abietis* (Harris) (Hymenoptera: Diprionidae) after treating eggs with Neemix 4.5 EC at a dose rate of 90 ppm.

In *C. claratris* we detected variations in the residual activities between the laboratory and greenhouse experiments. With 100% mortality the impact of fresh residues of NeemAzal-TS® (at 7 ml/ lw) on L1 survival was identical under greenhouse and laboratory conditions. However, in the greenhouse the residual toxicity of NeemAzal-TS® started to decline much faster than under laboratory conditions. In the laboratory residues of 0.1% Neemix-45 on cotton seedlings were highly effective for 10-11 days for *F. occidentalis* larvae, while only for 5 and 3-4 days in the greenhouse and outside, respectively (Ascher *et al.,* 2000). Stokes and Redfern (1982) found that the azadirachtin content was reduced by approximately 50% after seven days of exposure to sunlight. Hence, most likely the reduced mortalities in our greenhouse compared to the laboratory experiments can be

attributed to photo and thermo degradation of the active ingredients of the neem product.

In our study, we found more than 50% mortality in L1 five days after a systemic application of NeemAzal-MD 5 at the highest dose rate tested. Thereafter, mortality levels sharply declined. In a microcosm experiment, Thoeming *et al.* (2003) reported 90% corrected mortality in L1 of *F. occidentalis* six days after soil drenching of bean seedlings with NeemAzal-TS® at a dose rate 100 mg / lw, and these effects persisted for up to six days. Under controlled and greenhouse conditions, soil applications of chrysanthemums with 0.4% crude neem extract caused high mortality in late instars of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and the effect lasted for three weeks (Larew *et al.,* 1985). According to Lowery *et al.* (1993) the extent of systemic activity in neem depends on the insect species and host plants. Hence, the reasons for the differences in systemic effects of neem over time are probably due to varying host plants, protocols, substrates and insect species tested. In their study with *F. occidentalis* Thoeming *et al.* (2003) observed significantly lower mortality in thrips larvae in soils with a high organic matter content, and Pussemeier (2000) indicated that soil organic matter can absorb the active compounds of neem, thereby reducing their efficacy against plant-sucking insects. Thoeming *et al.* (2003) further indicated that rapid uptake and distribution of azadirachtin is enhanced at high concentrations available in the rhizosphere. The substrate used in this study consisted of approximately 29% organic matter and thus possibly affected the efficacy of

NeemAzal-MD 5, especially at the low dose rates. The high amount of organic matter content probably also caused a slow release of the active ingredients of neem, thus explaining the delayed effects in the lower compared to the higher dose rates tested.

In our study, neither a single soil drench application of NeemAzal-MD 5 nor fresh residues of NeemAzal-TS® sufficed to inhibit oviposition in *C. claratris*. Ascher *et al.* (2000) reported that foliar and soil treatments of cotton seedlings with NSKE and Azatin did not deter oviposition of *F. occidentalis* and *T. tabaci*. Likewise, Larew *et al.* (1985) observed that neem soil drenching did not affect oviposition in *L. trifolii* on chrysanthemums under controlled and greenhouse conditions. However, inhibitory effects of neem products on oviposition have been documented in different insect orders, i.e., Orthoptera, Heteroptera, Homoptera, Hymenoptera, Lepidoptera and Diptera (Saxena, 1989; Singh, 1993; Schmutterer, 1995; Isman, 1996).

Highly potent insecticidal activity of spinosad was observed against all tested life-stages of *C. claratris* (i.e., L1, L2 and adults) both through direct and residual contact toxicity and regardless of the dose rates used. The affected thrips showed tremors, involuntary movements and paralysis and ultimately died within 24-48 h. The topically treated thrips, as well as those exposed to spinosad residues of different ages did not feed, suggesting the toxicity was mainly caused through contact effects. Jones *et al.* (2002) recorded 100% mortality in adult *F. occidentalis* 24 h after a spinosad application irrespective of the dose rate and both as a

consequence of direct and residual contact toxicities. Similarly, in greenhouse experiments with cucumber and tomatoes Nawrocka (2002) recorded 100% mortality in *F. occidentalis* at two spinosad dose rates. In addition to thrips, spinosad can cause high mortality in other economically important pests, e.g. diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) (Hill and Foster, 2000), cabbage looper *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Liu *et al.,* 1999) and Caribean fruit fly *Anastrepha suspense* (Loew) (Diptera: Tephritidae) (King and Hennessey, 1996). As in the case of neem, we did not detect strong ovicidal effects of spinosad, thus *C. claratris* eggs could accomplish their embryonic development. However, due to the high residual toxicity of spinosad all emerged larvae died immediately after coming into contact with the treated leaflets on which the eggs were deposited. Adán *et al.* (1996) reported lack of toxicity of spinosad on the egg development in Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), whereas Medina *et al.* (2001) detected slight effects of spinosad on the development of eggs of the green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), corroborating results of this study. Spinosad residues are subject to photo degradation with half-lives of three to seven days in the field (Boyd and Boethel, 1998, Ruberson and Tillman, 1999). In our study after one week in the laboratory and greenhouse spinosad still caused 100% mortality in all life-stages of *C. claratris*.

In conclusion our results show that both tested neem products have a significant impact on the L1 of *C. claratris*, preventing the formation of F1 adults. High efficacy and much faster effects of spinosad against larvae and adults of *C. claratris*, coupled with its longer persistence can help to suppress the rapid population build-up of *C. claratris*, particularly under greenhouse conditions. Hence both bio-pesticides can become important components in IPM programs against *C. claratris*, which is currently threatening tomato production in central Thailand. More research is underway to explore the possibilities for further promoting the systemic effects of neem, to evaluate the potential resistance development in *C. claratris* against spinosad and effects of spinosad and neem on important natural enemies of the thrips.

6 General discussion

The findings of this study not only indicate that *C. claratris* is an important pest of tomatoes in Thailand, but also that it has the potential to threaten tomato elsewhere in the tropics. The main reasons for this are its high reproductive potential, the female-biased sex ratio, short life cycle, adoption to high temperatures (chapter 2), and most importantly the fact that it can transmit CaCV-AIT (chapter 4). Consequently, integrated control strategies for *C. claratris* are urgently needed, and data from this study on population dynamics and monitoring (chapter 3) and the control potential of bio-pesticides (chapter 5) may contribute to their development.

Even though several thrips species like *T. palmi*, *S. dorsalis, T. parvispinus* Karny, *T. tabaci* and *Haplothrips floricola* Priesner have been recorded on various economically important crops in Thailand (Bansiddhi and Poonchaisri, 1991), they are of minor importance on tomatoes. Nagai *et al.* (1988) reported that *T. palmi*, the most serious thrip pest of vegetables in Southeast Asia, cannot complete its life cycle on tomatoes. In contrast *C. claratris* is the predominant thrips species on tomatoes in Thailand (Murai *et al.,* 2000; Rodmui, 2002). In addition to tomatoes, *C. claratris* has also been recorded on several vegetable crops in Thailand (Bansiddhi and Poonchaisri, 1991; Murai *et al.,* 2000; Rodmui, 2002). The better adaptation to high temperatures of *C. claratris* compared to other thrips species occurring in Asia (chapter 2), coupled with its vector competence for the CaCV-AIT (chapter 4) suggest that it has the potential to become an important thrips pest

throughout tropical Asia. In addition, with 16-18ºC the lower thermal thresholds of *C. claratris* (chapter 2) indicate that it might even thrive under more temperate conditions. Hence, efficient plant quarantine measures are vital to prevent a spread of *C. claratris* in Southeast Asia and beyond.

Our findings revealed that *C. claratris* can transmit CaCV-AIT. This introduces a new vector to the presently existing pool of ten thrips vectors (Ullman *et al.,* 2002). In recent years in Thailand tospoviruses are increasingly becoming a major constraint for the production of many economically important crops. For instance *Groundnut bud necrosis virus* (GBNV) has become a major threat for groundnut production (Wongkaew and Chuapong, 1997). Moreover, incidences of *Watermelon silver mottle virus* (WSMV), CaCV and *Melon yellow spot virus* (MYSV) have been recorded on important vegetable species in Thailand like watermelon, cucumber, pepper and melon (Oraprapai Gajanandana [Biotec, Scienec park, Bagkok, Thailand] and Pissawan Chiemsombat [Kasetsart University, Kamphaeng Saen, Thailand], personal communication). Since 2002, this CaCV has emerged as an important disease on tomatoes, causing high losses in central and north-eastern regions of Thailand (Pissawan Chiemsombat, [Kasetsart University, Kamphaeng Saen, Thailand], personal communication). In the greenhouse studies (chapter 4) very often virus infections in young tomatoes resulted in the death of the plants prior to fruit setting. Infected mature tomato plants were able to produce fruits, but with abnormalities like ring spots. Such

damaged fruits are hard to sell on Thai markets, and if only at considerably lower prices.

In general, tospoviruses possess a broad host range (German *et al.,* 1992). In addition to crops they often also infect weeds, which then can become important sources for the viruses, as well as for their vectors (Hobbs *et al.,* 1993; Chatzivassiliou and Boubourakas, 2001). In Thailand, Wongkaew and Chuapong (1997) serologically proved that the presence of GBNV in the common weed species *Spilanthus paniculata* Wall. ex DC. (Asteraceae), *Physalis minima*, *Synedrella nodiflora* (L.) (Asteraceae) and *Cleome viscosa* L. (Capparaceae). Presently, nothing is known about additional host plants for CaCV-AIT, be they crops or weeds. However, such information is urgently needed to identify the sources of CaCV-AIT outside the greenhouse environment.

Very often thrips vectors have the competence to transmit several tospovirus isolates (Ullman *et al.,* 2002). Thus, in future studies the potential ability of *C. claratris* to vector serologically closely related tospoviruses like GNBV and WSMV or other tospoviruses should be investigated. Moreover, in future it may be possible to directly monitor viruliferuos *C. claratris* by detecting the virus inside the thrips body by means of Cocktail ELISA (Resende *et al.,* 1991) or RT-PCR (Weekes *et al.,* 1996), which would help to predict the virus incidences within a crop.

Results of chapters 3 and 5 will be helpful for the development of integrated management programs for *C. claratris*. Minimization of primary invasion of thrips

and their secondary spread within the crop are of crucial importance for the establishment of control strategies against *C. claratris*. Findings from chapter 3 showed that the net type (pore size 0.18 mm) used in the greenhouses at AIT could not prevent a thrips infestation and subsequent virus infection. Hence, nets with smaller pore size are essential to efficiently exclude a thrips invasion. For instance a net with a 0.05 mm pore size can reduce a thrips invasion in the AIT greenhouses without negatively affecting the microclimatic conditions required for tomato production (Harmanto, AIT/ Hanover University, personal communication). The results of chapter 3 also indicated that thrips infestation started one week post-planting of the tomato seedlings into the greenhouse. However, initial sources for *C. claratris* and this tospovirus (name) remained to be identified. Results from chapter 5 showed that spinosad can cause very high mortality in all foliar-dwelling life stages of *C. claratris.* Thus, weekly spraying of spinosad, starting immediately after transplanting the tomatoes into the greenhouses, will greatly reduce the initial invasion as well as the secondary spread of *C. claratris*. However, the sole reliance on one chemical greatly increases the risk of resistance development in the pest (Williams and Dennehy, 1996; Broadbent and Pree, 1997), and resistance development of insects against spinosad has already been reported (Ahmad *et al.,* 2002; Young *et al.,* 2002). In addition, our results revealed that neem can exert a substantial impact on first instar larvae of *C. claratris*. In a similar study with the western flower thrips *Frankliniella occidentalis* (Pergande) Thoeming *et al.* (2003) recorded strong contact effects of neem on the soil-dwelling life stages of the

thrips. As *C. claratris* also prefers to pupate in the soil, in future studies the impact of neem on the soil-dwelling life stages should also be evaluated.

In conclusion, the results presented in this work show that *C. claratris* has a high potential to directly and indirectly, the latter through vectoring CaCV, damage tomatoes under protected cultivation in central Thailand. Moreover, its microclimatic preferences indicate that *C. claratris* can become a major thrips pest in tropical Southeast Asia. The here presented data on population dynamics and control with bio-pesticides can provide baseline information for the development of monitoring and integrated pest management programs, respectively.

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