

Control of thrips
(*Ceratothripoides claratrix* (Shumsher))
by using UV absorbing films, nets
and reflective mulches

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Abstract

Protected cultivation in the tropics is permanently challenged with both high heat and humidity on the one hand and the risk of arthropod pests' infestation on the other hand. Cladding the sidewalls of greenhouses with porous nets can considerably improve ventilation and reduce heat and humidity overload; however, crops within these greenhouses are vulnerable for the immigration of herbivorous insect pests, especially such tiny and mobile insects as thrips. Hence, ventilation efficiency is conflicting with insect impermeability ("thrips tightness") of greenhouse covering materials. Additional properties of the greenhouse constructions or supplementing measures are desired to compensate for the poorness of physical exclusion. One environmentally friendly promising solution is to hinder the immigration of insects through disturbing their visual abilities. Insects with optical orientation strongly rely upon solar ultraviolet radiation (UV) (ranging from 340 to 390 nm) for navigation, dispersal and host location. Hence, modifying the natural UV portion in the ambient solar spectrum, by either eliminating UV transmittance into netted greenhouses or by excessive reflection from mulched ground, can cause visual disorders and disturb pest orientation and host recognition behaviour. Therefore, in the herein study we investigated the effects of visual disturbance caused by modifying solar UV intensity inside netted greenhouses on the immigration, distribution and population growth of *Ceratothripoides claratris*, a destructive thrips pest species of tomato plants in Central Thailand. Moreover, the incidence and spread of thrips transmitted Capsicum chlorosis virus (CaCV) was considered. All experiments were realized in greenhouses at the Asian Institute of Technology (AIT) from 2005 to 2007, within the frame of the DFG research group FOR 431 "Protected cultivation – an approach to sustainable vegetable production in the humid tropics".

In the first series of experiments, the effect of UV free environments on thrips flight and dispersal behaviour was studied. The transmittance of UV into the greenhouses was drastically reduced by means of UV absorbing plastic films (roof cover) and nets (sidewall cladding). Consequently, greenhouses with different internal UV intensity regimes were constructed using both, either UV absorbing or transmitting plastic films and nets in different combinations. UVA measurements confirmed the almost complete exclusion of the solar UV in greenhouses, where UV absorbing plastics and

nets were combined. This effect was slightly impaired when UV transmitting net was used at the sidewalls. When UV transmitting plastics were combined with the UV absorbing nets the UV transmission was not significantly reduced compared to the conventional UV transmitting greenhouses with neither UV absorbing plastic films nor nets. The results clearly demonstrate the superior UV exclusion effect by UV absorbing plastic roof cover than by sidewall nets.

To evaluate the response of *C. claratris* to either higher or lower UV intensity regimes, every two greenhouses clad with either UV absorbing and/or transmitting plastic roofs and net sidewalls were connected by a black compartment, which served as artificially release unit for the thrips. The results demonstrated a clear preference of *C. claratris* to compartments with higher UV intensities.

The preference and exclusion effects for thrips by UV manipulating greenhouses were further studied with four independent greenhouse constructions with same combinations of cladding materials as described above. With sticky cards exposed outside close to the sidewall nets, a fewer number of thrips were trapped at UV absorbing greenhouses, which suggests a lower attraction of the thrips to these greenhouse constructions, compared to those with UV transmitting covers. Subsequently, lower numbers of *C. claratris* were observed inside UV poor greenhouses on traps adjacent to the inner net walls as well as on exposed tomato plants, clearly showing a strong reduction of thrips penetration through the nets in relation to the decrease of inner UV intensity. The combined effect of the UV absorbing plastic and net materials resulted in a remarkable thrips free zone inside this greenhouse type compared to high thrips infestation and virus infection of plants in greenhouses clad with UV transmitting plastic covers. The opening of entrances for additional ventilation purposes impaired the excellent protection effect of UV absorbing greenhouse type, even though a significant protective effect was maintained in comparison to the UV transmitting covers. The thrips exclusion properties of conventional UV transmitting constructions could be improved simply by extending the UV absorbing plastic roof outside and upright to the sidewalls like a collar. The so established UV poor buffer zone around the greenhouses considerably reduced movement of thrips towards the sidewall nets and immigration through this barrier.

In addition to the deterrent effect, the dispersal of *C. claratris* under different regimes was studied. Thrips were released on single plants (simulation of artificial introduced hot spots) under UV absorbing covers compared to UV permeable conditions. In general, the dispersion potential of *C. claratris* was low regardless of the UV light regimes. Most of the released thrips tended to remain on plants adjacent to the release plants. However, under the UV deficient conditions shorter dispersal distances could be recorded and different slopes in the regression analysis indicated higher clumping rates around the release points compared to “normal” UV conditions.

In the second sequence of experiments, possible deterrent effects of a reflective mulch film on the immigration of thrips was investigated. Silver mulch film reflecting approximately 30% of the incoming solar UV was used to cover the ground inside and/or outside conventional UV transmitting greenhouses. Results showed a delayed infestation of thrips compared to greenhouses with black mulch inside, which reflected only about 5% of UV. However, the delayed thrips immigration did not result in long term control of thrips population growth in the greenhouses.

Infection intensity and spread of CaCV with time were closely associated with the temporal and spatial dynamics of the thrips vector, hence, the reduction of thrips infestation under poor UV regimes or by reflective mulches resulted in a decrease in the number of virus infected plants and stabilized yield considerably.

In conclusion, the herein presented results strongly suggest that the manipulation of the visual orientation of thrips offers a promising control strategy for that pest in protected cultivation systems in the tropics.

Keywords: UV absorbing plastics and nets, reflective mulch, tropical greenhouse constructions, thrips *Ceratothripoides claratris*, immigration, dispersal, Capsicum Chlorosis-Virus (CaCV).

Zusammenfassung

Geschützte Anbausysteme in den humiden Tropen sind mit zwei wesentlichen Herausforderungen konfrontiert: Kontrolle des internen Klimas mit hohen Wärmelasten und extremen Luftfeuchtigkeiten sowie Vermeidung der Einwanderung und/oder Einschleppung von Schadinsekten, die nach Etablierung im geschlossenen System aufgrund hoher Reproduktionsraten in einem quasi feindfreien Raum extremes Schadpotential aufbauen können. Die Abdeckung der Seitenwände tropischer Gewächshäuser erfolgt in der Regel mit Netzen. Diese bieten eine physikalische Barriere bei gleichzeitiger Möglichkeit intensiver Ventilation. Je durchgängiger die Netze sind, desto günstiger können die inneren klimatischen Bedingungen gesteuert werden. Auf der anderen Seite reduziert sich die Ausschlussleistung von Netzen gegenüber Schadinsekten mit steigender Maschenweite. Insbesondere für extreme kleine und mobile Schädlinge wie Thripse bilden Ausschluss ("thripsdicht") und Ventilation einen unvereinbaren Zielkonflikt. Gefragt sind zusätzliche Eigenschaften der Gewächshauskonstruktionen oder ergänzende Maßnahmen, um die Durchlässigkeit der für eine Klimasteuerung geeigneten Netze für Schädlinge zu kompensieren.

Eine vielversprechende Möglichkeit die Einwanderung von Schädlingen zu beeinflussen ist die Störung ihrer optischen Orientierung. Viele Insektenarten, insbesondere Phytophage, nutzen für ihre optische Orientierung, für die Navigation bei Langstreckenflügen, bei kleinräumigen Wanderbewegungen (Dispersionen) oder bei der Erkennung von Wirtspflanzen nicht nur das Differenzieren von Formen und Kontrasten, sondern auch Farbsehen und hier auch den kurzwelligen ultravioletten (UV) Anteil (zwischen 340 – 390 nm) des Lichtspektrums.

Eine gezielte Manipulation des UV Anteiles im Lichtspektrum, beispielsweise durch Ausschalten der UV Einstrahlung in die hier diskutierten Netzgewächshäuser oder durch überproportional hohe Reflektion von bodendeckenden Mulchfolien kann das Orientierungsverhalten von Schädlingen nachhaltig stören. Deshalb wurde in der hier vorgelegten Studie der Einfluss modifizierter UV Einstrahlung in tropische Gewächshäuser untersucht, wobei Einwanderung, Verteilung und Populationswachstum von *Ceratothripoides claratris*, einer unter den beschriebenen

Anbaubedingungen an Tomatenkulturen äußerst destruktiven Thripsart im Vordergrund standen. Des Weiteren wurde das Auftreten und die Verbreitung des Tospovirus Capsicum chlorosis virus (CaCV), das von dieser Thripsart übertragen wird, berücksichtigt. Alle Experimente fanden in Versuchsgewächshäusern auf dem Campus des Asian Institute of Technology (AIT) zwischen 2005 to 2007 statt. Das Forschungsprojekt gehörte zum Programm der DFG-Forschergruppe FOR 431 "Protected cultivation – an approach to sustainable vegetable production in the humid tropics".

In der ersten Serie von Experimenten wurde der Einfluss UV armer "Flugräume" auf Flugaktivität und Verteilung (Wahlverhalten) von *C. claratris* untersucht. Die UV Einstrahlung in Gewächshauskonstruktionen wurde durch den Einsatz UV absorbierender Dachfolien und Netze an den Seitenwänden der Häuser reduziert. Durch unterschiedliche Kombinationen jeweils UV absorbierender und UV durchlässiger Folien und Netze konnten in Versuchsgewächshäusern abgestufte UV Strahlungsintensitäten erzeugt und deren Effekte verglichen werden. Die differenzierten UV Anteile wurden durch Messungen der UV Strahlung erfasst. Diese Messungen bestätigten, dass bei Kombination von UV absorbierenden Dachfolien mit UV absorbierenden Netzen an den Seitenwänden eine nahezu vollständige Ausschaltung der internen UV Strahlung möglich ist. Mit UV durchlässigen Netzen an den Seitenwänden bei absorbierenden Dachfolien war die Filterwirkung nur leicht vermindert, hingegen deutlich bei der Kombination von durchlässigen Folien mit absorbierenden Netzen. Keine Filterwirkung ergab die Kombination von UV durchlässigen Materialien für Dach und Seitenwände.

Zur Überprüfung des Wahlverhaltens von *C. claratris* wurden unterschiedliche Kleingewächshäuser (Kombinationen der Eindeckungen wie oben beschrieben) jeweils in Paaren miteinander so verbunden, dass sich zwischen unterschiedlichen Häusern (Flugräumen) ein beidseitig zu öffnender, abgedunkelter Käfig ("release box") für die Freilassung der Thripse befand. Diese konnten nach Freisetzung im Startkäfig ihre Orientierung frei wählen. Die Ergebnisse zeigten eine eindeutige und gestufte Präferenz von *C. claratris* entsprechend der UV Intensität in den Flugräumen. Die Ausschlussleistung der unterschiedlichen Häuser wurde danach unter miniaturisierten aber realistischen Bedingungen mit nicht kombinierten,

individuellen Versuchseinheiten (Kleingewächshäuser) weiter verfolgt. Dazu wurden einseitig fängige Klebetafeln entweder außen auf den Netzen oder innen an der Seitenwand (Klebeflächen jeweils nach außen gerichtet) exponiert. Es zeigte sich, sowohl bei der Zuwanderung von außen auf die Seitennetze, als auch bei der Passage der Thripse durch die Netze anhand der geringen Fangzahlen auf den Fallen eine hohe Ausschlussleistung der UV absorbierenden Konstruktionen. Bestätigt wurden diese Befunde durch die geringe Flugaktivität von *C. claratris* innerhalb der UV armen Gewächshäuser, dokumentiert durch die Fangzahlen auf zentral exponierten Klebefallen und den Befall auf exponierten Tomatenpflanzen. Der kombinierte Effekt von UV absorbierenden Dachfolien mit absorbierenden Netzen ergab jeweils weitgehend thripsfreie Gewächshäuser im Vergleich zu hohen Befallsdichten und auch Virus-Infektionen von Pflanzen in Gewächshäusern mit UV durchlässigen Folien. Eine zusätzliche Öffnung der Gewächshäuser an den Stirnseiten (Türen) zur weiteren Verbesserung der Klimatisierung unter extremen Bedingungen verhinderte zwar den vollständigen Ausschluss von *C. claratris* durch die UV absorbierenden Gewächshaustypen, trotzdem konnte auch unter diesen Bedingungen eine signifikante Reduzierung der Befallszahlen im Zentrum der Häuser im Vergleich zu UV durchlässigen Bedeckungen erreicht werden. Die Barriereleistung konventioneller UV durchlässiger Gewächshäuser konnte zudem durch eine einfache Zusatzkonstruktion deutlich verbessert werden. Verlängerte Dachüberstände mit UV absorbierenden Folien können bei dem hohen Sonnenstand in den Tropen zu UV armen Zonen rund um die Gewächshäuser führen, welche wiederum die Dispersion der Thripse in Richtung der Netzseiten und damit auch die Immigration durch die Netze deutlich reduzieren.

Zusätzlich zu dem abschreckenden Einfluss geringer UV Intensitäten wurde die Dispersion von *C. claratris* unter verschiedenen UV Bedingungen untersucht. Dazu wurden Thripse auf einzelnen Pflanzen freigelassen, um punktförmige Einschleppungen zu simulieren. Die Dispersionsneigung von *C. claratris* erwies sich generell als recht gering, viele der freigelassenen Individuen entfernten sich nur langsam von den Startpflanzen. Trotzdem konnten unter UV absorbierenden Bedingungen die kürzesten Verbreitungsdistanzen bestimmt werden und

Regressionsanalysen zeigten die intensivste Klumpung um die Freilassungspunkte bei dieser Gewächshausvariante.

Eine zweite Versuchsreihe beschäftigte sich mit abschreckenden Effekten einer reflektierenden Mulchfolie. Diese silberimprägnierte Folie reflektierte ca. 30% der einfallenden UV Strahlung. Die Folien wurden als Bodenbedeckung entweder flächendeckend oder als Streifen um UV durchlässige Gewächshäuser ausgelegt und mit schwarzen Mulchfolien verglichen. Die Ergebnisse zeigten eine deutliche Verzögerung der Besiedlung der Häuser mit *C. claratris* in den Varianten mit den reflektierenden Folien. Trotz der verzögerten Immigration konnte jedoch keine signifikante Reduktion des Populationswachstums von *C. claratris* in diesen Häusern erreicht werden.

Die Intensität initialer Infektionen von Tomatenpflanzen mit dem Capsicum Chlorosis Virus (CaCV) und dessen Verbreitung mit dem Verlauf der Vegetationsperiode korrelierte eng mit der räumlich/zeitlichen Dynamik des Vektors. Folgerichtig ergaben sich geringere Virusprobleme unter UV armen Bedingungen und bei dem Einsatz reflektierender Mulchfolien.

Zusammenfassend betrachtet zeigen die hier präsentierten Ergebnisse, dass eine Manipulation der visuellen Orientierung von Thripsen, insbesondere die Ausschaltung der UV-Strahlung, vielversprechende Möglichkeiten eröffnet, diese extrem schwierig zu bekämpfenden Schädlinge auch in geschützten Anbausystem der Tropen effektiv und umweltschonend zu kontrollieren.

Stichworte: UV absorbierende Folien und Netze, reflektierende Mulche, Tropengewächshäuser, der Thrips *Ceratothripoides claratris*, Immigration, Dispersion, Capsicum Chlorosis-Virus (CaCV).

TABLE OF CONTENTS

Abstract	i
Zusammenfassung	iv
Abbreviations	x
1 General introduction	1
<i>Ceratothripoides claratris</i>	7
Insect vision and host location.....	10
2 Effects of UV deficiency on the flight and immigration of thrips <i>Ceratothripoides claratris</i> into tomato greenhouses in the tropics	14
2.1 Introduction	14
2.2 General materials and methods	15
2.2.1 Study location	15
2.2.2 Thrips rearing	16
2.2.3 Plants.....	16
2.2.4 Plastic films and nets.....	17
2.2.5 Basic greenhouse setups	17
2.2.6 Measurement of climatic parameters	17
2.2.7 Statistical analysis	19
2.3 Light transmission and other climatic parameters inside the greenhouses clad with different combinations of UV transmitting and UV absorbing plastics and nets	20
2.4 Paired choice experiments with small experimental greenhouses	22
2.4.1 Materials and methods	22
2.4.2 Results.....	24
2.4.3 Discussion	24
2.5 Natural immigration of <i>Ceratothripoides claratris</i> to greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets.	28
2.5.1 Materials and methods	28
2.5.2 Results.....	31
2.5.3 Discussion	46
2.6 Effect of projecting roofs covered with UV absorbing plastic film to greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets on the immigration of <i>Ceratothripoides claratris</i>	52
2.6.1 Materials and methods	52
2.6.2 Results.....	55

2.6.3	Discussion	58
2.7	Spatial and temporal distribution of <i>Ceratothripoides claratris</i> under the greenhouses clad with different combinations of UV transmitting and UV absorbing materials	61
2.7.1	Materials and methods	61
2.7.2	Results.....	64
2.7.3	Discussion	71
3	Effect of UV reflecting mulch film on the immigration of <i>Ceratothripoides claratris</i>	74
3.1	Introduction	74
3.2	Materials and method	75
3.3	Results	78
3.4	Discussion.....	86
4	General discussion.....	90
	References.....	101
	Acknowledgement	116

Abbreviations

BI	Black mulch
CaCV	Capsicum chlorosis virus
DAR	Day after release
DAS – ELISA	Double-antibody sandwich enzyme-linked immunosorbent assay
IPM	Integrated pest management
L:D	Relation of light to darkness
L1	First larval instar
L2	Second larval instar
Nab	Ultraviolet absorbing net
Ntr	Ultraviolet transmitting net
Pab	Ultraviolet absorbing plastic
PAR	Photosynthetic active radiation
Ptr	Ultraviolet transmitting plastic
SAS	Statistical analysis system
SD	Standard deviation
SE	Standard error
Si	Silver mulch
UV	Ultraviolet radiation

1 General introduction

Raising concerns about the sustainability of agricultural production, in particular the health risks of consumers and environmental pollutions demand the development and improvement of integrated pest management (IPM) systems aiming to use bio-control measures as the first option and to reduce the use of synthetic pesticides as much as possible. The control of pests with an IPM toolbox also can help to reduce the risk of high selection pressure for pests' tolerance or resistance to a few safe and effective insecticides and thus to prolong their efficient life-time. Prevention is usually more effective than remedial control of the pest. Recently, there have been increasing interests in growing high value crops like tomatoes under protected environments, i.e. various growing tunnel and greenhouse types adopted to different climatic conditions (Jensen and Malter 1995, Krizek et al. 2005, Jones 2008).

Horticultural practices under protected systems such as well-designed greenhouses provide great flexibility to growers in planning the cropping season and production regardless of adverse ambient environmental conditions, i.e. heavy rainfalls, hails, hoarfrost, heavy wind and others (Jensen and Malter 1995, Antignus 2000, Antignus 2007). Inputs (irrigation and fertilization) in such systems can be optimally controlled by growers and hence high crop quality can be achieved in a cost-effective manner. Moreover, screen-, and glasshouses provide opportunities to prevent the immigration of insect pests, and/or to establish bio-control strategies with natural enemies, which can help to waive or at least to considerably reduce the intensity of insecticide spraying.

A main problem of protected cultivations in hot periods in the Mediterranean and in the humid tropics is the high energy (heat) load of such systems (Nielsen 2002, Tanny et al. 2003, Harmanto et al. 2006). High heat load inside the greenhouses can cause stress to plant growth and reproduction. At the same time, plants may be especially under risks from pest and pathogen damages once introduced because heat and humidity loads inside the greenhouses are known to favour the development of most well heat adapted pests such as thrips and whiteflies (Premachandra et al. 2004) and fungal diseases. Hence, proper regulation of

temperature and humidity inside greenhouse crop stands is necessary to avoid heat damage and to establish appropriate evapotranspiration conditions for optimal water and nutrition balances of crops (Tanny et al. 2003, Harmanto et al. 2006) and also to affect the environmental conditions for pests and diseases. Moreover, greenhouses are considered by themselves as areas free of natural enemies, hence without natural regulation. Therefore, it is also extremely necessary to reduce the immigration of or artificial infestation with detrimental pests, especially herbivorous arthropods.

Netted greenhouses with plastic covers and net sidewalls can serve as an alternative solution for closed glass- or plastic-houses in the hot and humid tropics to improve the air circulation and prevent the immigration of pest insects. Different greenhouse structures and their covering materials can manipulate the transmission of solar radiation and ventilation (Giacomelli and Roberts 1993, Bailey et al. 2003), and therefore the microclimatic conditions, e.g. temperature, humidity and air flow inside the greenhouses and thus affecting crop performance (plant physiology) as well as the environmental conditions for pests and diseases. The selection of greenhouse cladding materials has to compromise among several aspects to achieve optimum conditions for plant growth as well as pest and pathogen exclusion, leading to optimal crop production systems in terms of crops quality, i.e. physical quality and acceptable pest and diseases infestations and permissible, low pesticide residues, high yields and strong market benefits.

Nets as greenhouse sidewalls are physical barriers able to control the immigration of insects. The efficiency is defined by the relation of the size of the immigrating insects to the mesh size. Rather big pest insects such as lepidoteran pests, adult leafminers and even winged adult aphids can be successfully excluded by small mesh sizes, meaning porous material. However, in order to prevent the immigration of such small insects like thrips, extremely tight nets with fine mesh sizes are necessary. Unfortunately, reducing the mesh porosities will inhibit the proper air exchange between inside and outside the greenhouses, increasing the inner temperature and humidity within crop, which will not only affect plant growth but also favour, in particular, fungal pathogens. Reducing the mesh size, however, may open inlet ports for immigration of the small sized insects like thrips and whiteflies. These insect pests are usually especially destructive since they often not only damage by their feeding

activity but also vector virus diseases. To overcome the tightness – ventilation dilemma of cladding materials, it seems necessary to improve the ‘barrier’ efficiency of nets or roof films with additional features such as killing (e.g. net impregnated with insecticides) or insect behaviour manipulating properties (e.g. deterrent compounds, disturbing visual orientation). In particular, the latter is in accordance with IPM philosophy. A promising effort is to manipulate insects’ orientation by disturbing the visual based crop selection process. Changing the “normal” light properties especially in the short wavelength portion of the light spectrum can facilitate this approach.

The ultraviolet radiation (UV) components of the solar radiation spectrum is said to play an important role for insect behaviour including orientation, navigation, feeding, and interaction between sexes (Raviv and Antignus 2004, Antignus 2007). Too low or no UV reflectance from host plants affects herbivorous insects’ orientation and recognition properties. However, too high reflection of UV from a ground surface also can repel the approach and landing of those insects to and onto their host plants. Changing of UV on and around the crop areas may interfere with insects’ ability to take off, to initiate orientation flight and to detect and locate the crop plants.

The filtration (absorption) of UV around or within greenhouses will impede insects’ flight to navigate and locate the crop inside those greenhouses. Greenhouse materials, plastic films and nets, impregnated with UV absorbing additives into the raw materials, can filter out the UV from solar light. The principle of UV absorbing materials is to block the transmission of UV (200 - 400 nm), but not to interfere with the transmission of the visible light spectrum/or photosynthetically active radiation (PAR) (400 – 700 nm) (Antignus et al. 1996, Antignus 2000, Costa et al. 2002, Antignus 2007). Absorbing compounds to the short ultraviolet wavelengths were traditionally added into the polyethylene films to increase the stability of the films (Krizek et al. 2005) or to prevent the petal blackening of greenhouse roses (Antignus 2007). The combination of UV absorbing plastic films on the roofs and nets on the sidewalls serves as physical and optical barriers to the immigration of insect into greenhouses. Therefore, cladding the greenhouses with UV absorbing plastic films and nets will allow increasing the size of net porosities, leading to the improvement of the air circulation and ventilation, while still achieving high efficacy of insect exclusion.

The use of polyethylene films and nets with UV absorbing properties has gained attention as a promising tool in IPM strategies to control of diseases and insect pests. The efficiency is dependent on the UV exclusion property of the materials and the dependence of the target insects on UV for visual orientation. Several recent studies have reported about the use of UV absorbing plastic films and nets to prevent the immigration of economically important insect pests including whiteflies (*Bemisia tabaci* and *Trialeurodes vaporariorum*) (Antignus et al. 1996, Antignus et al. 1998, Costa and Robb 1999, Antignus 2000, Costa et al. 2002, Mutwiwa et al. 2005, Doukas and Payne 2007a), thrips (*Frankliniella occidentalis*) (Costa and Robb 1999, Kigathi 2005), aphids (*Aphis gossypii*), leaf miners (*Liriomyza trifolii*), red spider mite (*Tetranychus telarius*) (Antignus et al. 1998) and moths (*Laphigma sp.*) (Antignus 2000).

Not only the light spectrum around plant stands is important for insect orientation, the different reflection patterns from plants and ground also decide whether insects will recognize and alight on the host plants. Artificially increased UV reflectance around or inside crop stands using reflective mulches impregnated with aluminium can disturb the host selection behaviour of the pests. Such UV reflective mulches delayed the immigration of whiteflies (*Bemisia sp.*) (Brust 2000, Summers and Stapleton 2002a, Summers et al. 2004), aphids (*A. gossypii*) (Summers et al. 2004), leave hopper (*Dalbulus maidis*) (Summers and Stapleton 2002b), thrips including *Thrips spp.* (McLaren and Fraser 2001, Van Toor et al. 2004) and *Frankliniella spp.* (Stavisky et al. 2002, Reitz et al. 2003, Riley and Pappu 2004).

However, thus far in almost all studies reporting about UV effects of films and mulches, a detailed measurement of the actual amount of UV reduction or reflectance inside the greenhouses or/and in the field was missing. The spectral transmittance of greenhouse cladding materials and reflectance of mulches, if available, were mainly measured in the laboratory. Subsequently, the effects of UV absorbing plastics and nets and reflective mulches were only quantified by measurement of pest numbers or virus infections in case of vectors. Moreover, most of these studies were performed in the Middle East or temperate regions but not under the climatic conditions of the humid tropics.

Ceratothripoides claratris is the main insect pest among others heavily damaging tomato in central Thailand, especially in protected cultivation systems. This insect species is well adapted to the high heat/humidity conditions in tropical netted greenhouses (Premachandra et al. 2004). The short life cycle and fast development result in fast population built-up after infestation (immigration). *C. claratris* feeds on the leaves, and as typical for thrips, it opens single cell of the leaf parenchyma with the mandible and sucks out the cell content with a dine tube built by the maxilla. Feeding from cell to cell results in a typical destruction of leaf tissues (feeding scars) leading to a loss of photosynthetic active tissues and heavy infestations finally cause wilting of the whole plant. Furthermore, *C. claratris* is an efficient vector of a tospovirus, the Capsicum Chlorosis Virus (CaCV), leading to severe plant damages and fast losses of the whole crop (Premachandra et al. 2004, Premachandra et al. 2005a).

Management of thrips is usually difficult because of several reasons: (1) their tiny sizes and cryptic feeding behaviours in the crevices of flowers, bracts and leaf sheaths making them difficult to be detected at the beginning of the infestation (Lewis 1997a, Jensen 2000, Morse and Hoddle 2006), and for insecticides to reach to the individuals, (2) their life cycle with soil passage stages of the prepupa and pupa giving a reservoir not being affected by foliar treatments, (3) their fast development and short life cycle especially in the warm environments inside the greenhouses (Lewis 1997a), and (4) their fast transmission of virus after short feeding periods. Hence, the selection of appropriate and in time control methods is very crucial for the success of the control. The demand for safe control measures with regards to farmers' and consumers' health and environmental damages complicates the situation. Presently, the management of *C. claratris* can be successful with some fast degrading bio-pesticides. Safe control on young tomato plants can be achieved with Neem treatments of the soil (systemic Neem effects) (Thoeming and Poehling 2006); on older plants, Spinosad and Aradizachtin are efficient (Premachandra et al. 2005b). However, under high infestation pressure more broad spectrum synthetic pesticides such as the Pyrethroid Cypermethrin are used by the farmers. Regarding IPM approaches, insecticides should only be applied if finally necessary. Such strategies have also the advantage to prolong the lifetime efficacy of insecticides and to prevent

resistant development in the pests. Biotechnical measures such as the use of UV absorbing cladding materials for greenhouse designs promise an alternative strategy in management the infestation of this thrips on tomato plants. If successful this approach will not only inhibit thrips immigration to the crop but also provide the possibility to increase the net's hole sizes for the netted greenhouses leading to a great improvement of the microclimates inside the greenhouses under tropical conditions. First pilot trials about potential of that approach were demonstrated by Kumar and Poehling (2006).

In the herein studies, effects of UV absorbing plastic films (roofs) and nets (sidewalls) were comprehensively investigated for their abilities in preventing thrips, *C. claratris*, infestation in tomato greenhouse in Central Thailand. Within this purpose, 1) Pair-wise choice experiments were performed to test the preference of *C. claratris* to different UV intensities; 2) Greenhouses clad with different combinations of UV absorbing and transmitting plastics and nets were compared for their deterrent potentials to the landing of thrips on the outer greenhouses' net sidewalls and its penetration into greenhouses; 3) The effects of additional extended UV absorbing roof structures influencing the UV intensities around the greenhouse constructions on flight and immigration behaviours of *C. claratris* were using artificial releases of *C. claratris* from outside; 4) The effects of different UV intensity conditions on the spatial dispersal of *C. claratris* from the first artificial infested points was studied.

In addition, a suspected insect repelling effect of reflective mulch materials was also investigated in combination with the properties of netted greenhouses. Effects of mulches arranged as ground cover either inside the greenhouses and/or as an outside surrounding margin were compared.

In conclusion, the objectives of the present study were:

- To study the effects of manipulation of UV intensity on the orientation, immigration and dispersal of *C. claratris* using UV absorbing plastic films, nets and UV reflective mulches.
- To study the population dynamics of *C. claratris* on tomato plants after its introduction into netted greenhouses with different UV intensities of internal light conditions and its ability to transmit CaCV tospovirus.

Ceratothripoides claratris

Biology and ecology

Ceratothripoides claratris (Shumsher) (Thysanoptera: Thripidae) has been reported in Thailand since 1992 (Okajima et al. 1992) and was declared as a thrips pest species in the tropical regions in 1998 (Mound and Kibby 1998). However, not until 1999, this thrips species was for the first time reported damaging tomato. This is the only thrips species that causes severe damages to leaves and stems but not fruits of tomato in fields in Central Thailand; damage was also reported from Malaysia. So far, there has not been any other report on the presence and occurrence of this thrips species in any other country (Murai et al. 2000).

Similar to other thrips species in the order Thysanoptera, *C. claratris* shares general biological characteristics of herbivorous thrips. Thrips is a minute and slender insect, usually a few millimetres long, and generally yellow, brown, or black in colour (Lewis 1997b, Morse and Hoddle 2006). It is considered among the stealthiest insect invaders because of its small size and cryptic habits (Moritz 1997, Morse and Hoddle 2006). *C. claratris* belongs to the family Thripidae, which gathers most of the relatively few serious crop pest thrips species out of a total of 8000 defined and undefined thrips species in the order Thysanoptera (Lewis 1997b, Mound 1997).

Intensive studies to understand the biological characteristics, damage severity and management of *C. claratris* have been carried out in Thailand since 2002 (Premachandra et al. 2004, Premachandra et al. 2005b, Premachandra et al. 2005c, Thoeming and Poehling 2006, Kumar and Poehling 2006). Most of adults and larvae of *C. claratris* were found to aggregate on the lower leaves of tomato plants. Subsequently, the infestation gradually moved upward to higher leaves. Adults were observed on flowers and fruits at a late stage of infestation only if leaves were hardened and deteriorated by heavy thrips infestation; however, no severe damage was noticed. Hardly ever this thrips species was found on buds (Premachandra et al. 2005c). Severe infestation at the beginning of tomato planting in greenhouses can cause complete death of the plants after seven weeks planting (Premachandra et al. 2004). *C. claratris* was also recorded damaging cucurbit crops, melon and tomato but not eggplant and chilli pepper (Murai et al. 2000). Other hosts including pumpkin,

cowpea, yard long bean and chilli were also reported as suitable hosts for *C. claratris* (Premachandra et al. 2004). Apart from *C. claratris*, *Thrips palmi* was also observed damaging tomato in Southeast Asia, Japan and Central America. However, this thrips species is not considered as the pest of tomato since it could not complete its lifecycle on tomato plants (Terry 1997, Murai et al. 2000). Thus, *C. claratris* is the most important thrips on tomato in Thailand and maybe in neighbouring countries as well (Murai et al. 2000). In awareness of the actual fast worldwide spreading of the Western Flower thrips *F. occidentalis* from its original distribution in western North America (Mound 1997, Kirk and Terry 2003), the further dispersion and distribution of *C. claratris* should be carefully observed.

C. claratris, like other thrips species in the suborder Terebrantia, has six developmental stages, including egg, two larval instars, prepupa and pupa, and adult (Premachandra et al. 2004). Smooth shelled eggs are inserted into plant tissues with a saw-like ovipositor (Lewis 1997c, Moritz 1997). Larvae feed on leaves. The late second larval instars drop off plants and pupate in the soil. At the optimum temperature for their development (30°C), which was determined by Premachandra et al. (2004), it took about 9 days for eggs to develop to adults, consisting of approximately 3 days for eggs, 2 days for first instars, more than 1 day for second instars and more than 2 days for prepupa and pupa. This temperature was also the best temperature for female fecundity in terms of the total number of eggs deposited as well as the mean daily eggs laid by both virgin and inseminated females. At this temperature, a female can live around twelve days and lay on average 9 to 10 eggs per day, whereas they can live longer but produce much fewer eggs at 25°C. At 40°C the longevities of both males and females were affected and females failed to reproduce. The sex ratio of offspring was strongly temperature-dependent. Female offspring biased at 30 – 35°C, whereas more males were produced at 25°C (Premachandra et al. 2004). According to Kirk (1997), in warm climatic environments, thrips can continue breeding throughout the year without an inactive period.

Virus association

C. claratris was described as vector of a tospovirus of the serogroup IV, which serologically and genetically closely resembles a recently described Capsicum

Chlorosis Virus (CaCV) on tomatoes (Premachandra et al. 2005a). Hence, *C. claratris* is added into a group of scarcely 13 thrips species as plant virus vectors (Campbell et al. 2008) out of more than definite 5000 thrips species (Ullman et al. 1997, Mound 2004, Mound 2005, Morse and Hoddle 2006). To be successful in transmitting virus, thrips must acquire virus during the feeding of first instars or early second instars (Nagata et al. 1999, Mound 2004, Mound 2005, Premachandra et al. 2005a, Morse and Hoddle 2006). The virus acquisition and transmission potential of thrips is significantly reduced with larval aging. The second instars of *C. claratris* that acquires virus at early first instars are ready to transmit virus, however, the transmission is more efficient as these viruliferous thrips develop into adults. Both male and female adults, who have similar virus acquisition history, have equal possibilities to transmit virus (Premachandra et al. 2005a). The vector competence of tospovirus is thrips species-dependent (Wijkamp et al. 1995), and also varies between different populations of the same species (Chatzivassiliou et al. 1999, Mound 2005). Even in a same thrips population, the virus acquisition and transmission potentials can vary among individuals (Halaweh and Poehling, personal communication, 2008). These authors raised the question of genetic involvement in virus vector competence of *C. claratris*. Thus, not every individual is able to acquire and transmit the virus although having fed on heavily virus infected plants at early first larval stage if a specific genetic trait is not present.

Management

Presently, management of *C. claratris* is successful with so called “bio-insecticides”, e.g. Spinosad, Avermectins, compounds derived from soil microorganism, and botanical insecticides, especially Neem. Synthetic insecticides also effectively control this thrips species, e.g. Cypermethrin. However, aiming at a sustainable greenhouse production, bio- and botanical insecticides should be preferred to synthetic insecticides if possible. For instance, biweekly repeated application of a water soluble Neem product containing 17% Azadiractin A or Neem pellets containing 0.01 - 0.5% Azadiractin A to the soil at the transplanting time of the tomato plants caused more than 85% and 71% corrected mortalities, respectively, of all thrips developmental stages under the greenhouse conditions in Thailand, which can be compared to the efficacy of the synthetic insecticides, Cypermethrin (Thoeming and Poehling 2006).

Foliar application of Spinosad caused 100% mortality of all developmental stages of *C. claratris* (Premachandra et al. 2005b).

Several other non-chemical control tactics have been proven to successfully control many thrips species. These alternatives include repellent plant volatiles (Koschier et al. 2007); trap plants (Buitenhuis et al. 2007); entomopathogenic fungi (Ekesi et al. 1998, Jacobson et al. 2001, Maniania et al. 2001, Maniania et al. 2003, Ugine et al. 2005a, Ugine et al. 2005b, Fiedler and Sosnowska 2007); entomopathogenic nematodes (Lim et al. 2001, Ebssa et al. 2001, Premachandra et al. 2003, Lim and Van Driesche 2004a, Lim and Van Driesche 2004b, Lim and Van Driesche 2004c, Ebssa et al. 2004a, Ebssa et al. 2004b); predatory bugs (Cocuzza et al. 1997, Scott Brown et al. 1999, Van Laerhoven et al. 2000, Sanchez and Lacasa 2002, Deligeorgidis 2002, Shipp and Wang 2003, Baez et al. 2004, Xu et al. 2006); predatory mites (Faraji et al. 2002, Shipp and Wang 2003, Walzer et al. 2004, Berndt et al. 2004); and UV absorbing plastic films and nets as greenhouse covering (Costa and Robb 1999, Kigathi 2005) and UV reflecting mulches (McLaren and Fraser 2001, Stavisky et al. 2002, Reitz et al. 2003, Van Toor et al. 2004, Riley and Pappu 2004). However, with *C. claratris*, only entomopathogenic fungi (Panyasiri et al. 2007) and the mentioned physical UV exclusion methods have been examined in first pilot experiments by Kumar and Poehling (2006). Since the herein study focuses on the effect of manipulated UV on the visual behaviour of *C. claratris*, some important basic aspects of insect vision and the mechanism that UV affects on insect's visual ability are reviewed more in detail in the following section.

Insect vision and host location

Host location behaviour of herbivorous insects is a complex activity, which is closely related to their migration and dispersal behaviour. Many stimuli including colours, shapes, sizes (at the angles that the hosts relatively appear to insect vision) and volatiles associated with the hosts have been reported to be involved in host finding behaviour of insects (Terry 1997, Giurfa and Menzel 1997, Kimmerle and Egelhaaf 2000). These visual and olfactory stimuli, either individuals or in mixture, are the primary cues used by insects to orientate to their host plants. Most of the studies on insect vision and host location have been performed with several species of the two

insect orders, Hymenoptera and Lepidoptera (Briscoe and Chittka 2001) in relation to flower recognition (Möller 2002). Hymenoptera are the best investigated group, of which several species from different habitats were examined (Briscoe and Chittka 2001). Although insects possess relatively simple nervous systems, they have sophisticated visual abilities, which are in some features quite similar to those of vertebrates. Their compound eyes, which have a high temporal but rather low spatial resolution, are adapted to resolve fast motions (sophisticated flyers). They also allow differentiation of colours, polarised lights and geometric patterns (Giurfa and Menzel 1997).

The visual mechanisms that insects use to orientate and navigate to the host plants are complex; and many mechanisms have been suggested to explain host detection abilities. For the here described study, host detection ability of herbivorous insects is discussed from the viewpoint of colour differentiation and/or reaction to the brightness of ambient or reflected light. Regarding colour vision, not only the colours of the target itself but also the colour contrast of targets with background components (water, soil, rocks, and others) helps plant visiting insects to distinguish between the targets and the surrounding environments. The crucial factors enabling the insect to distinguish between hosts and non-hosts are hue of colours, the dominant wavelength remitted from host's surface; the saturation and purity of the hue; the brightness, total energy, and percentage reflectance at peak wavelengths. The hue preference is species dependent; however, within a species males and females have similar responses to the same colours (Terry 1997). In thrips, colour appears to be a primarily important cue. Colour alone can navigate them to the hosts without receiving any volatiles emitted from the hosts (Terry 1997, Teulon et al. 1999, Davidson et al. 2006, Berry et al. 2006).

Colour vision is only possible if more than one photoreceptor type with different spectral sensitivities is available. Up to six photoreceptor types are described for insects, which are species dependent and help the insects to adapt to their specific living environments and feeding habitats. Different parts of eyes are often equipped with receptors of different spectral sensitivities. Spectral range, which can be recognized by an insect, positively correlates with the number of photoreceptor types in the compound eyes. The more photoreceptor types are available, the broader is

the spectral wavelength range that the insect can recognize and vice versa. Despite of this variability, most arthropod species except ants, even those living in entirely different ecological niches, possess very similar sets of UV (λ_{\max} ~350 nm), blue (λ_{\max} ~440 nm), and green (λ_{\max} ~530 nm) photoreceptors. Having only two types of photoreceptors is also common in insects (Briscoe and Chittka 2001). In all cases, the UV receptors are always present. For instance, *F. occidentalis* has only two photoreceptors, which are sensitive at UV (365 nm) and green-yellow (540 nm) wavelengths (Matteson et al. 1992) and so do the greenhouse whiteflies, *T. vaporariorum* (Mellor et al. 1997).

The UV receptors in insect eyes are highly sensitive allowing them to recognize even a very small amount of UV reflecting from object surfaces (host plant) and to discriminate target objects by brightness ratio of UV reflecting from the target vs. the ambient radiation (Möller 2002). The UV reflectance from plant surfaces attracts visiting insects. Regarding flowers, the UV reflecting patterns attract flower visiting insects such as bees, which visit the flower to sample pollen, on the other hand plants are favoured from pollination. Many flowers have specific UV reflecting patterns, making important structures especially visible for insects, e.g. from the petals, which are invisible to humans but visible to insects (Bellingham et al. 1997, Gronquist et al. 2001, Möller 2002, Johnsen et al. 2006). Such important structures for nutrition flower visiting insects can then be clearly detected from distance. Another example is the discrimination of leaves and flowers or even leaf structures. Leaves, depend on their structures (wax and hair), reflect different certain amounts of UV under the polarized light (Holmes and Keiller 2002, Grant et al. 2003, Johnsen et al. 2006).

Although UV is crucial for discrimination of the objects, UV receptors alone do not assure for the success of object discrimination. A contrast mechanism with a second photoreceptor type is necessary to guarantee host recognition. Möller (2002) reported that the ideal host recognition involved the contrast mechanism of UV and green receptors in insect eyes. UV receptors alone or the contrast mechanism between UV and blue photoreceptors did not result in reliable host recognition. Hence, with the exclusion of UV wavelength from the light spectrum, only one important receptor involves in receiving the colour; or contrast discrimination process

is not stimulated. Consequently, many authors like Raviv and Antignus (2004) and Antignus (2007) reviewed the effect of UV on the behaviour of insects and suggested that the UV component of the solar spectrum, either individual or in mixture with other visible radiation, plays an important role in insect behaviours including orientation, navigation, feeding, and interaction between sexes.

The relative reflectance of UV wavelengths (350 to 390 nm) from the ground around the plant canopy in relation to the crop itself is also important for determining whether anthophilous and polyphagous herbivorous thrips species will alight on a host (Matteson and Terry 1992, Terry 1997, Lewis 1997b). Very high UV reflectance from the ground can cause repellence of herbivorous insects during host selection (Greenough et al. 1990). On the one hand, the magnitude of such effects depends on the intensity of reflection, for instance low reflectance of UV (less than 35 per cent) does not inhibit the immigration of *F. occidentalis* to the crop stands (see Terry 1997). On the other hand, the specificity of the insects for distinct feeding sites is important. It was shown that anthophilous thrips are much more affected by UV reflectance from background than grass-feeding thrips (Terry 1997). This might be explained by the fact that different herbivorous insects have “developed” different responses to colours in relation to their preferred feeding sites. Since anthophilous thrips are attracted to colours and complex reflection pattern of light components that match to flowers, intensive UV reflection from backgrounds may much more severely affect their abilities to discriminate targets compared to grass- and foliage-feeding species with more restricted needs for colour differentiation (Matteson and Terry 1992, Antignus 2000).

2 Effects of UV deficiency on the flight and immigration of thrips *Ceratothripoides claratris* into tomato greenhouses in the tropics

2.1 Introduction

In the previous section, the important role of UV portion in the solar spectrum for insect orientation, host finding and alighting has been discussed. Reducing the transmission of UV into protected cultivation constructions such as greenhouses or tunnels is expected to alter the immigration and flight behaviour of insect pests. Several publications have reported the remarkable effect of UV absorbing materials for excluding various economically important insects from UV poor environments, including whiteflies (*B. tabaci*), thrips (*F. occidentalis*), aphids (*A. gossypii*), leaf miners (*L. trifoli*) and moths (*Laphigma sp.*) (Antignus et al. 1998, Antignus 2000, Costa et al. 2002, Kigathi 2005, Kumar and Poehling 2006). These insects showed clear preference for UV rich environments in contrast to the areas with low UV intensities. Limitation of insect infestation and reduction of their population development and spreading were not only lower the direct feeding damage but also significantly decrease insect born virus infections. Therefore, it seems worth to study such an approach of manipulation the UV for “soft” control of *C. claratris*, one of the most devastating pests in tropical tomato greenhouses.

Considering the particular situation of protected cultivation systems in the hot and temporary humid tropics, principally netted greenhouses are the construction of choice. Cooling systems for entirely closed greenhouses are expensive. Complete exclusion of thrips penetrating through the net sidewalls is difficult since thrips, *C. claratris*, is a very tiny insect; hence, very fine net size is required for preventing their penetration. Only nets with holes smaller than 78 mesh size are proposed as “thrips-safe” compared to 52 mesh nets efficiently excluding whiteflies and aphids and 40 mesh nets, which cannot be passed by even bigger insects such as butterflies and flies. However, 78 mesh nets cause enormous reduction (up to 50%) in air exchange between inner and outer greenhouse environments, accompanied by

an increase of internal temperature and humidity, that is detrimental for plant growth (problems with evaporation and water balance) and that favours fungal growth. Therefore, as a compromise for achieving a convenient internal microclimate and considerable insect exclusion ability, the 52 mesh net was recommended for cladding greenhouses in the tropics. However, a considerable number of thrips, *C. claratris*, was still found invading greenhouses covered with this mesh size net (Harmanto et al. 2006).

The implementation of UV filtration concept for 52 mesh net may provide a promising improvement of exclusion ability of this net type to thrips while maintaining a reasonable air exchange and a convenient inner microclimate in the protected cultivation systems. A thorough understanding how the manipulation of the UV range in the solar spectrum by using UV absorbing cladding materials affects the population dynamics of *C. claratris* and the microclimatic conditions inside such greenhouse constructions is a prerequisite for improving and finally implementing innovative and sustainable pest control measures.

Hence, in this study, several experiments have been carried out aiming (1) to test the principle UV preference of *C. claratris* in paired choice experiments; (2) to investigate the attraction and immigration of thrips into greenhouse constructions clad with different combinations of UV absorbing and transmitting plastic films and nets, taken into consideration for both closed and opened-door greenhouse types; (3) to study the effect of extended UV absorbing roof films (as a kind of projecting roof that creates a margin of UV deficiency around the greenhouses) on the immigration of *C. claratris*; (4) to examine the effect of different UV conditions to the spatial and temporal dispersal of *C. claratris* after being accidentally introduced into the greenhouses.

2.2 General materials and methods

2.2.1 Study location

All experiments were conducted in a greenhouse complex of the Leibniz University Hannover – AIT joint research project “Protected cultivation - An approach to

sustainable vegetable production in the humid tropics” in Asian Institute of Technology, Pathumthani, Thailand.

2.2.2 Thrips rearing

C. claratris was reared directly on tomato plants in plexi glass cages in a climate chamber at a temperature of $30 \pm 1^\circ\text{C}$, a relative humidity of $60 \pm 5\%$ and a photoperiod of 12L:12D. The cages' upper panels were replaced with thrips proof net (Sefar Petex 07-64/45, Sefar AG, Switzerland) to improve the air exchange inside cages. *C. claratris* adult females were first collected from an infested tomato field in the neighbourhood of the greenhouses. In the laboratory, thrips were allowed to lay eggs for about 4 hours on insect free fresh leaves. Thereafter, adult females were removed. The leaves with deposited eggs were incubated in Petri dishes (8.5 cm in diameter) with the bottom covered with 0.5 cm a mixture of Plaster of Paris and charcoal (ratio 9:1). This bottom layer was previously moistened with 2 mL of tap water to establish high moisture for keeping the leaves fresh. The Petri dishes were then kept in the climate chamber. The eclosion of eggs was checked from the third day after oviposition. Newly hatched first larval instars were collected using a fine painting brush No.1 and released on 3 week old potted tomato plants. These plants were then kept in cages for population development in the climate chamber. Intensive damaged plants were regularly replaced by fresh ones.

2.2.3 Plants

Tomato seeds, variety FM2260 (AVRDC, Shanhua, Taiwan), were sown individually in multiply hole plastic trays using Pindstrup compressed peat moss (Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and then kept in a nursery. Three week old seedlings were transplanted in small pots (15 cm in diameter) for insect rearing and bigger ones (30 cm in diameter) for greenhouse planting.

Thirty 3 week old tomato seedlings were transplanted in black plastic pots (30 cm in diameter) in each greenhouse (7.5 x 2 m) using a locally commercial growing substrate (Dinwondeekankasat, Ayutthaya, Thailand), containing of 28% organic compounds and a mineral fraction consisting of 30% sand, 39% silt and 31% clay. Plants were arranged in three rows. The distance between two adjacent plants was 40 cm. Plants were irrigated according to computer controlled dripping system. With the irrigation water plants were automatically fertigated with a mixed nutrient solution

containing of 2.5% concentration of a stock solution mixed from Kristallon™ (6:12:36:3% N:P:K:Mg + Micro) and Calcinit™ (15.5:19% N:Ca) (both Yara, Oslo, Norway) in a ratio of 70:30.

2.2.4 Plastic films and nets

Two types of plastic films and nets, which were similar to those studied by Kumar and Poehling (2006), were used in this study. The two plastic films used for covering greenhouse roof were an UV absorbing film (Sun Selector Diffused Antivirus®, Ginegar Plastic Product Ltd, Kibbutz, Israel) and an UV transmitting film (PE-1A, RKWAG, Worms, Germany). The two net products for covering the greenhouse sidewalls were Bionet® (UV absorbing net) and Anti Insect® (UV transmitting net) (50 mesh) (both Klayman Meteor Ltd., Petah-Tikva, Israel). Spectral transmission characteristic of these materials were measured in the laboratory using a PerkinElmer Lambda 900 UV/VIS/NIR spectrophotometer (PerkinElmer Life and Analytical Science, Boston, MA) (Figure 1).

2.2.5 Basic greenhouse setups

The greenhouses were constructed using steel framework (7.5 m in length x 2 m in width x 2.5 m in height (in the centre)). The two plastic film and net types mentioned above were combined to construct the following four basic greenhouses with plastic roofs and vertical net sidewalls (up to 2 m height) for the whole studies: (i) UV transmitting plastic film and net (Ptr - Ntr), (ii) UV absorbing plastic film and net (Pab - Nab), (iii) UV absorbing plastic film and UV transmitting net (Pab - Ntr), and (iv) UV transmitting plastic film and UV absorbing net (Ptr - Nab) (Figure 2). Specific greenhouse arrangement and orientation were further described in experiment section.

2.2.6 Measurement of climatic parameters

Radiometer UV sensors (Indium Sensor, Neuenhagen, Germany) were installed at the height of 0.5 m above the ground inside greenhouses to measure the UV intensity. The greenhouses were also equipped with Pyranometers (solar meters) (Kipp & Zonen B.V., Delft, Netherland) at the same height (0.5 m above the ground) to measure the global solar radiation. Dry and wet thermocouples were also installed

at 2 m above the ground to measure the temperature and humidity inside greenhouses. Data from all sensors were automatically transferred to and recorded in a low-power consumption data logger for every 5 minutes (Institute of Horticulture and Biosystem Engineering, Leibniz University Hannover, Germany).

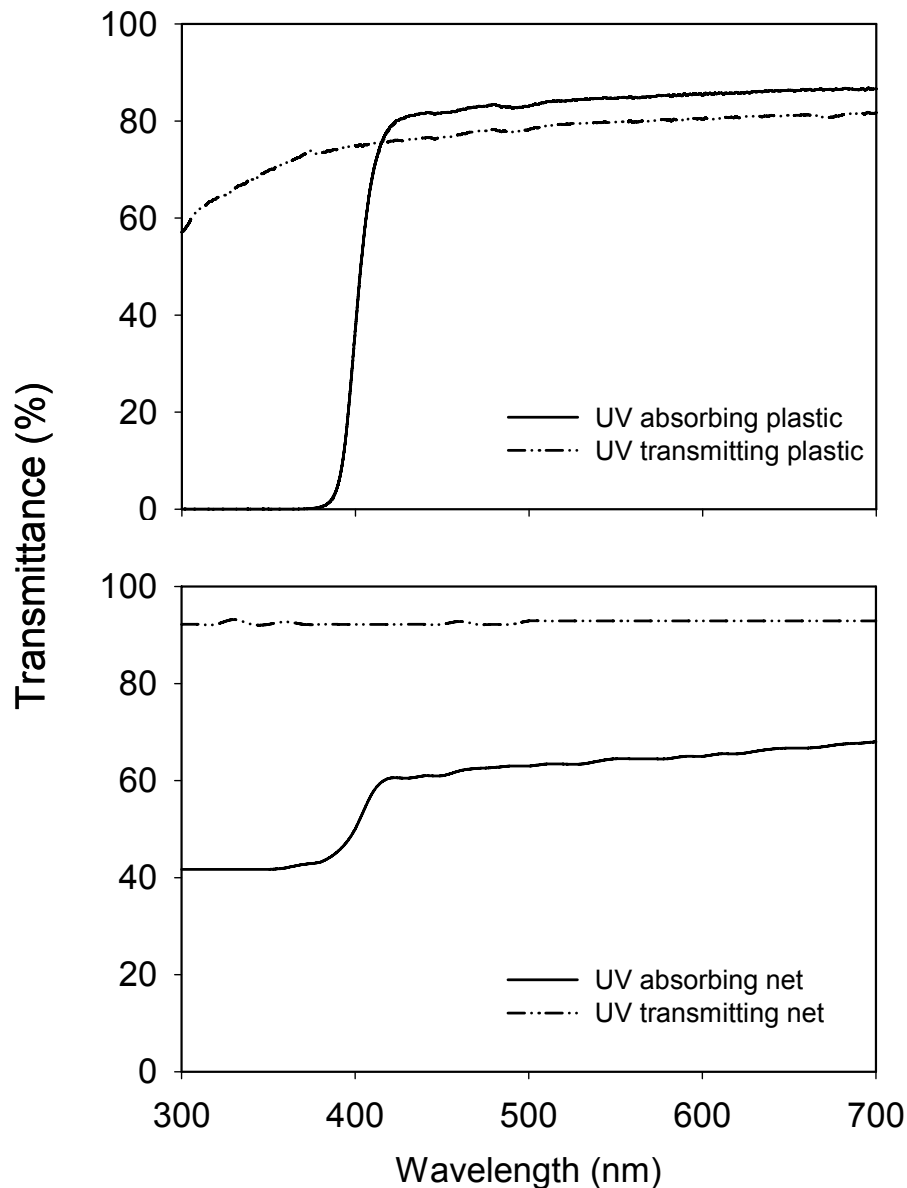


Figure 1. Spectral transmission of UV absorbing plastic (Sun Selector Diffused Anti Virus[®], Ginegar Plastic Products Ltd., Israel), UV transmitting plastic (PE-1A, RKW AG, Germany), UV absorbing (Bionet) and UV transmitting (anti-insect) nets (50 mesh, Klayman Meteor Ltd., Petah-Tikva, Israel) measured with a PerkinElmer Lambda 900 UV/VIS/NIR spectrophotometer (Kumar and Poehling 2006).

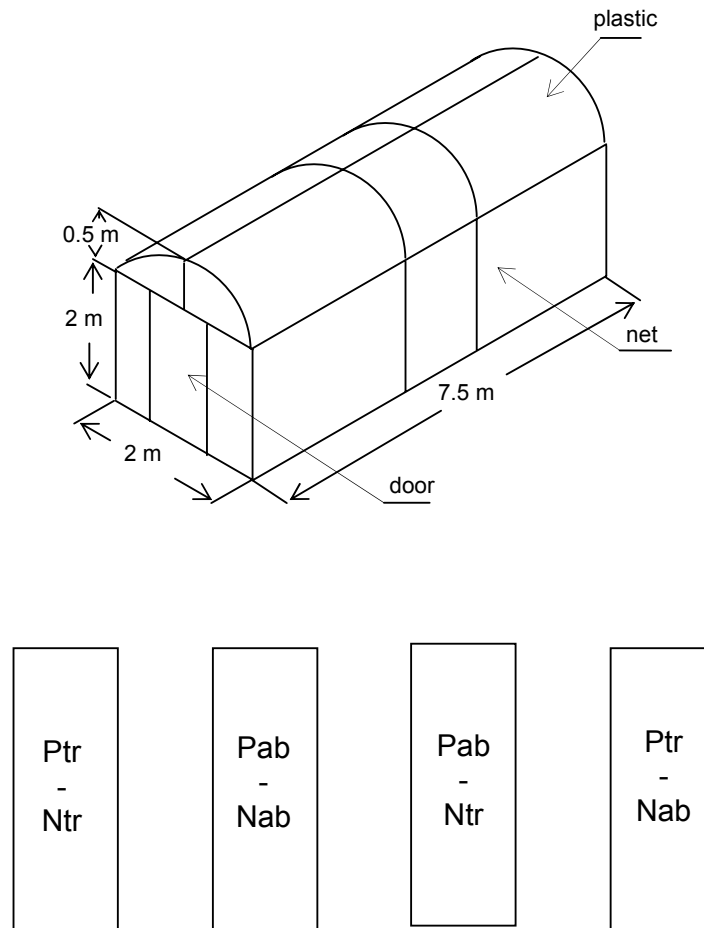


Figure 2. Greenhouse structure (upper) and four basic greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets (under). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

2.2.7 Statistical analysis

All data were tested for normal distribution using UNIVARIATE procedure in SAS. If the assumption of normal distribution was proved, TTEST option in SAS was applied to compare the means of every two samples in the paired choice comparison, and supplemental effect of projecting roof experiments. If the homogeneity of variances was achieved, the pooled test was selected; otherwise Satterthwaite test was used when the assumption was violated. When the normal distribution of the samples was not revealed, data were subjected to one-side exact WILCOXON two sample test option in SAS.

For more than two samples comparisons, after being subjected to UNIVARIATE test, data was checked for homogeneity of variance using the HOVTEST = LEVENE option of SAS version 8 and pooled only when variance homogeneity could be assumed. Subsequently, data was analyzed using the MIXED procedure option in SAS only when the normal distribution and variance homogeneity assumptions were satisfied. The mixed linear model is generalized from the standard linear model used in the GLM procedure. The mixed linear model fits a variety of mixed linear models to data, and thus provides more flexibility in data modelling than the GLM procedure does. In case of a significant difference between greenhouses, the means number of thrips on traps or tomato plants of different greenhouses were compared by the DIFF/Tukey option on the LSMEANS statement. When the normal distribution and/or variance homogeneity of the samples was violated, data were subjected to nonparametric test option in SAS. In all cases, the significant value was set at $P < 0.05$.

2.3 Light transmission and other climatic parameters inside the greenhouses clad with different combinations of UV transmitting and UV absorbing plastics and nets

UVA intensities inside greenhouses were significantly dependent on the type of plastic film and net used, of which the plastic type gave more pronounced effect (Figure 3A). The greenhouse clad with UV absorbing plastic film and net (Pab – Nab) filtered almost all UV from the global radiation. The UV absorbent effect was significantly reduced when UV absorbing plastic was combined with UV transmitting net (Pab – Ntr). The highest UV intensities were measured in the greenhouses covered with UV transmitting plastic.

On the other hand, the total global solar radiation was not affected under different plastic cover types (Figure 3B). Temperature inside the greenhouse covered with UV absorbing plastic and net (Pab - Nab) was slightly higher than that in other greenhouses (Figure 3C). Relative humidity values in different greenhouses fluctuated over the experimental periods, giving no clearly different trends (Figure 3D).

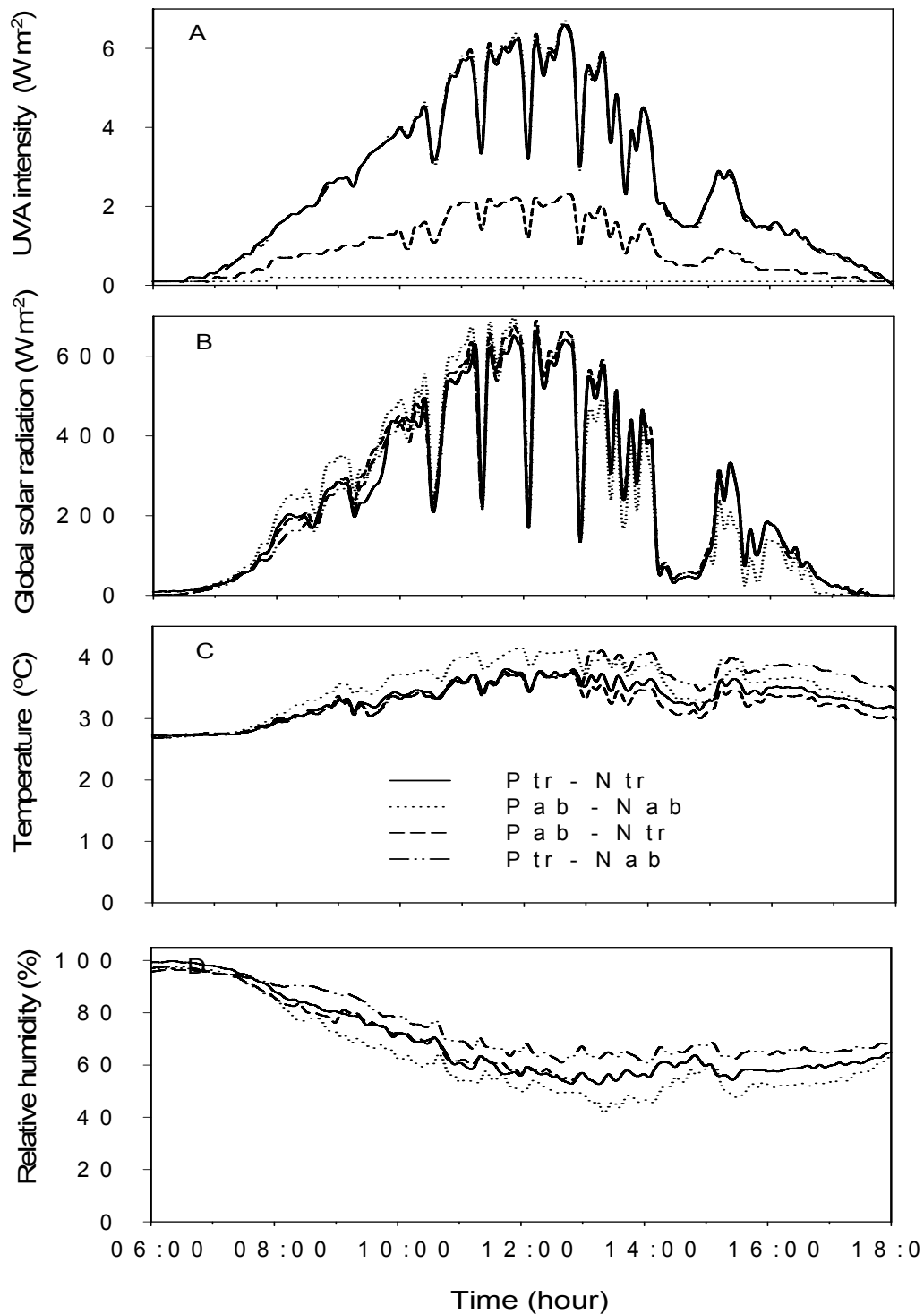


Figure 3. UVA intensity (W m^{-2}) (A), global solar radiation (W m^{-2}) (B), temperature ($^{\circ}\text{C}$) (C), and relative humidity (%) (D) inside greenhouses clad with different UV transmitting and absorbing plastics and nets (one day data plotted as example). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

2.4 Paired choice experiments with small experimental greenhouses

2.4.1 Materials and methods

The choice experiment was conducted in four paired choice comparisons to test the effect of different UV intensities on orientation behaviour of *C. claratris*. Choice chambers consisted of two greenhouse compartments (3 m in length x 2 m in width x 2.5 m in height) clad with different combinations of UV transmitting and absorbing plastics and nets, which were connected through a middle black plastic cladding compartment (1.5 m in length x 2 m in width x 2.5 m in height) (Figure 4). Another layer of UV absorbing plastic film was covered outside the black film to protect it from UV degradation. This black plastic cladding compartment, which was opened (1 x 1 m) to the two connected greenhouse compartments, formed a dark area for releasing of *C. claratris*. Released thrips were free to fly towards the greenhouse compartments of interest.

Three paired comparisons were conducted between the greenhouse compartments made of UV transmitting plastic film and net (Ptr - Ntr) with (1) UV absorbing plastic film and net (Pab - Nab); (2) UV absorbing plastic film and UV transmitting net (Pab - Ntr); and (3) UV transmitting plastic film and UV absorbing net (Ptr - Nab). The fourth set was to compare between UV absorbing plastic film and net (Pab - Nab) and UV absorbing plastic film and UV transmitting net (Pab - Ntr) (Figure 4).

Three hundred *C. claratris* adults of unknown age were collected from the rearing cages into plastic vials (2 cm in diameter x 4 cm in height) using an aspirator. The collected *C. claratris* were starved for 2 hours before releasing in experiment. The sex ratio of the thrips population was determined every experimental day by sampling of 100 thrips from stock culture resulting in a ratio of 2 to 3 females per male. Subsequently, these vials of *C. claratris* were placed on a stand (0.5 m in height) in the middle of the black compartments and opened for release of thrips. The releasing time was always around 11 am on the experimental dates.

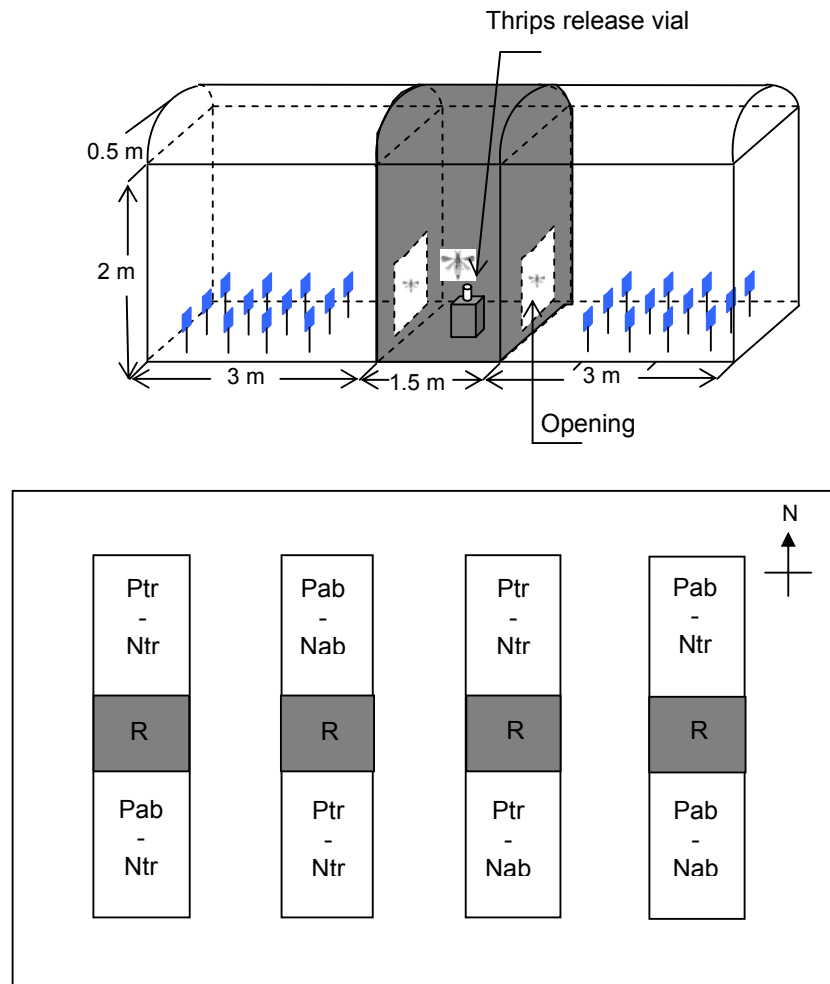


Figure 4. Experimental design to study the effect of different UV intensities on orientation behaviour of *C. claratris* in choice experiments. R: Release chamber, Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

The recapture of *C. claratris* in each greenhouse compartment was performed with (1) sticky blue traps (12 x 15 cm) positioned vertically at 0.5 m above the ground and/or (2) three week old tomato trap plants. Every three traps or plants were arranged in four rows at distances of 1, 1.5, 2 and 2.5 m from the black compartment, resulting in a total number of twelve traps or plants in each greenhouse compartment. Sticky traps were collected six hours after releasing of thrips and subsequently checked in the laboratory for thrips under binocular (Krüss. A. K., Krüss Optronic, Germany, magnification: 15 x 4). The numbers of thrips recaptured on trap plants

were counted directly on the plants in greenhouses at six hours following the release of thrips. The release and recapture trial using sticky blue traps as attractant was repeated six times, whereas that using plants was repeated four times over time.

2.4.2 Results

C. claratris showed consistently less preference for the greenhouse compartments receiving lower UV intensities irrespective of the trap types (blue traps or plants) (Figure 5). This trend was more obvious when blue traps were used as attractant. Consistently, in the experiment using blue traps for recapture, significantly fewer number of *C. claratris* dispersed into the greenhouse compartments receiving lower UV intensities of all tested combinations, i.e. Pab - Nab vs. Ptr - Ntr ($t_{6,17} = -2.80$, $P = 0.0302$); Pab - Ntr vs. Ptr - Ntr ($t_{10} = 2.72$, $P = 0.0216$); Ptr - Nab vs. Ptr - Ntr ($t_{10} = 3.47$, $P = 0.006$); and Pab - Nab vs. Pab - Ntr ($t_{5,74} = -3.38$; $P = 0.0159$) (Figure 5A). When tomato plants were used as attractants inside the greenhouse compartments, the same trend was quite obvious in treatments with Ptr - Ntr type of recapture arena (Figure 5B). Consistently, higher number of thrips was recaptured in the Ptr - Ntr compared to its paired compartments with lower UV intensities (Pab - Nab, Pab - Ntr, Ptr - Nab), however, the only statistically significant difference was determined for the combination Ptr - Ntr vs. Pab - Ntr. ($t_6 = 2.64$; $P = 0.0386$). In contrast to the trend and to the sticky trap experiments, higher number of thrips was found at the compartment Pab - Nab compared to its pair (Pab - Ntr) when tomato plants were used as trap. However, this reverse result in the comparison Pab - Nab vs. Pab - Ntr with plant recapture was not significant ($t_6 = 1.65$; $P = 0.1495$) (Figure 5B).

2.4.3 Discussion

C. claratris apparently preferred the greenhouse compartments that received higher UV intensities (Figure 5A and B). A significant reduction in the recapture of *C. claratris* both on traps and tomato plants could always be observed in greenhouse compartments that block more UV. This result supported other studies that reported the preference to UV of insects such as western flower thrips, whiteflies, and aphids. Costa and Robb (1999) reported a clear flight preference of western flower thrips *F. occidentalis* to higher UV levels in choice experiments with plastic tunnels. Later, Kigathi (2005) further corroborated these findings for *F. occidentalis* by choice

experiment in laboratory and small greenhouse flight arenas. *Caliothrips phaseoli* also evidently favoured in choice situations areas with UVA compared to the areas blocking this spectrum (Mazza et al. 2002). Preference for richer UV environments was also reported for the whiteflies, *Bemisia argentifolii* (Costa and Robb 1999), and *T. vaporarium* (Costa et al. 2002, Mutwiwa et al. 2005, Doukas and Payne 2007a) when tested in choice situations.

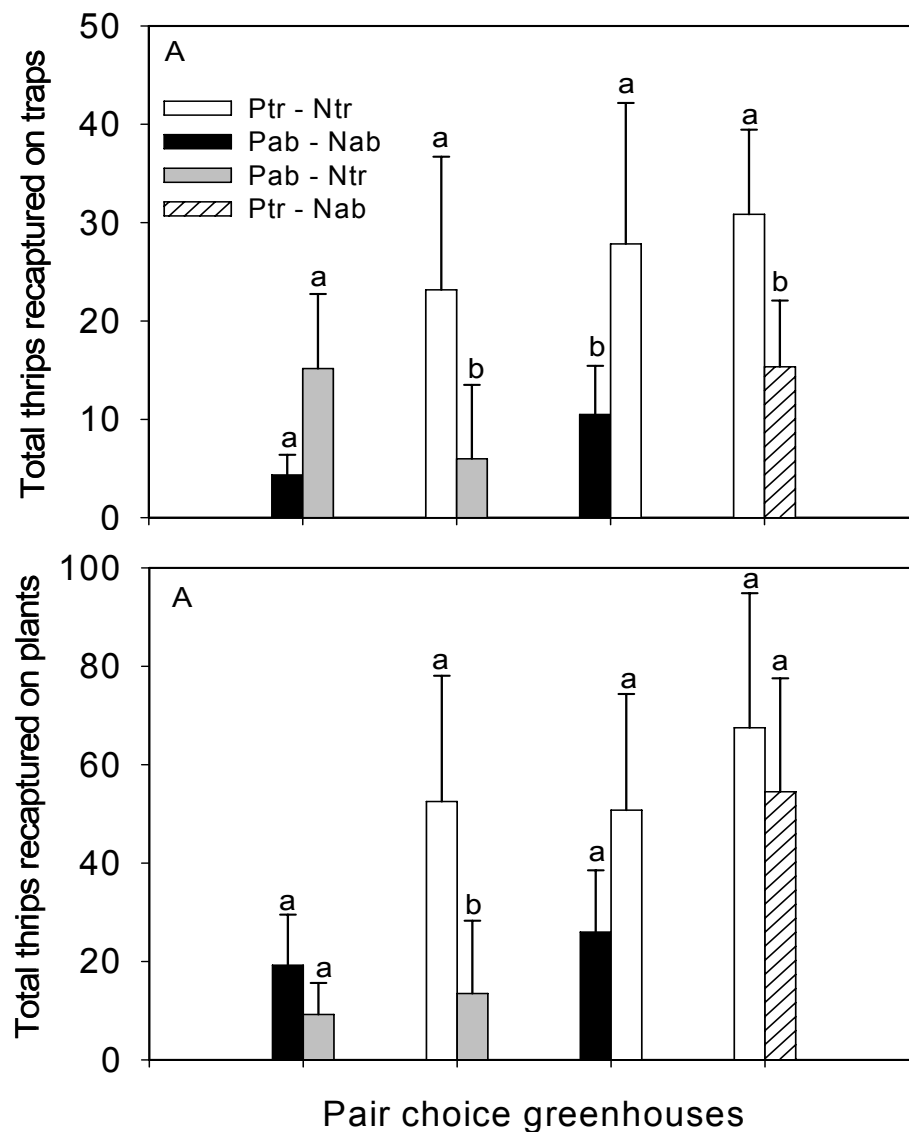


Figure 5. Recapture of *C. claratris* in paired choice experiments with blue sticky traps (A) and tomato plants (B). Mean (\pm SD) in the same group in each graph followed by the same letter are not significantly different (T-test, $P < 0.05$, $n = 6$ for experiment with trap, and $n = 4$ for experiment with plant). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

The underlying mechanism explained why thrips show such a strong bias to UV richer environments is still discussing and speculative. In the choice experiments, thrips must first be stimulated to take off from the release platform and select a specific direction for flight. It can be assumed that this first step is not completely controlled by the far distant targets (traps or plants). Therefore, it is liable that for this first orientating flight activity the stimulation of UV receptors in the compound eyes was mainly resulted from ambient light excitement. Mellor et al. (1997) provided evidence that UV receptors are especially numerous in the dorsal eye region of the whiteflies, *T. vaporariorum*, and other herbivorous insects, which also suggests an important role of incoming UV for the orientation mechanism of insects. Assuming a similar visual system of *C. claratris* like *F. occidentalis* with two photoreceptors, which are sensitive at a UV wavelength (365 nm) and a green yellow wavelength (540 nm) (Matteson et al. 1992), it seems that the UV receptors were not excited by ambient light conditions lacking the UV part, and thus did not trigger the dispersal flight of thrips to the UV excluding environments. Unfortunately, there are no detailed studies to further elucidate this mechanism. Concerning the attractiveness of the targets, it is generally accepted that UV reflection from plant surfaces plays an important role in making plants visible to herbivorous insects and thus direct their flight (Möller 2002). This can enhance the attraction of insects to traps and plants in the UV rich areas.

However, still a certain proportion of *C. claratris* dispersed into the UV deficient constructions, even into the Pab – Nab, of the flight chambers in both experiments. Other choice studies also always found a small proportion of insects, e.g. whiteflies, *T. vaporarium*, dispersing into the areas with no UV (Costa et al. 2002, Mutwiwa et al. 2005). Apart from a certain probability for random movements under such artificial conditions (Doukas and Payne 2007a), it can be concluded that thrips ability to locate blue traps and plants is unlikely a sole function of the UV in the ambient light or reflectance from objects. Flight triggered by other biotic (e.g. olfactory stimuli) and abiotic (e.g. air movement) factors should be considered as well.

Apart from being excited by visual stimuli and abiotic factors especially, olfactory stimuli (volatile signal compounds) may be involved in navigation control of dispersing insects. Plant odour may play a role as attractant to insects. However, odour stimuli were found to have minor effects on the flight direction of thrips than

colour stimuli as observed in western flower thrips *F. occidentalis* (Teulon et al. 1999, Davidson et al. 2006) and New Zealand flower thrips *Thrips obscuratus* (Berry et al. 2006).

The only discrepancy in the otherwise regular trend in dispersal flight of thrips was recorded for the choice comparison between the strongly UV deficient Pab – Nab construction and the slightly UV richer Pab – Ntr treatment using plants as attractants. Fewer thrips was found in the latter construction (Pab – Ntr), although this difference was not significant. It is unlikely that plants under the weak UV condition were less attractive to *C. claratris* than those under the very poor UV condition. A possible explanation is that apart from immigration processes from the start compartment into the adjacent flight chambers, a possible loss of thrips across the nets to outside should also be considered. Especially during the first flight activity, some random landing on the sidewall nets are likely, and thrips migration through the non-‘barrier’ UV transmitting net should be comparatively high. The relatively low recapture of *C. claratris* in the similar greenhouse compartment (Pab – Ntr) compared to the Pab – Nab when the paired choice chambers were set up between these compartments and the Ptr – Ntr may corroborate this hypothesis.

Higher rates of recapture using plants compared to blue traps can be attributed to a number of factors. First, the different light reflection from plants and traps may cause different attraction effects to released *C. claratris*. The peak reflectance for green leaves (at c.a. 550 nm) (Grant et al. 2003, Liu et al. 2006, Johnsen et al. 2006) seems to be closer to the peak sensitivity of the first receptor in thrips eyes compared to the wavelength reflected from the blue card (c.a. 440 nm, our measurement). Second, the motion of objects, e.g. movement of plants in wind, is also an important factor for visual detection (Giurfa and Menzel 1997, Kimmerle and Egelhaaf 2000). And third, plant odour may also play an important role in final selection and acceptance of the target.

In conclusion, the results of this study suggest that *C. claratris* also expresses a distinct preference to high UV intensities for flight behaviour, and avoids areas where UV was excluded. This favours further researches to understand the potential control effect of UV absorbing materials to immigration and flight behaviour of the thrips,

C. claratris, in the netted greenhouses used in protected cultivation systems in the tropics.

2.5 Natural immigration of *Ceratothripoides claratris* to greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets

2.5.1 Materials and methods

Natural immigration of C. claratris into different clad closed-door greenhouses

The four basic greenhouses constructed from different combinations of UV transmitting and absorbing plastics and nets (for detail see sections 2.2.4 and 2.2.5) were used in this study. These greenhouses were orientated East-West along their length and arranged 4 m apart from each other to avoid shading effects from adjacent greenhouses (Figure 6). Each greenhouse contained thirty potted plants (for detail see 2.2.3). Greenhouse doors were closed during the entire experimental period.

Transparent plexiglass traps (12 x 15 cm) glued one side with clear glue (Insect glue, My success, Bangkok, Thailand) were arranged at 2 cm distance from both outer and inner surfaces of the net sidewalls to catch thrips migrating towards the outer net surface and those passing inside the greenhouses (Figure 7). Traps were arranged at three different heights at 0.5, 1 and 1.5 m above the ground in a distance of 1 m between adjacent traps, compiling 18 traps at one height and a total of 54 traps at the outer net surface of each greenhouse. Similarly, 48 traps were installed at the same height and distance on the inner net walls with the sticky surfaces facing outward to the net to catch immigrating thrips crossing through the net sidewalls. The first traps were mounted one week after tomatoes were planted in the greenhouses. They were collected and replaced with new traps weekly for 6 weeks. Numbers of *C. claratris* trapped were counted on every trap individually under the binocular (Krüss. A. K Krüss Optronic, Germany; magnification: 15 x 3) in the laboratory.

Population dynamics of *C. claratris* on tomato plants inside greenhouses were investigated weekly for 7 weeks after planting of tomatoes. The numbers of adults, first and second larval instars of *C. claratris* were directly counted on ten randomly selected plants in each greenhouse. The counting was repeated for the same selected plants over the whole investigation period. In the last week of the investigation, week 7 after planting, young leaves of sampled plants were collected and analysed with DAS-ELISA test in the laboratory to detect virus infections.

The experiment was repeated three times over time throughout the year 2006, i.e. from February to April, from May to July, and from September to November. Greenhouses were sanitised and sprayed with Spinosad™ (1.5 ml L⁻¹) after each experimental replication. Greenhouses' nets and plastic covers were washed with high pressure water to remove dust prior to the next replication of the experiment.

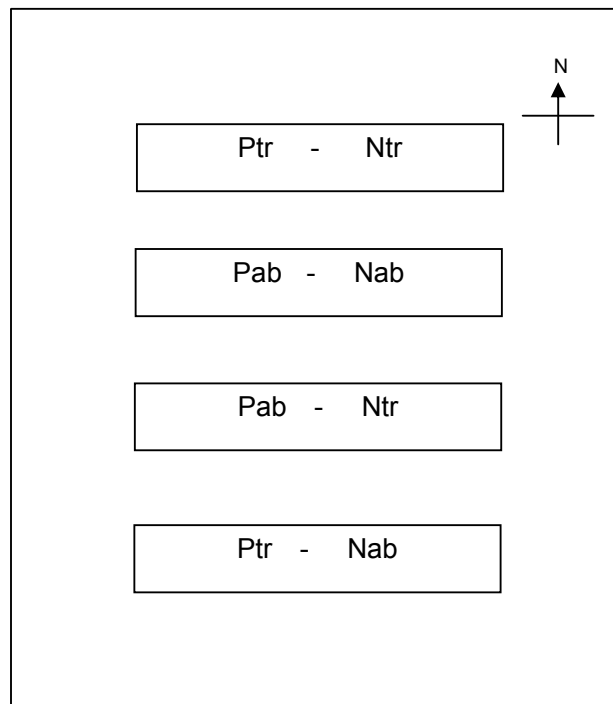


Figure 6. Experimental setups used to study natural immigration of *C. claratris* to closed-door greenhouses clad with different UV transmitting and absorbing plastics and nets. Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.



Figure 7. Greenhouse structure and experimental set up viewed from outside (left) and inside (right).

Natural immigration of C. claratris to different clad opened-door greenhouses

The same experiment was set up as described above, except that the two front doors (1 m in width x 2 m in height) of each greenhouse were opened daily from 8 am to 4 pm throughout the experimental period (Figure 8). Greenhouses were oriented North – South. Within the framework of this experiment, only the population dynamics of *C. claratris* on tomatoes and the plant virus incidence were investigated for 5 weeks after planting. Experiment was repeated twice over time, i.e. from September to October and from November to December, 2006.

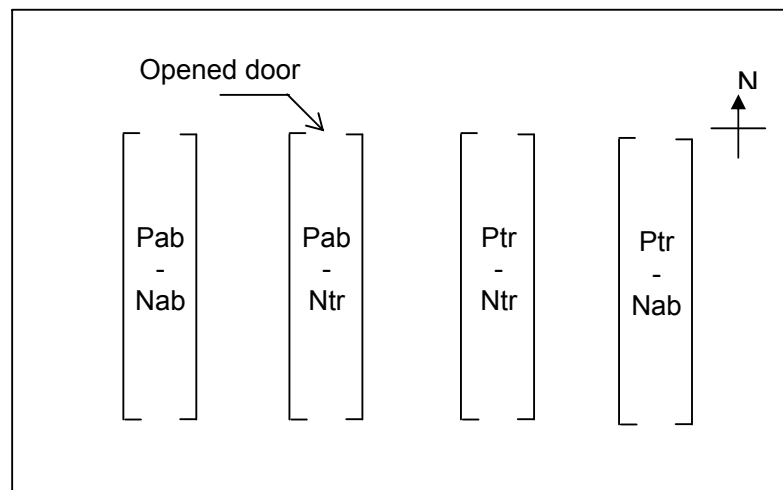


Figure 8. Experimental setups used to study natural immigration of *C. claratris* to the opened-door greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets. Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Virus detection by double-antibody enzyme-linked immunosorbent assay (DAS-ELISA)

Infection of tomato plants with CaCV-AIT tospovirus was verified using double-antibody sandwich enzyme-linked immunosorbent assay (DAS - ELISA) according to the procedure described by Premachandra et al. (2005a). A mixture of poly- and monoclonal antibodies (AGDIA[®] Inc., Elkhart, IN, USA. Cat. No. SRA 61500) originally developed to detect *Watermelon silver mottle virus* (WSMoV) and *Groundnut bud necrosis virus* (GBNV) was used for the detection of CaCV following the supplier's protocols.

2.5.2 Results

Attractiveness of greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets to C. claratris

Replication 1 (from February to April, 2006). The captures of thrips on the outer net sidewalls were significantly different at greenhouses clad with different plastic and net types across the experimental period ($F_3 = 9.27$, $P < 0.0001$) (Figure 9A). Until 4 weeks after planting, significantly fewer thrips were caught at the outer net walls of the two greenhouses covered with UV absorbing plastic (Pab) compared to those covered with UV transmitting plastic (Ptr) regardless the net types, although not many *C. claratris* were trapped at all greenhouses during this time period. Subsequently, the capture of *C. claratris* gradually increased towards the end of the experiment at all greenhouse types (GH * time: $F_{15} = 8.67$, $P < 0.0001$). While the capture of *C. claratris* at the greenhouse type Pab – Nab remained at a least level, there was a shift in thrips capture at the greenhouse type Pab – Ntr. Highest thrips numbers were captured at this greenhouse type compared to others on the last investigation time, week 7 after planting.

The penetration of *C. claratris* into greenhouses was strongly affected by both greenhouse covering plastic and net types (Figure 9B). The combination of Pab – Nab proved to be the best protective greenhouse structure against thrips immigration. Until six weeks after planting only a very few *C. claratris* was caught on the traps inside this greenhouse. This number was far lower than those captured inside other

greenhouses, although the captures on the outer net walls were more or less similar between different treatments (Figure 9A). On the other hand, the penetration intensity of thrips into the greenhouse type Pab – Ntr was not impaired. The penetration rate of thrips *C. claratris* into this greenhouse can be compared to the greenhouse type Ptr – Nab. The highest number of *C. claratris* was found on traps at the inner net walls of the greenhouse type Ptr – Ntr across the experimental period ($F_3 = 137.42$, $P < 0.0001$). Data from the last monitoring date in this greenhouse was not plotted in the graph because the excessive capture inside compared to that outside was supposed resulting from the dispersal of thrips from inside owing to the high density on plants inside. The average penetration efficiency (%) of thrips, calculated as the ratio (percentage) of thrips caught on traps inside compared to outside traps, was 20.7, 0.9, 18.2 and 14.2 at the greenhouse type Ptr – Ntr, Pab – Nab, Pab – Ntr, and Ptr – Nab, respectively. Accordingly to the significant increase in the capture of *C. claratris* at the outer net walls across the experimental period the number of thrips caught inside also significantly increased over time (GH * time: $F_{15} = 100.42$, $P < 0.0001$).

Replication 2 (from May to July, 2006). Similar to the first experiment, significantly fewer *C. claratris* were found on traps at the outer net walls of the greenhouses covered with UV absorbing plastic (Pab) compared to the captures at the greenhouse type Ptr – Ntr (GH: $F_3 = 88.19$, $P < 0.0001$) (Figure 10A). In this experiment, the UV absorbing net also expressed its superior effect against thrips shown by the low number of thrips captured at the greenhouse type Ptr – Nab. Although the capture of thrips at all greenhouses decreased after week 4 post-planting and did not recover until the last investigation in week 7 after planting (GH * time: $F_{15} = 22.96$, $P < 0.0001$), the traps exposed on greenhouse type Ptr – Ntr always contained the highest numbers of thrips compared to other treatments during the first 6 weeks period.

Accordingly, a very low number of *C. claratris* was found on the traps inside the three greenhouses covered with either UV absorbing plastic and/or net (Figure 10B). Significantly lower numbers of thrips penetrating into these greenhouses compared to that immigrating into the greenhouse type Ptr – Ntr (GH: $F_3 = 47.69$, $P < 0.0001$). From week 5 after planting, no thrips was trapped inside the greenhouses covered

with UV absorbing plastic. In addition, a poor capture of thrips inside the greenhouse type Ptr – Nab was recorded. This situation did not change until week 7 after planting. The average penetration efficiency of thrips was again highest in the greenhouse Ptr – Ntr (17.4%), followed by the greenhouse Ptr – Nab (7.1%). Penetration rate did not exceed 2% in the greenhouses clad with UV absorbing plastic (Pab– Nab, Pab – Ntr).

Replication 3 (from September to November, 2006). Replication 3 corroborated results from other two previous replications. First, statistically significant differences were recorded in the capture of thrips at all greenhouses (GH: $F_3 = 19.21$, $P < 0.0001$ for traps outside; GH: $F_3 = 27.46$, $P < 0.0001$ for traps inside) and over time (GH * time: $F_{15} = 16.14$, $P < 0.0001$ for traps outside; GH * time: $F_{15} = 12.87$, $P < 0.0001$ for traps inside). Second, the UV absorbing plastic showed again the highest potential in reducing the exposure of the greenhouses and plants to thrips immigration. Although lower total numbers of thrips were captured both at the outer and inner net walls in this replication, the data trend matched that from the first replication (Figure 11A and B). Until week 4 after planting, significantly fewer thrips were trapped at the outer net walls of the greenhouses covered with UV absorbing plastic. A dramatic increase in the captures on the greenhouse Pab – Ntr was recorded in the last 2 weeks of the investigation, week 6 and 7 after planting. No *C. claratris* was found penetrating inside the greenhouse Pab – Nab although a few individuals were trapped at the outer net walls. The highest penetration rate was recorded at the greenhouse Ptr – Ntr (111.6%), followed by the Pab – Ntr (59.1%) and Ptr – Nab (39%).

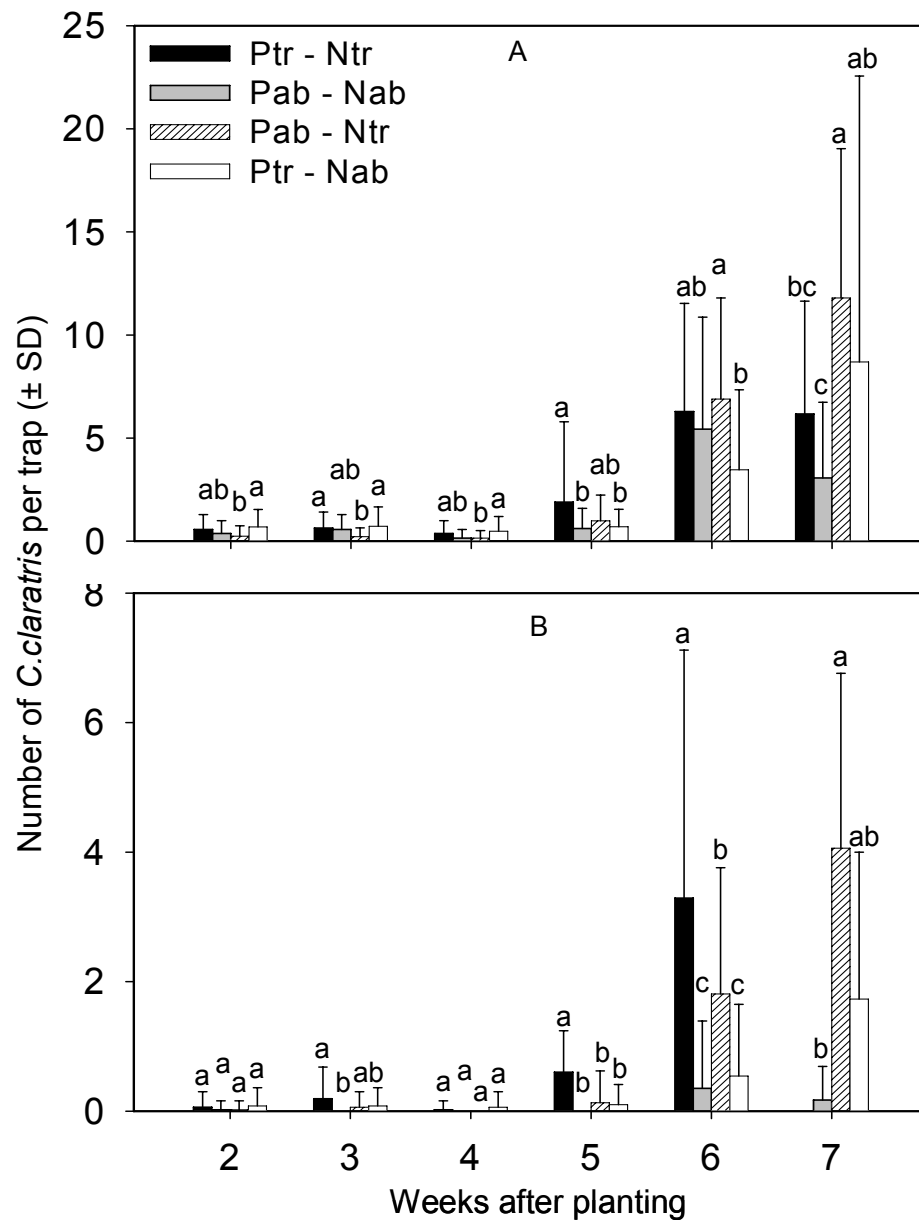


Figure 9. Trapping of thrips *C. claratris* on traps outside (A) and inside (B) greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 1 (from February to April, 2006). Mean numbers of *C. claratris* per trap (\pm SD) in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

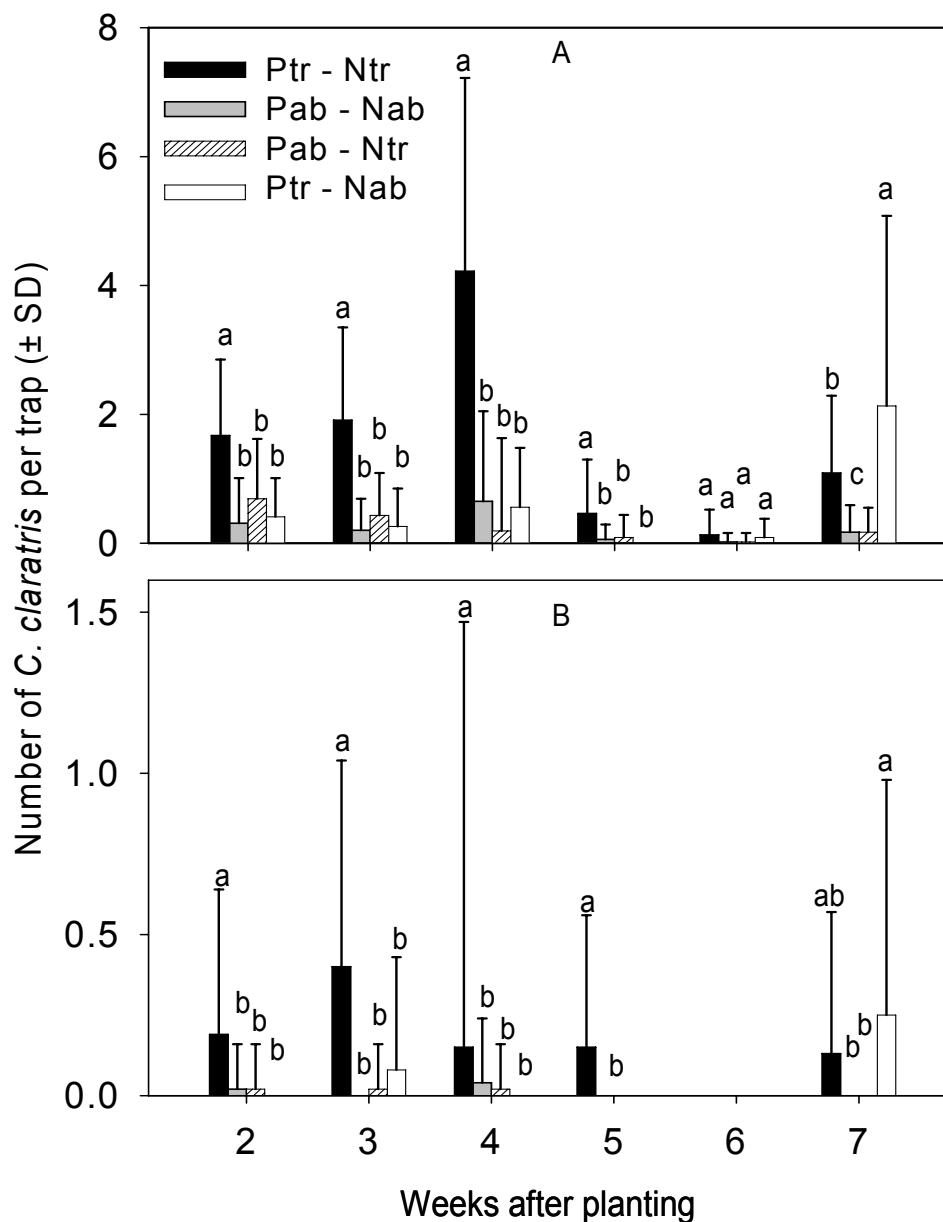


Figure 10. Trapping of thrips *C. claratris* on traps outside (A) and inside (B) greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 2 (from May to July, 2006). Mean numbers of *C. claratris* per trap (\pm SD) in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

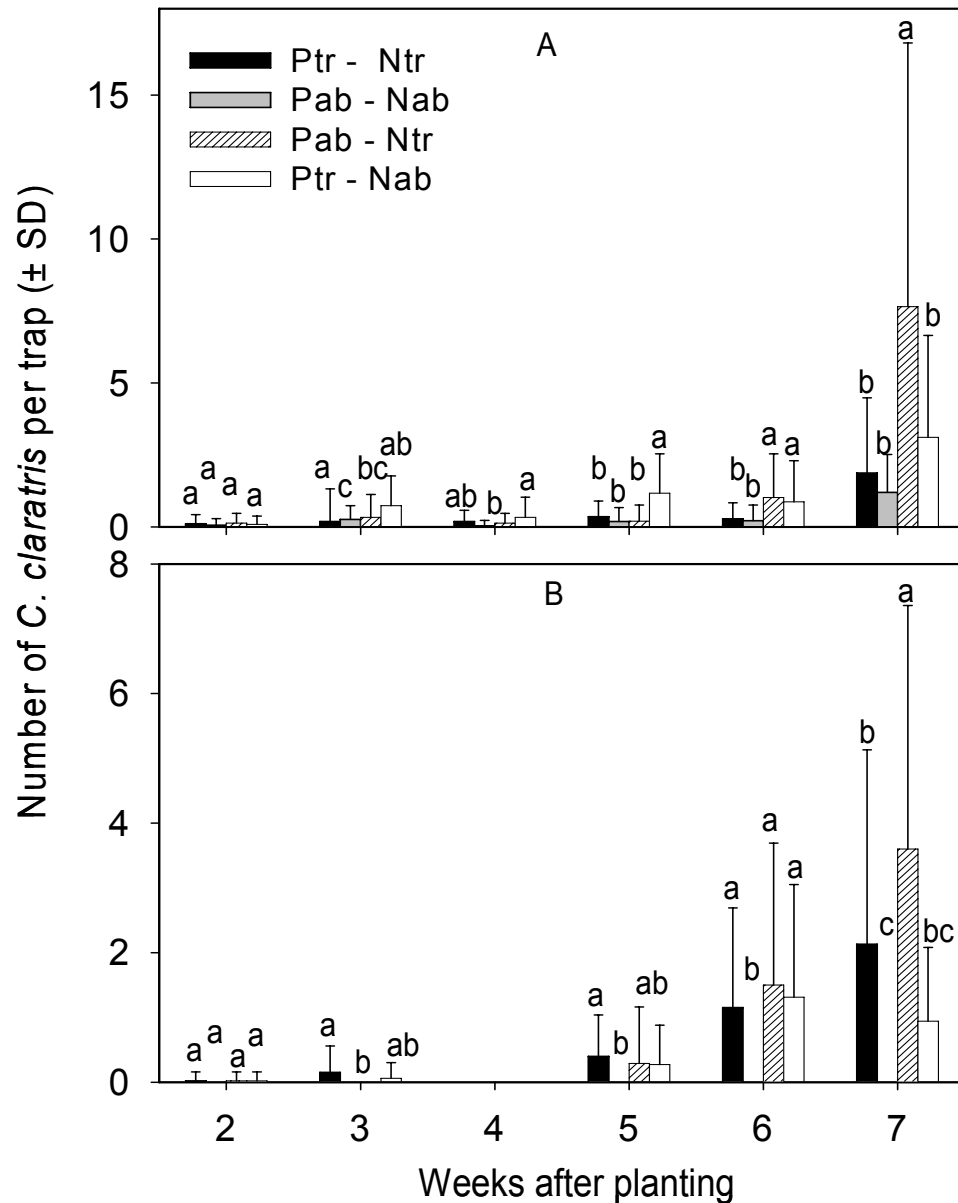


Figure 11. Trapping of *C. claratris* on traps outside (A) and inside (B) greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 3 (from September to November, 2006). Mean numbers of *C. claratris* per trap (\pm SD) in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Immigration and population development of C. claratris on tomato plants inside closed-door greenhouses

Replication 1 (from February to April, 2006). The greenhouse type Pab – Nab proved to be the best protective structure against *C. claratris*. In accordance to the low number of thrips trapped both outside and inside net walls, none of the tomato plants was found to be infested in this greenhouse type (Figure 12). On the contrary, plants in the greenhouse Ptr – Ntr were severely infested and damaged. The greenhouses covered with either the UV absorbing plastic (Pab – Ntr) or net (Ptr – Nab) also did not provide efficient protection. The earliest infestation was recorded in the greenhouses covered with UV transmitting plastic. At week 2 after planting 40% of the plants investigated were already infested in the greenhouse Ptr – Ntr. The infestation quickly spread over this greenhouse within 2 weeks. The plants in the greenhouse Pab – Ntr were infested a week later compared to plants under UV transmitting covers, however, the infestation level quickly reached the same damage level as in the greenhouse Ptr – Nab. Six weeks after planting, 100% of plants controlled were infested in both greenhouses (Figure 12A).

Accordingly, the highest thrips population was recorded in the greenhouse type Ptr – Ntr over the whole experimental period (GH: $F_3 = 34.09$, $P < 0.0001$ for adult; $F_3 = 43.71$, $P < 0.0001$ for L1; $F_3 = 41.63$, $P < 0.0001$ for L2) (Figure 12B, C, and D). The thrips population in this greenhouse steadily built up until week 4 after planting, and reached a density of about 20 adults, 50 L1, and 100 L2 per plant. Subsequently, these numbers dramatically increased and caused total plants dry death 3 weeks later, i.e. week 7 after planting. The infestations in the greenhouses types Pab – Ntr and Ptr – Nab were less severe. No significant difference was recorded in thrips populations in these two greenhouses. Thrips populations dramatically increased over time in all infested greenhouses (GH * time: $F_{15} = 32.25$, $P < 0.0001$ for adult; $F_{15} = 21.31$, $P < 0.0001$ for L1; $F_{15} = 38.95$, $P < 0.0001$ for L2).

Replication 2 (from May to July, 2006). Likewise, the greenhouse type Pab – Nab was totally protected from thrips immigration (Figure 13). Interestingly, none of the plants in the greenhouse type Pab – Ntr was infested, too. The infestation in the other two greenhouses Ptr – Ntr and Ptr – Nab was also less severe than in the

previous replication. Not before the last week of the observation period, week 7 after planting, 100% of the plants were infested with *C. claratris*. The damage was equivalent in these two greenhouse types (Figure 13A).

The thrips populations inside greenhouses with UV transmitting plastic cover increased exponentially over time (GH * time: $F_{15} = 7.57$, $P < 0.0001$ for adult; $F_{15} = 5.34$, $P < 0.0001$ for L1; and $F_{15} = 9.49$, $P < 0.0001$ for L2) (Figure 13B, C, and D). Significant differences were determined among the thrips populations in the greenhouses covered with different plastic types, but not between the two with the same plastic roof material.

Replication 3 (from September to November, 2006). Consistent to the other two replications, the plants inside the greenhouse Pab – Nab were again free of thrips and any sign of thrips damage (Figure 14). Heavy infestations were observed in the greenhouses made from UV transmitting plastic. These greenhouse types were entirely infested two weeks after planting. Infestation in the Pab – Ntr remained at 40% of plants until week 5 after planting before dramatically increasing a week later (Figure 14A).

Although the population dynamics of *C. claratris* in all greenhouses fluctuated over the experimental period, a general trend of a significant lower thrips population in greenhouse Pab – Ntr could be observed compared to those in the greenhouses clad with UV transmitting plastic (GH: $F_3 = 21.83$, $P < 0.0001$ for adult, $F_3 = 21.70$, $P < 0.0001$ for L1, and $F_3 = 21.38$, $P < 0.0001$ for L2) (Figure 14B, C, and D). No significant difference was determined among the thrips population in the two greenhouses covered with the same UV transmitting plastic.

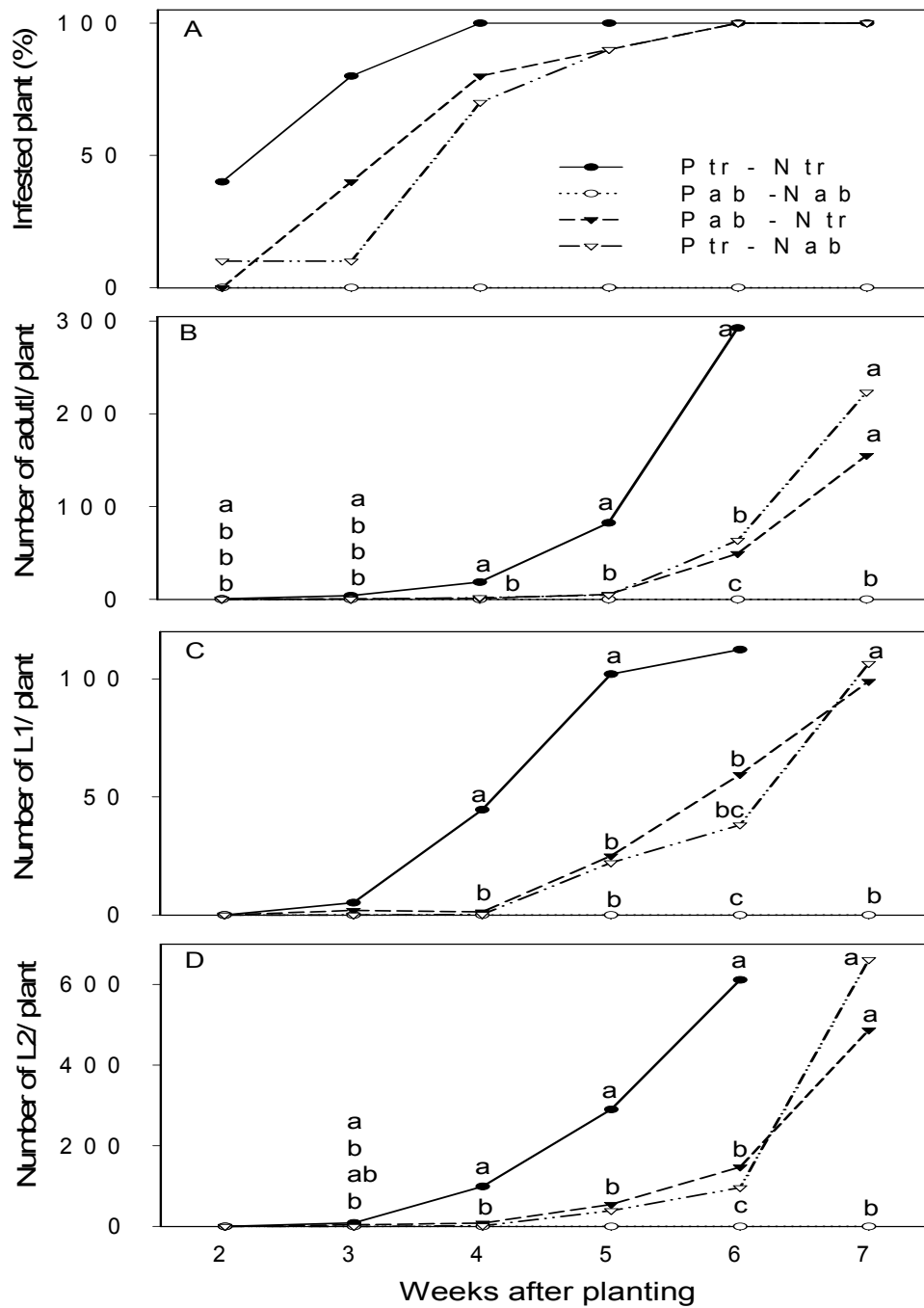


Figure 12. Plant infestation (A) and population development of *C. claratris* adults (B) first larvae (C) and second larvae (D) on tomato plants inside closed-door greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 1 (from February to April, 2006). Mean individual numbers of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

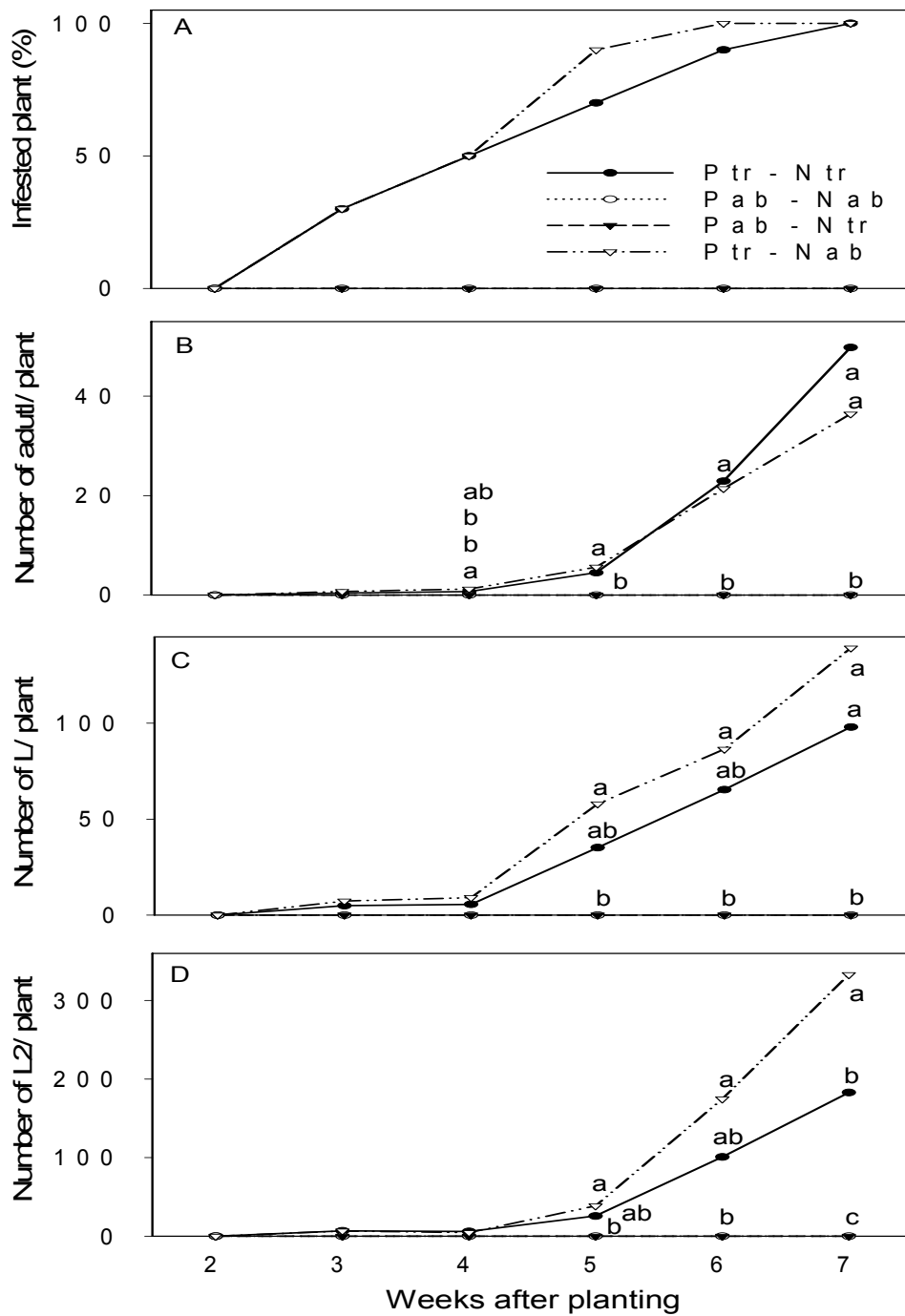


Figure 13. Plant infestation (A) and population development of *C. claratris* adults (B), first larvae (C) and second larvae (D) on tomato plants inside closed-door greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 2 (from May to July, 2006). Mean individual numbers of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

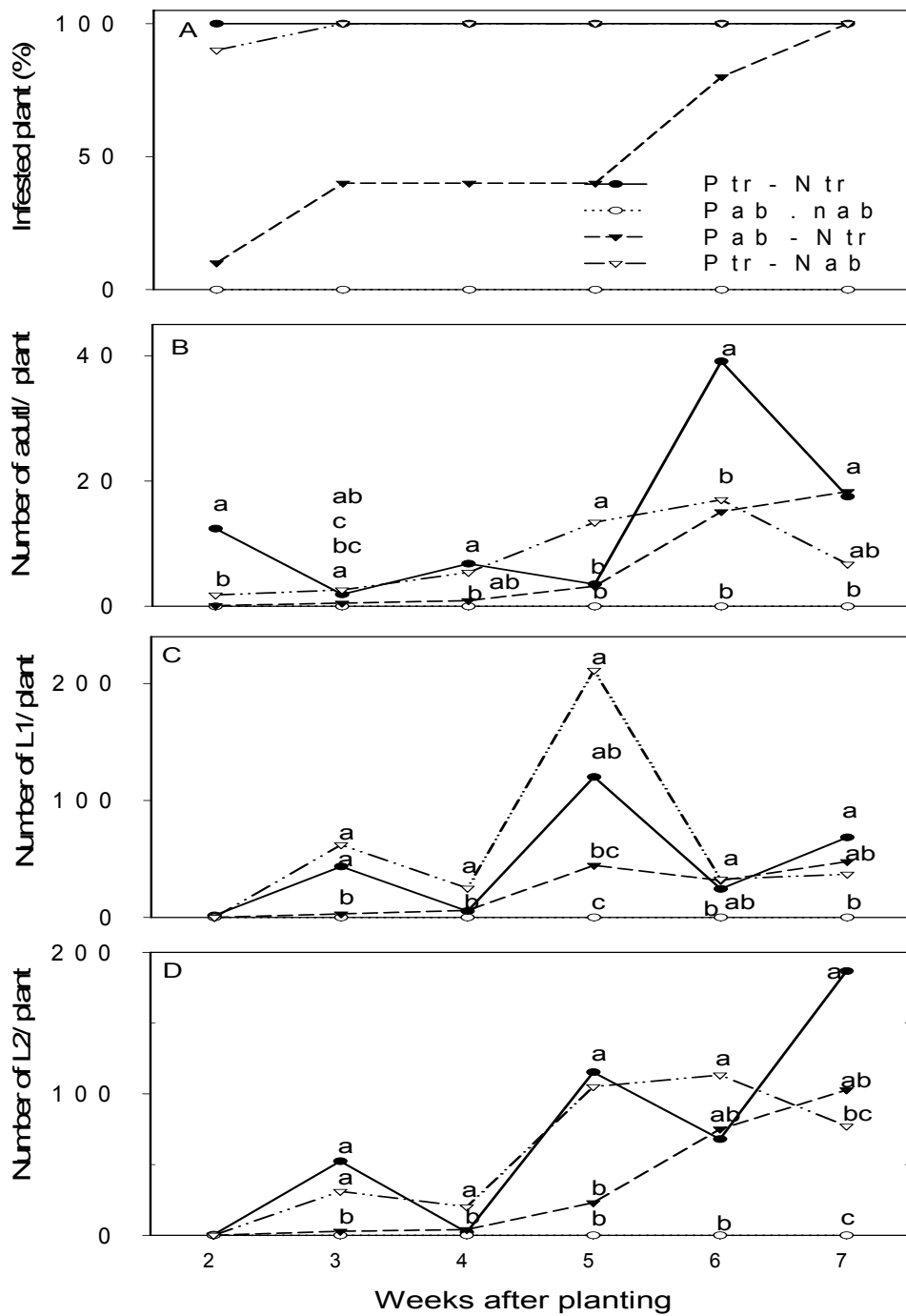


Figure 14. Plant infestation (A) and population development of *C. claratris* adults (B), first larvae (C) and second larvae (D) on tomato plants inside closed-door greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 3 (from September to November, 2006). Mean individual numbers of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Virus infection. Virus infection pattern clearly resembles thrips infestation. Plants in the greenhouse type Ptr – Ntr were most severely infected with CaCV, whereas plants in the Pab - Nab never showed any virus infection. The second heavy infection could be observed in the greenhouse Ptr – Nab (Table 1). The Pab - Ntr treatment was only heavily infected in the first replication. Concerning the time periods, the most severe infection was recorded in the first replication, from February to April, when 100% of thrips infested plants in all greenhouses were virus infected. The lowest infestation levels of plants were measured in the second replication, from May to July with a maximum of 20% virus incidence. In the third replication, from September to November, a virus incidence of 40% was detected in the greenhouse Ptr – Nab and 80% in that house completely covered with UV transmitting materials (Ptr – Ntr). No virus infected plants were found in the greenhouse Pab – Ntr.

Table 1. Virus incidence of tomato plants in different closed-door greenhouses clad with different UV transmitting and absorbing plastics and nets throughout the year 2006 at week 7 after planting.

Greenhouse	Virus incidence (%)		
	Replication 1 (February – April)	Replication 2 (May – July)	Replication 3 (September – November)
Ptr - Ntr	100	20	80
Pab - Nab	0	0	0
Pab - Nab	100	0	0
Ptr - Nab	100	20	40

Note: Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Immigration and population development of C. claratris on tomato plants inside opened-door greenhouses

Replication 1 (from September to October, 2006). With doors opened, the remarkable anti-thrips effect of the greenhouse type Pab – Nab was slightly impaired but still pronounced (Figure 15). Two weeks after planting, 20% of the investigated plants were infested with thrips (Figure 15A). However, at the same time thrips had

already spread completely over the greenhouses covered with UV transmitting plastic (Ptr – Ntr and Ptr – Nab). In the Pab – Nab treatment, thrips spread slowly and reached a 50% infestation rate not before week 5 after planting. A distinct delay in thrips infestation was also observed in the greenhouse type Pab – Ntr compared to the greenhouses covered with UV transmitting plastic.

As a consequence, thrips densities in greenhouses covered with UV absorbing plastic were significantly lower than in those covered with UV transmitting plastic (GH: $F_3 = 53.30$, $P < 0.0001$ for adult; $F_3 = 25.73$, $P < 0.0001$ for L1; and $F_3 = 38.39$, $P < 0.0001$ L2) (Figure 15B, C, and D). Although smaller thrips densities were obviously recorded in the greenhouse type Pab – Nab compared to that with UV transmitting net (Pab – Ntr), no significant difference was found. Thrips populations in both greenhouses covered with UV transmitting plastic increased significantly during the investigation period (GH * time: $F_9 = 11.34$, $P < 0.0001$ for adult; $F_9 = 5.52$, $P < 0.0001$ for L1, and $F_9 = 5.74$, $P < 0.0001$ for L2). However, a significantly lower thrips density was recorded in the greenhouse with UV absorbing net (Ptr – Nab).

Replication 2 (from November to December, 2006). Very similar thrips population and plant infestation trends could be observed among the greenhouses like in replication 1 (Figure 16). In this replication, a complete protection of tomato plants from thrips infestation in the greenhouse type Pab – Nab could be achieved until 4 weeks after planting.

Virus infection. Although a certain proportion of investigated plants was infested by *C. claratris*, almost no virus incidence was detected in the greenhouses covered with UV absorbing plastics up to 5 weeks after planting, except that about 10% of the plants were infected in the greenhouse Pab - Ntr (Table 2). On the other hand virus infections in greenhouses with UV-transmitting roofs were rather high, ranging from 90% to 100% in greenhouse Ptr - Ntr and 70% - 50% in Ptr - Nab in the first and second replication, respectively.

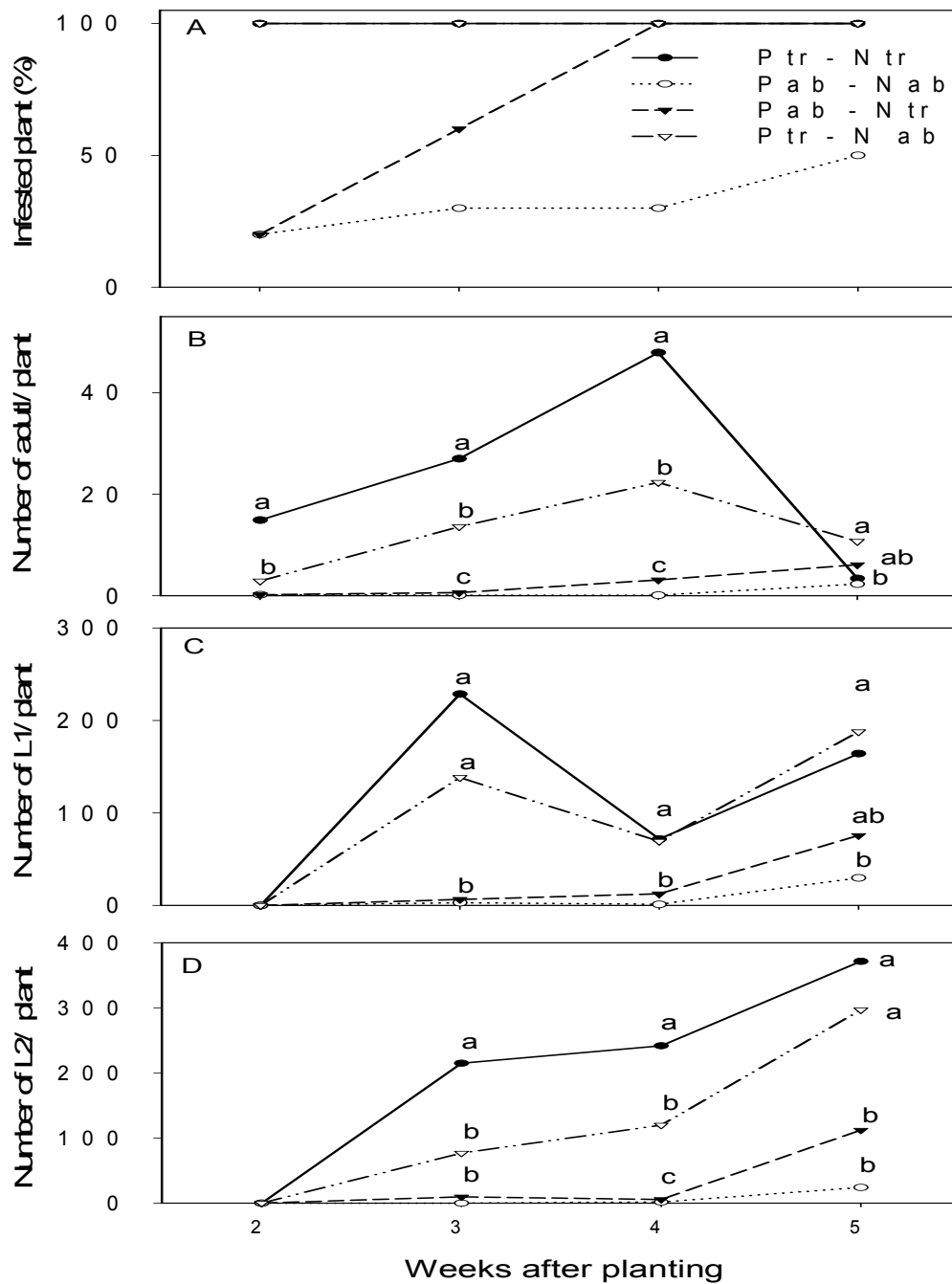


Figure 15. Plant infestation (A) and population development of *C. claratris* adults (B), first (C) and second larval instars (D) on tomato plants inside opened-door greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 1 (from September to October, 2006). Mean individual number of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

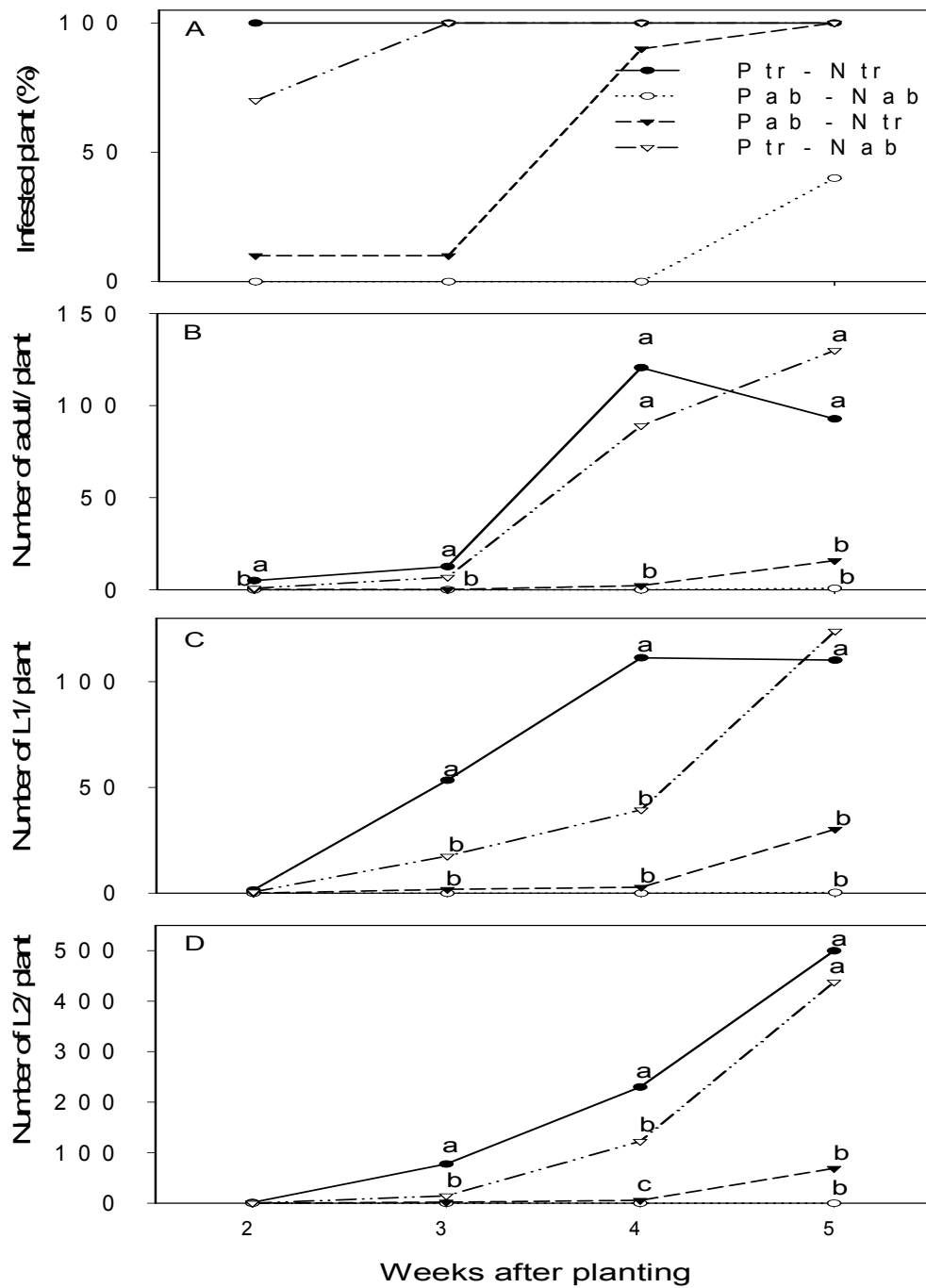


Figure 16. Plant infestation (A) and population development of *C. claratris* adults (B), first (C) and second larval instars (D) on tomato plants inside opened-door greenhouses clad with different UV transmitting and absorbing plastics and nets in replication 2 (from November to December, 2006). Mean individual number of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter is not significantly different (LS-mean, Tukey's test, $P < 0.05$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Table 2. Virus infection of tomato plants in opened-door greenhouses clad with different UV transmitting and absorbing plastics and nets at 5 weeks after planting.

Greenhouse	Virus incidence (%)	
	Replication 1 (September to October)	Replication 2 (November to December)
Ptr - Ntr	90	100
Pab - Nab	0	0
Pab - Ntr	10	0
Ptr - Nab	70	50

Note: Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

2.5.3 Discussion

Attractiveness and prevention of greenhouses clad with different UV transmitting and absorbing plastics and nets to *C. claratris*

The results clearly showed that the absorption of UV radiation from the ambient light spectrum significantly affected the orientation and host location abilities of *C. claratris*. This effect extended a short distance outside the manipulated environment, since even the migration of thrips to external parts of net walls was impaired. A fewer number of thrips was trapped at the outer net walls of the greenhouses covered with UV absorbing plastic compared to that of the greenhouses covered with UV transmitting plastic. In addition, fewer numbers of thrips passed through the nets to immigrate into the former to infest the plants. These results corroborate previous findings from Antignus et al. (2001) and Kumar and Poehling (2006) about the immigration response of the whiteflies, *B. argentifolii*, *B. tabaci*, the aphids, *A. gossypii*, and the thrips, *C. claratris*, towards and into greenhouses different in UV light transmittance. The less attraction of the greenhouses with low UV intensities to whiteflies (Antignus et al. 2001, Kumar and Poehling 2006), aphids and thrips (Kumar and Poehling 2006) compared to the rich UV areas was reported throughout the experimental period.

The combination of UV absorbing plastic and net (Pab – Nab) resulted in the most remarkable anti-thrips effect of the greenhouse. These two UV absorbing material types mutually supplemented each other to protect the greenhouses from immigration of the thrips. This combination not only obstructed thrips to find plants from a distance, but also impeded them to penetrate via the net walls. Almost no thrips individuals were trapped at the inner net walls, despite a certain number of thrips was found on the outer traps at this greenhouse type. The distinct gradient of thrips distribution across this border is obvious between outside and inside traps.

On the other hand, it was remarkable that *C. claratris* dramatically changed its preference towards the greenhouse type Pab – Ntr after five weeks of experiment in the present study. High numbers of thrips were caught on the sidewalls of this greenhouse. This phenomenon was observed in two out of three experimental replications. An explanation for that change could be related to the nutritional status of the host plants. Heavily infested plants within the UV transmitting greenhouses were overcrowded and damaged in that phase, leading to high numbers of thrips searching outside greenhouses for host plants. Whereas the plants in greenhouse covered with UV absorbing plastic and transmitting net (Pab – Ntr) were in a much better condition and located visually and by stimulating olfactory signals. The higher attraction of more healthy and only moderate infested plants could overweight the inhibitory effect of the UV deficient environment. A similar argument can be given for the increase in thrips capture around the greenhouse made from UV absorbing plastic and net (Pab – Nab) at the same time. However, the increase in capture of thrips at this greenhouse's net walls was not as pronounced as at the Pab-Ntr, presumably because of the additionally visual barrier function of the UV absorbing net. On the other hand, this reduced protective property of these greenhouses was not observed with low surrounding thrips pressure as recorded in the second replication.

Reduced efficiency in preventing thrips immigration has been reported in the greenhouse solely covered with UV absorbing nets (Antignus et al. 1998). While 50 mesh UV absorbing net (Bionet) effectively prevented the penetration of several insects including the whiteflies, *B. argentifolii*, the aphids, *A. gossypii*, the leafminers, *L. trifolii*, and the red mites, *T. telarius*, this net type failed to restrict the immigration

of western flower thrips, *F. occidentalis*, and the rust mites, *V. lycopersici*. The mesh-size of the net in relation to the size of the target insect and the UV incomplete filtration properties of the net were attributed for this failure (Antignus et al. 1998).

Immigration and population development of C. claratris on tomato plants inside greenhouses with different UV intensities

The efficiency of the greenhouse constructions in insect exclusion is strongly dependent on the filtration properties of plastic roof and net sidewall materials to UV intensity. In the present study, the greenhouses covered with UV absorbing plastic and net most successfully protected tomatoes against thrips immigration. None of the tomato plants in this greenhouse type was infested with *C. claratris*. However, the anti-thrips effect of this construction was significantly impaired if either the UV absorbing plastic roof or net was replaced by an UV transmitting one, or when the entrances were opened during the cultivated period, resulting from higher amount of UV radiation penetrated to the inner parts of the enclosed rooms. The combination of UV absorbing and transmitting materials in the greenhouse's designs did especially retard the initial thrips infestation and population build up to some extents with a more pronounced effect if absorbing plastics were used. However, the combination of Pab – Ntr also can protect plants as well as the combination Pab – Nab only if the outer thrips population pressure was not too high. These results agree with the recently published studies of Doukas and Payne (2007a). These authors reported that the anti-whitefly (*T. vaporariorum*) effect of the UV absorbing plastic tunnel was significantly impaired if the complete UV protection area was reduced by cladding only part (more than 20%) of the sidewalls with UV transmitting plastic. The low filtration effect of the 50 mesh net covering greenhouse to the UV radiation also failed to prevent the immigration of *F. occidentalis* although this greenhouse type successfully excluded several other insects including the whiteflies, *B. argentifolii*, and the aphids, *A. gossypii* (Antignus et al. 1998).

Greenhouse constructions in the tropics need plastic roofs to protect the crops from heavy rainfalls but cannot completely be closed by plastics, which would be most effective against insect immigration. They require intensive air ventilation to achieve sufficient heat exchange. One possible solution is to use fans in combining with the

plastic film construction but such constructions are expensive. Houses with plastic roofs and netted sidewalls are cheap, however, during hot seasons additional ventilation is necessary, which can be achieved by partial opening of the sidewalls or by opening larger entrances on two opposite sides of the houses. In our study, we choose this second option. Even with the opened entrances, the constructions with UV absorbing covering plastics on roof and nets on the sidewalls (Pab – Nab) exhibit excellent anti-thrips effects. Similar to the finding of Antignus et al. (2001), the infestation started from the plants at the entrance. Similarly, a partial decrease of efficacy could be observed in relation to material combinations. In the here study, higher preventive effect was also found at the opened-door greenhouse made from UV absorbing plastic and transmitting net (Pab – Ntr) compared to those with UV transmitting plastic roofs. Thrips population in the latter built up very fast. The results agree with the findings of Kumar and Poehling (2006), which reported profound exclusion effects of the netted greenhouses covered with UV absorbing plastic on roof to *C. claratris*, *B. tabaci* and *A. gossypii* irrespective of the opening period of the doors. Similar findings are reported from other studies. Antignus et al (2001) showed that opened-door greenhouses covered with UV absorbing plastic and UV transmitting nets were still superior to houses with UV transmitting plastics against whiteflies, *B. argentifolii*, but could not achieve a complete and durable protection. Costa et al. (2002) reported that densities of aphids, *A. gossypii*, and thrips, *F. occidentalis*, were restricted under the side-opened greenhouses covered with plastics blocking the transmission of UV light up to 380 nm, however, the effect was inconsistent, and the same greenhouse design failed in protection against the whiteflies, *T. vaporariorum*.

Virus infection in greenhouse constructions clad with different combinations of UV transmitting and absorbing plastics and nets

The spreading of virus disease is closely associated with the distribution of its insect vector. In accordance with the infestation levels of thrips in different greenhouse constructions tested, plants in the greenhouse covered with UV absorbing plastic and net (Pab – Nab) were successfully protected from thrips and virus infection. On the contrary, plants within greenhouses covered with UV transmitting plastic and net (Ptr – Ntr) suffered from fast and intensive virus spread all over the greenhouse area,

resulting in 100% virus infection rates. The heavy virus infection in this greenhouse type caused a total loss of the harvest in both closed- and opened-door greenhouse designs one month after planting. More limited virus infection was recorded in the greenhouse types that partially filtered UV light either via the plastic roof (Pab – Ntr) or netted sidewalls (Ptr – Nab) with a more pronounced efficiency in the former. Lower infections of tomato plants in these greenhouses compared to the UV transmissive constructions could slow down the development of detrimental virus effects until the first harvest. The delay in thrips infestation and the consequent restriction in thrips population development may be the reasons for the limitation in virus infection in these greenhouse types although thrips was also found spreading all over the greenhouses but slower compared to the UV transmitting cladding greenhouses. The older plants inside greenhouse Pab – Ntr at the time of infestation with thrips and virus may be more resistant to virus infection than those in greenhouse Ptr – Ntr, which was infested few weeks earlier. This hypothesis was supported by several other studies on the relation between plant age and virus infection (e.g. Moriones et al. 1998, Wang et al. 1999, Hernan and John 2001, Mandal et al. 2007).

The number of virus particles inoculated to the plants by the vectors among others is the most important factor influencing the success of inoculation and systemic spreading of the virus. Hence, the proportion of viruliferous thrips individuals in the infesting population represents the potential of that population to transmit the virus. For instance, six viruliferous adults of *F. occidentalis* per plant were determined as the minimum number required for the successful transmission and symptom development of tomato spotted wilt virus (TSWV) in several chrysanthemum cultivars (van de Wetering et al. 1999). In the here study, we did not control the percentage of viruliferous immigrants in the different treatments, however, we hypothesised that the UV manipulation will not influence the ratio of viruliferous vs. non-viruliferous thrips, and that simply the higher thrips population in the greenhouse covered with UV transmitting plastic and net (Ptr – Ntr) may be responsible for higher numbers of viruliferous individuals and the higher virus load of the crop. Antignus et al. (2001) also argued in their experiments that mainly the UV deficient area limited vector immigration but did not alter the ability of viruliferous insects to transmit the virus.

The complex interaction among plants, virus-borne insects and virus determines the success or failure of insect infestation, virus infection and virus spreading. Apart from the direct interaction between each of the partners involved, i.e. plant vs. virus, plant vs. insect, insect vs. virus, insect and virus may indirectly affect each other infection/infestation to plants via shared host plant defence reaction, because they both excite the defence reaction in plants once infested/infected. Presumably, the induced defence reaction of plants to one infested/infected factor (insect or virus) will restrain the infection/infestation of the followers (virus or insect). On the other hand, to successfully establish on that plant, virus and insect must overcome the defence mechanism in plants. Since virus needs its insect vectors to spread, several evidences showed that while encountering with the defence mechanism induced plants, virus may also interfere the defence response of plants to its insect vectors so that promoting the development of the vectors, eventually for spreading of virus itself (Belluire et al. 2005). Many insects were found preferring the virus infected plant over the healthy one and/or developing and reproducing faster with higher survival rate of the offspring on the former compared to the latter, e.g. thrips, *F. occidentalis* (Bautista et al. 1995, Maris et al. 2004, Belluire et al. 2005, Stumpf and Kennedy 2007), *F. fusca* (Stumpf and Kennedy 2005), aphids, *Aphis fabae* (Kennedy 1951), silver whiteflies, *B. agentifolii* (Mayer et al. 2002), meadow spittlebugs, *Philaenus spumarius* (Eubanks et al. 2005). These all findings hypothesised that virus infection has improved plant/food quality for its insect vector. However, in most of the cases, this hypothesis was qualitatively rendered based on the attraction and performance of the insects on plants but the actual change in the plant tissues was not quantified. Among a very few detailed studies, Eigenbrode et al. (2002) described the enrichment of plant kairomone volatiles in the virus infected plants, which thus attract more insects. Moreover, the yellow colour of the virus infected plants was also argued to attract more thrips (Yudin et al. 1987). The attractiveness, high development rate and fast spreading of thrips in the greenhouse type Ptr – Ntr in the present study may not only be the function of UV light regime but also result from the increased plant attraction caused by virus infection. The presence of high numbers of virus infected plants may also be another explanation for the observed late increase in attractiveness of thrips towards the greenhouse Pab – Ntr.

The strong preventive effect of UV filtration on the spreading of the virus was also well documented from other studies. The spread of tomato yellow leaf curl virus in tomato crops was sharply limited by restriction in the abundance of its vector, the whiteflies, *B. argentifolii*, in culture tunnels clad with UV absorbing material (Antignus et al. 1998, Antignus et al. 2001). Similarly, a strong reduction in tospovirus infection (CaCV) was recorded from poor UV intensity greenhouses in relation to the reduction of abundance of the vector *C. claratris* (Kumar and Poehling 2006).

2.6 Effect of projecting roofs covered with UV absorbing plastic film to greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets on the immigration of *Ceratohripoides claratris*

2.6.1 Materials and methods

'Forced' immigration of *C. claratris*. The four greenhouses clad with different plastic films and nets previously used to examine natural immigration of *C. claratris* (for more detail see Figure 6) were modified for this study. While the north-oriented side of the greenhouses remained unmodified, the southern roof sides were protruded 0.5 m longitudinally and upright to the sidewalls of the greenhouses using a steel frame and covered with UV absorbing plastic film (Figure 17 and Figure 18). This additional roof structure aimed to increase the "room" with reduced UV intensity over the greenhouse area like an additional buffer zone. These greenhouse structures were used to study the supportive effect of an external UV absorbing roof to greenhouses clad with different types of roof plastics and sidewall nets against the immigration of *C. claratris*.

For this purpose, thrips were artificially released from the two sides with different roof projecting designs of each greenhouse and their subsequent immigrations into that greenhouse were compared. Cages (1 m x 0.5 m x 0.5 m) with an opened side (1 m x 0.5 m), and UV transmitting plastic cover (upper side), while other sides clad with black plastic film served as release station (Figure 19). Cages were placed in the middle of the longitudinal greenhouse's sidewalls, 0.5 m above the ground and with

the opened side attached to the sidewalls (Figure 17 and figure 18). 250 laboratory-reared adults of *C. claratris* of unknown age were released into each cage from a plastic vial (3 cm in diameter and 5 cm in height), resulting in a total number of 500 thrips released at each greenhouse. The sex ratio of thrips was estimated for 100 thrips from the same rearing culture for every release given an average ratio of approximately 2 to 3 females to 1 male.

Prior to the release, thrips were marked with either green or orange fluorescent dusts (Radiant Colour N.V., Belgium) to discriminate between thrips released from the cages at the northern and southern sidewalls of each greenhouse. For marking of the thrips, the fluorescent dust was at first sucked to coat the inner wall of a suction tube (5 mm in diameter and 15 mm in length) of an aspirator with a thin layer. Subsequently, this aspirator was used to collect thrips from the rearing cages. The collected thrips was marked while being sucked through the dust coated tube. This technique allowed uniformly marking high number of thrips with a sufficient amount of dust to detect later, and avoiding too much marked dust on thrips, which may results in interfering thrips physical strength and consequently alter the flight ability of thrips.

The releases of thrips were usually carried out at around 11 am. Inside every greenhouse, 30 blue sticky traps (12 x 15 cm) were arranged in a grid form and at 50 cm above the ground in order to capture the thrips entering the greenhouses. The sticky traps were collected six hours after releasing the thrips. Subsequently, the number of thrips on the traps was counted under a binocular (Krüss. A. K., Krüss Optronic, Germany, magnification: 15 x 4) in the dark laboratory. A handheld UV lamp (UVP, Inc. 20066 W. Upland, CA) was used to verify the fluorescent dye colour on thrips (Figure 19). Experiments were repeated three times.

Free flight/Short-distance immigration of C. claratris. Similar greenhouse structures with projecting roof as described above were used. However, the release cages were posited 1 m away from the greenhouses' sidewalls. The UV transmitting covered upper side of the cages was replaced with black plastic film in order to direct thrips vision towards the only light source from the opening side directed towards the greenhouse.

Inside and outside the greenhouses, under the projecting and normal roof areas, the UV intensities were measured using radiometer UVA sensors (Indium Sensor, Neuenhagen, Germany). The UVA sensors were placed on stands (50 cm height) in the middle of the greenhouses or at a distance of 20 cm from the outer net wall. Since only two UVA sensors were available, the measurements were conducted on three (consecutive) days with similar weather conditions.

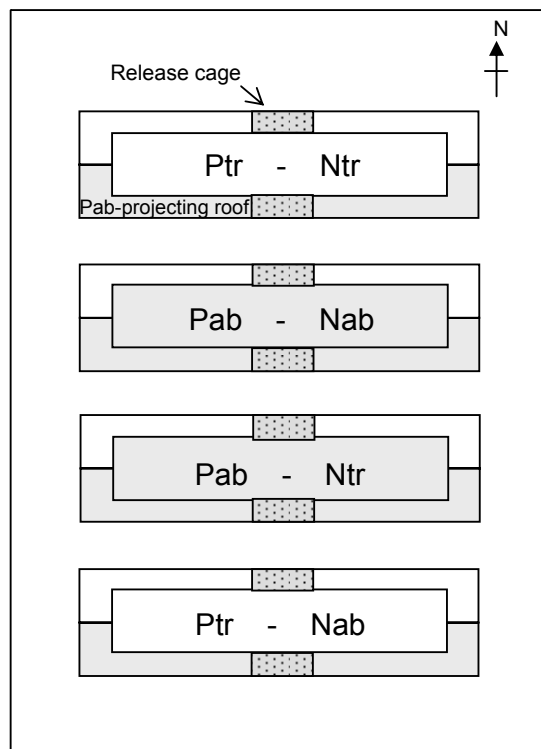


Figure 17. Experimental setup used to study the supplemental effect of projecting roofs to greenhouses clad with different UV transmitting and absorbing plastics and nets on the immigration of *C. claratris*, Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.



Figure 18. Experimental greenhouse design viewed from outside (left) and inside (right).

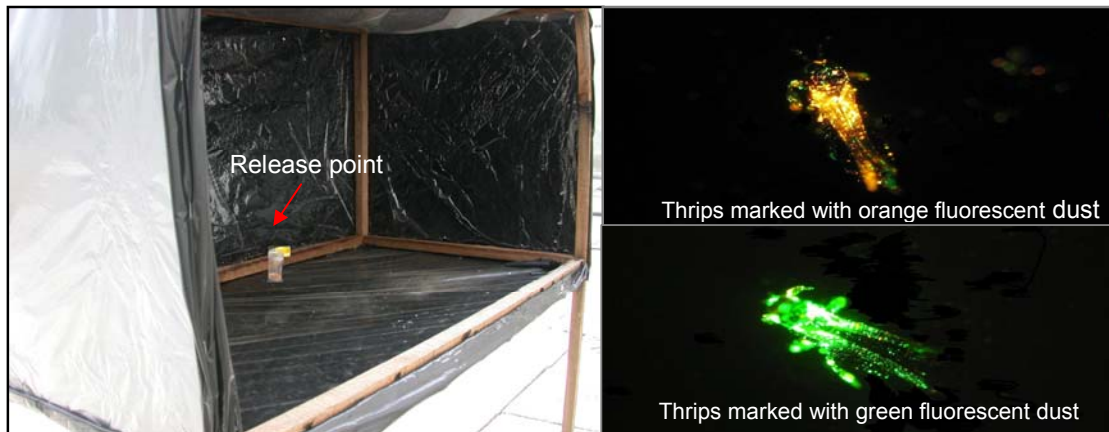


Figure 19. The cage used to release the thrips (left) and thrips marked with fluorescent dust under the microscope (400 x) illuminated with a UV lamp.

2.6.2 Results

Light transmission

The UV absorbing projecting roofs strongly reduced the ambient UV intensity (Figure 20). The UVA value measured under the projecting roof was approximately 3 W m^{-2} compared to about 10 W m^{-2} at the corresponding area which was not equipped with a projecting roof. However, the reduction in UV intensity caused by the projecting roof was less pronounced than that resulting from the cladding of UV absorbing plastic to the greenhouses. The UVA intensities were about 0.2, 1.8, and 7 W m^{-2} , inside the

greenhouses Pab - Nab and the Pab – Ntr and those covered with UV transmitting plastic (Ptr - Ntr and Ptr - Nab) respectively.

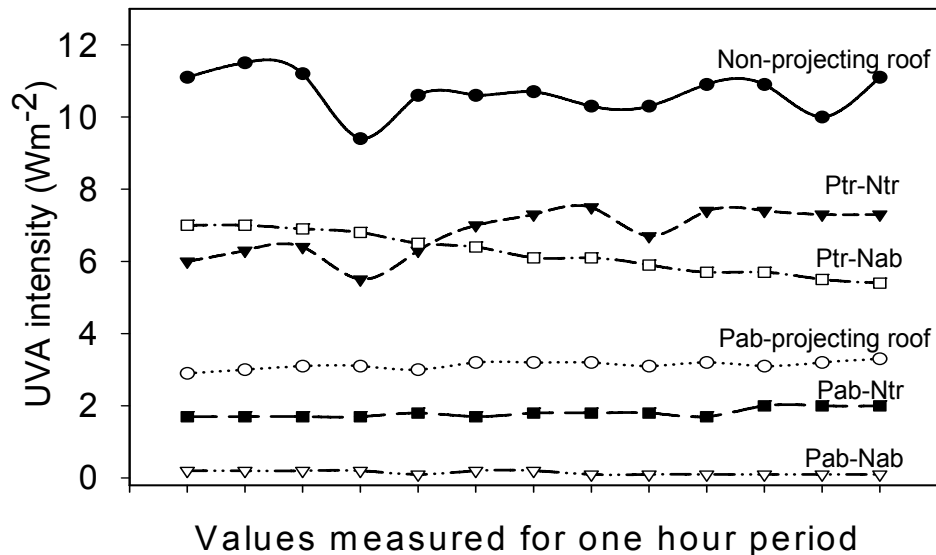


Figure 20. UVA intensities ($W m^{-2}$) measured for one hour at different parts of greenhouses (outside under the projecting roof and inside the greenhouses). Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net. (Values are measured at noon during three consecutive days).

Supplemental effect of UV absorbing projecting roofs to greenhouses clad with different combinations of UV transmitting and absorbing materials on the immigration of *C. claratris*

In all experiments with thrips release from cages closely contacted to the greenhouse sidewalls, consistent fewer thrips emigrated from cages under the projecting roof were captured inside all greenhouse types compared to the releases under the corresponding side with no UV absorbing roof extension (Figure 21A). With an exception of the Pab – Nab treatment this difference was highly significant in other cases ($P = 0.0024, 0.0079; 0.0035$ at the greenhouse Pab – Ntr, Ptr – Nab and Ptr – Ntr, respectively).

When the release cages were arranged in a distance of 1 m from the greenhouse sidewalls the supplemental anti-thrips effect of the projecting roof was only pronounced at the greenhouse clad with UV transmitting plastic and net (Figure 21B).

In this greenhouse, the number of thrips emigrated from release cages under the projecting roof (3.7) was significantly lower than from cages on the other side of the greenhouse (16.3) ($P = 0.0017$). In all other greenhouses no significant additional effect of the projecting roof was recorded but the immigration from both sides was significantly different to the Ptr - Ntr greenhouse.

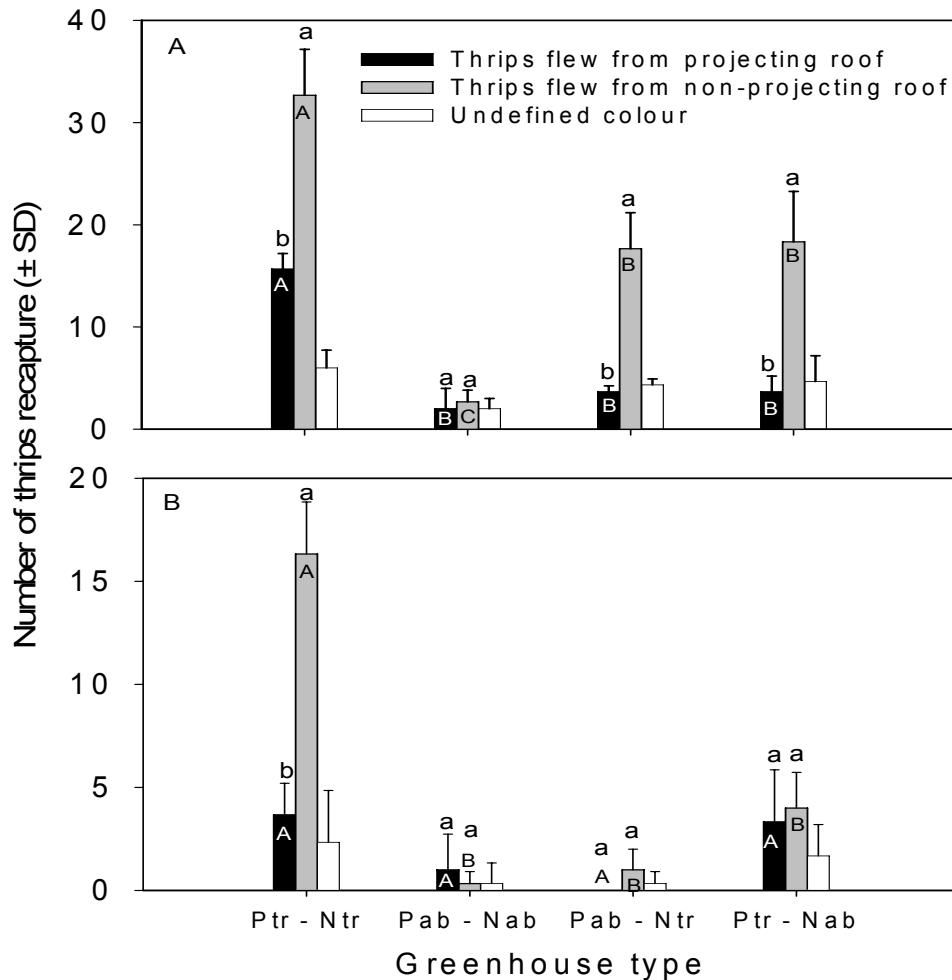


Figure 21. The immigration of thrips, *C. claratris*, into greenhouses clad with different combinations of UV transmitting and absorbing plastics and nets with an additional effect of extended UV absorbing roof. Thrips were released from cages either attached directly to (graph A) or positioned in a distance of 1 m (graph B) from the sidewalls. Within each pair of external roof design for a given greenhouse (lower case letters) or among the same external roof design of different greenhouses (upper case letters), different letters above or inside the bars indicate significant differences in immigration of thrips (T-test, $P < 0.05$, $n = 3$) and (Tukey's test, $P < 0.05$, $n = 3$), respectively. (The number of undefined colour thrips was not included in the statistical test).

In both experimental setups, the highest immigration of thrips was recorded inside the greenhouse clad with UV transmitting plastic and net (Ptr – Ntr). On the contrary, the lowest thrips number was consistently found inside the greenhouse covered with UV absorbing plastic and net (Pab – Nab), while intermediate immigration rates were recorded in the combinations of UV transmitting and UV absorbing cladding materials (Pab – Ntr and Ptr - Nab).

Unmarked thrips were also trapped inside all greenhouse types together with marked thrips. Their number was counted and indicated in the graphs as undefined source of thrips since they can be from either marked thrips populations who lost the marked dust or from natural sources outside the greenhouses. In all experiments the major proportion of the released thrips (more than 85% in most cases) has left the release plastic vials.

2.6.3 Discussion

The additional installation of projecting roofs clad with UV absorbing plastic resulted in a reduction of the UV intensity in the area underneath these structures approximately 70% of the ambient UV intensity. When the release cages were directly placed within this “room”, thrips were released into an environment with low UV intensity. They were expected to be attracted by the blue traps inside the greenhouses, separated by the net walls from release areas with either higher UV intensity (under UV transmitting covers) or lower UV intensity (under UV absorbing covers). Consistently, in all greenhouse types the immigration of thrips from the UV-reduced areas under the projecting roofs was significantly lower than from the sides without projecting roofs regardless of the UV properties inside the greenhouses. This effect was even pronounced in the conventional greenhouse types clad with UV transmitting plastic and net, which has “normal” high UV intensity. It is obvious that the orientation and colour recognition abilities of the thrips were strongly affected by the low UV intensity within the release environment even though the otherwise attractive traps were exposed in a short distance and on the same level to the release area. Additionally, the capability of thrips to orientate and to recognise the traps behind the net barrier of UV absorbing type decreased with overall UV intensity inside the greenhouses regardless of the both release conditions, i.e. under the

areas of poor UV intensity of the projecting roof and rich UV intensity of the non-projecting roof.

High proportions of released thrips (> 85%) left the release vials indicates that marking of thrips with fluorescent dust did not affect their physical properties and takeoff ability. Rhainds and Shipp (2004) also recognized no impact of fluorescent dust on the dispersal ability of *F. occidentalis* when comparing marked and unmarked thrips. Most of the immigrated thrips were captured on traps close to the net walls adjacent to the release cages, only a few individuals flew across the greenhouses. The source of the small numbers of unmarked thrips captured on traps in all greenhouse types could not be identified and it remained open whether they originated from external sources or were released individuals having lost the marking dust. Presumably, they belong to released thrips, which lost the marking dust during flight activity.

Many insect species dispersing by flight activity have distinct preference for environments with higher UV intensities compared to poorer UV intensity areas when given a choice (Costa and Robb 1999, Costa et al. 2002, Kigathy 2005, Mutwiwa et al. 2005, Doukas and Payne 2007a). This has also been validated recently for the thrips species, *C. claratris*, investigated in this study (for detail see section 2.4). However, in these choice experiments, the insects were released from a central dark release device allowing a free and not limited dispersal to environments with different UV light intensities. To the best of our knowledge, there is no information available yet on the movement behaviour of insects from a relatively weak UV intensity area (e.g. under the UV absorbing projecting roof) to an area of higher UV intensity (e.g. to the greenhouses clad with UV transmitting plastic) or to an area of lower UV intensity (e.g. to the greenhouses clad with UV absorbing plastic) like in the here study. Nevertheless, there is some evidences that the dispersal of insects is limited under the conditions of low UV intensities (Antignus et al. 2001, Chyzik et al. 2003, Mutwiwa et al. 2005). In experiments conducted by Costa and Robb (1999), the attempt to recapture released *F. occidentalis* under UV deficient conditions entirely failed, supporting the result of the experiment discussed here, that under the conditions of reduced UV intensities the orientation ability of the thrips species *C. claratris* is impaired. The discussion on the effects of UV deficient conditions on

dispersal of thrips will be resumed and continued more detailed in the following section (section 2.7).

The projecting roof also significantly reduced the immigration potential of the thrips into the greenhouse type covered with UV transmitting materials (Ptr - Ntr) when thrips was released in a distance of 1 m from the sidewalls. A much lower number of thrips released from the side with the projecting UV absorbing roof immigrated into this greenhouse compared to the individuals released from the other corresponding non-projecting roof side. Overall, the magnitude of the thrips repelling effect of the additional UV absorbing projecting roof of this greenhouse type can be compared to that of the greenhouses clad with UV absorbing plastic and/or net. It is likely that the reduction of the UV intensity under the projecting roof acts as a visual 'barrier' to *C. claratris*. *C. claratris* is obviously not able to cross this UV deficient barrier to the otherwise attractive blue colour traps even over that short distance. On the other hand, this 'barrier' did not considerably improve the effect of the greenhouses clad with either UV absorbing plastic or net in preventing the immigration of *C. claratris*. The total numbers of thrips recaptured inside these greenhouses were very low, regardless the source of release from the projecting roof or from the non-extended roof side of greenhouses, which indicates that the high repellent potential in these constructions mainly resulted from the UV absorbing properties of the greenhouses rather than from the additional effect of the projecting roofs. However, these results corroborated findings from earlier trials conducted in the framework (for detail see section 2.5).

Installation of the projecting roof provides another possibility in using UV absorbing plastics for control of thrips immigration besides cladding the entire greenhouses with this material.

2.7 Spatial and temporal distribution of *Ceratothripoides claratrix* under the greenhouses clad with different combinations of UV transmitting and UV absorbing materials

2.7.1 Materials and methods

The effect of UV on the dispersal ability of thrips was studied inside the four greenhouse types clad with different UV transmitting and absorbing materials previously used to examine the natural immigration of *C. claratrix* (for detail see Figure 6). The spatial dispersal of thrips over time was assessed from an initial infested point by artificially releasing thrips individuals on a plant at the one-end or in the middle of these greenhouses (Figure 22A, and B).

Thirty three-week-old potted tomato plants were arranged in a grid form in three rows with a distance of 0.7 m between pots and rows, measured from the centre of adjacent pots. One week after transplanting, one hundred *C. claratrix* adults of unknown age from the laboratory stock culture were released on the last plant in the middle row at the western end of the greenhouses (one-end release) (Figure 22A). In a second experiment, a same number of thrips was released on a plant in the middle of the greenhouses (Figure 22B). The sex ratio of thrips was estimated for 100 thrips from the same rearing culture for every release giving an average ratio of approximately 2 to 3 females to 1 male. Thrips were usually released in the morning around 8 am of the release day. The dispersal of thrips to other plants was investigated on day 1, 3 and 7 after release by direct counting the number of adult thrips on all plants in the greenhouses. Each experiment was repeated three times over time.

Prior and after an experimental repetition, greenhouses were sanitized and sprayed with Spinosad^M (1.5 mL L⁻¹). The surrounding area of experimental site was also cleaned and sprayed with Spinosad to limit the immigration of thrips from outside.

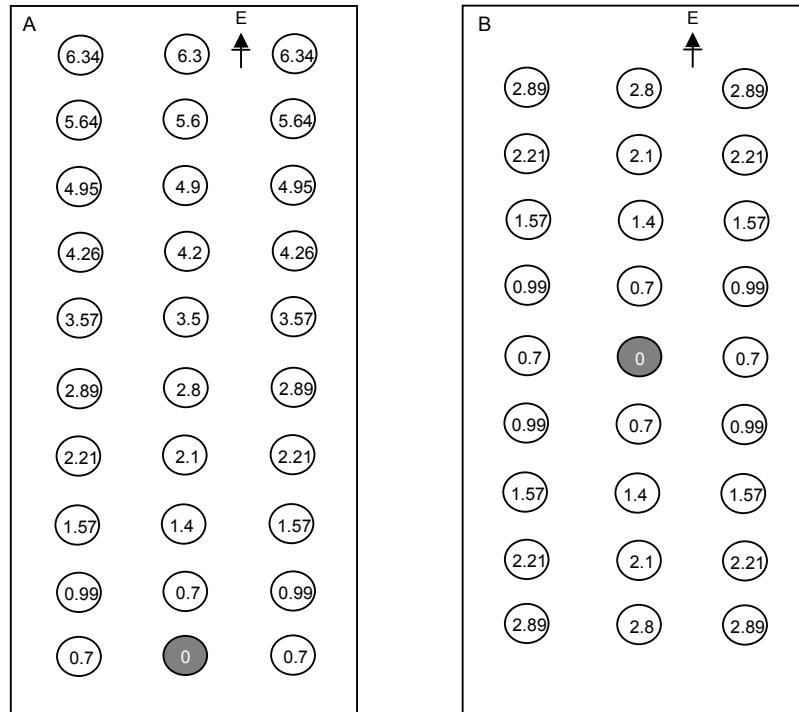


Figure 22. Experimental designs studying the dispersal of a thrips population from an initial infestation point. The gray points in diagram A (one end release) and B (middle release) represent for the release points of 100 thrips. The numbers in the circle show the relative distance (m) from the recapture plants to the initial infested plant (0 m).

Mean distance travelled by thrips

Assuming that released thrips dispersed randomly and independently from each other, with no drift effects influencing the direction of dispersal, and that the dispersal coefficient of thrips was constant over a period of observation, the distance moved by thrips was calculated by fitting to a formula given by Southwood and Henderson (2000).

$$D = \sum_{i=0}^y \frac{\mathcal{N}_i (x_{i+1}^2 - x_i^2)}{\sum_{i=0}^y \mathcal{N}_i (x_{i+1}^2 - x_i^2)} \frac{1}{2} (x_{i+1} + x_i)$$

Where D is the mean distance travelled by thrips over a period until the investigated day, \mathcal{N}_i is the mean number of thrips per plant recaptured at the same distance from the released plant, x_i is the distance from the released plant of the i^{th} distance, x_{i+1} is the outer distance to x_i , and y is the outer most distance.

Diffusion model predicting the dispersing density of *C. claratris* at different distances from the release point

Among several diffusion equations used to estimate the decline of insects' densities with distance from a release point, the exponential models provide the best fits to most field diffusion of insects (Taylor 1980, Southwood and Henderson 2000). The exponential model is a general form of most other diffusion equations (Taylor 1980, Turchin and Thoeny 1993). This model describes well the rapid decline of the density near the release point but extend infestation with small density ('long tail') to farther distance, which is usually observed in weak flying insects like thrips. In this study, the spatial dispersion of the thrips population between plants inside greenhouses was fitted to the well-known Taylor's decay exponential model (1978) (Taylor 1980, Southwood and Henderson 2000). The Taylor's decay exponential model is defined as:

$$\mathcal{N}(x) = a \exp(-bx)$$

Where \mathcal{N} is the thrips density at a distance x from the release plant; a is the initial density and b is the decay rate.

2.7.2 Results

Mean distance travelled. It is likely that the UV poor conditions did not support the dispersal of the thrips, *C. claratris*. Until day 7 after releasing (DAR), thrips individuals released under this condition (under the UV absorbing plastic covers) moved a shorter distance than those released under the richer UV light regimes (under UV transmitting plastic covers) during the same time period. In both experiments, where thrips were released either at the one-end or in the middle of the greenhouses, the lowest dispersing distances were recorded in the greenhouses clad with UV absorbing plastic and net (Pab - Nab). For instance, on day 7 after release the average distance travelled by thrips in this greenhouse was 0.79 and 0.56 m from the release point compared to more than 1 and 0.8 m in other greenhouses in one-end (Table 3) and middle release treatments (Table 4), respectively.

Table 3. Mean (\pm SD) distance travelled by thrips released at the one-end of the greenhouses clad with different UV transmitting and absorbing plastics and nets.

Greenhouse	Mean distance moved (m) during time intervals after release (day after release - DAR)		
	1DAR	3DAR	7DAR
Ptr – Ntr	0.67 \pm 0.13a	1.06 \pm 0.29a	1.48 \pm 0.29a
Pab – Nab	0.43 \pm 0.05a	0.68 \pm 0.15a	0.79 \pm 0.05b
Pab – Ntr	0.50 \pm 0.11a	0.71 \pm 0.09a	1.10 \pm 0.11ab
Ptr – Nab	0.47 \pm 0.01a	0.94 \pm 0.20a	1.11 \pm 0.11ab

Mean distance travelled inside different greenhouses for a given investigated day (columns) followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$, $n = 3$). Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

However, in the one-end release experiment, a significant difference could only be detected between the dispersing distances of thrips populations in the greenhouse types Pab – Nab and Ptr - Ntr on day 7 after release ($F_3 = 8.78$, $P = 0.0065$) (Table 3). Whereas after release of thrips in the middle of the greenhouses, the distances thrips dispersed in these greenhouses were significantly different throughout the

investigation period ($F_3 = 5.14$, $P = 0.0285$ for 1 DAR; $F_3 = 6.39$, $P = 0.0162$ for 3 DAR) (Table 4). Due to external infestation of thrips from natural sources outside, dispersal data from that treatment on 7 DAR in the greenhouse Ptr - Ntr was not included. At this time point, the dispersal distance of thrips in the greenhouse Pab-Nab was significantly shorter than in other greenhouses (Pab - Ntr and Ptr - Nab) ($F_3 = 10.33$, $P = 0.0114$). Although thrips in the greenhouse type Pab - Ntr dispersed with slightly shorter distance than under the UV transmitting covers, no significant difference could be determined among the distances dispersed by thrips in these greenhouses.

Table 4. Mean (\pm SD) distance travelled by thrips released in the middle of the greenhouses clad with different UV transmitting and absorbing plastics and nets.

Greenhouse	Mean distance moved (m) during time intervals after release (day after release - DAR)		
	1DAR	3DAR	7DAR
Ptr – Ntr	0.59 \pm 0.10a	0.86 \pm 0.16a	-
Pab – Nab	0.39 \pm 0.03b	0.47 \pm 0.08b	0.56 \pm 0.13b
Pab – Ntr	0.44 \pm 0.06ab	0.61 \pm 0.12ab	0.80 \pm 0.03a
Ptr – Ntr	0.52 \pm 0.07ab	0.69 \pm 0.04ab	0.87 \pm 0.07a

Mean distance inside different greenhouses for a given investigated day (columns) followed by the same letter are not significantly different (LS-mean, Tukey's test, $P < 0.05$, $n = 3$). Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Total dispersed distances. While the released thrips under the UV transmitting plastic covers (Ptr) could be recaptured as far as 3.57 m from the release point (one-end release) on the last observation day (7 DAR), they only reached a distance of 2.21 m when the UV inside greenhouse was filtered by both plastic and net (Pab - Nab) (Figure 23). In this experiment design, the farthest distance that thrips in the greenhouse type Pab - Ntr could move was 2.89 m. When thrips was released in the middle of the greenhouses, it could reach the plants far at the ends (2.8 and 2.89 m) of greenhouses with UV transmitting plastic covers, Ptr – Ntr and Ptr – Nab, on 3 and

7 DAR, respectively (Figure 24). Under the UV absorbing covers the longest distance thrips dispersed was up to 2.21 m from the central release point.

Due to the low number of thrips that dispersed and the strong variance in recapture rates of thrips on trap plants at the same distance, almost no significant difference in thrips recapture was calculated at a given recapture distance across the greenhouses. In all greenhouses, the distribution of thrips decreased in relation to the increase in the distance from the released plant.

Diffusion model. Significant negative relationships were detected between the densities of dispersing thrips and the dispersing distances (Table 5 and 6). The distribution of thrips decreased as increasing the distance from release plants. After the first day post-release, higher decay rates (the slopes of the regressions) were consistently calculated in the diffusion regressions of thrips in the greenhouse type Pab – Nab compared to other greenhouse types clad with UV transmitting plastics and/or nets. However, this difference was not statistically significant when thrips were released from one-end of the greenhouse. On the other hand, significant higher decay rates were always detected in dispersal of released thrips from middle of greenhouse the type Pab – Nab compared to Ptr – Ntr ($F_{3,8} = 12.11$; $P = 0.0024$ for 1 DAR; $F_{3,8} = 9$; $P = 0.0061$ for 3 DAR), and to others clad with either UV absorbing plastic or net but only on day 7 after releasing ($F_{2,6} = 8.75$; $P = 0.0166$). High values of the regression coefficient ($R^2 \sim 0.99$) and significant probability level (P value) of the model indicate that the exponential decay model described well the dispersal behaviour of thrips in this study.

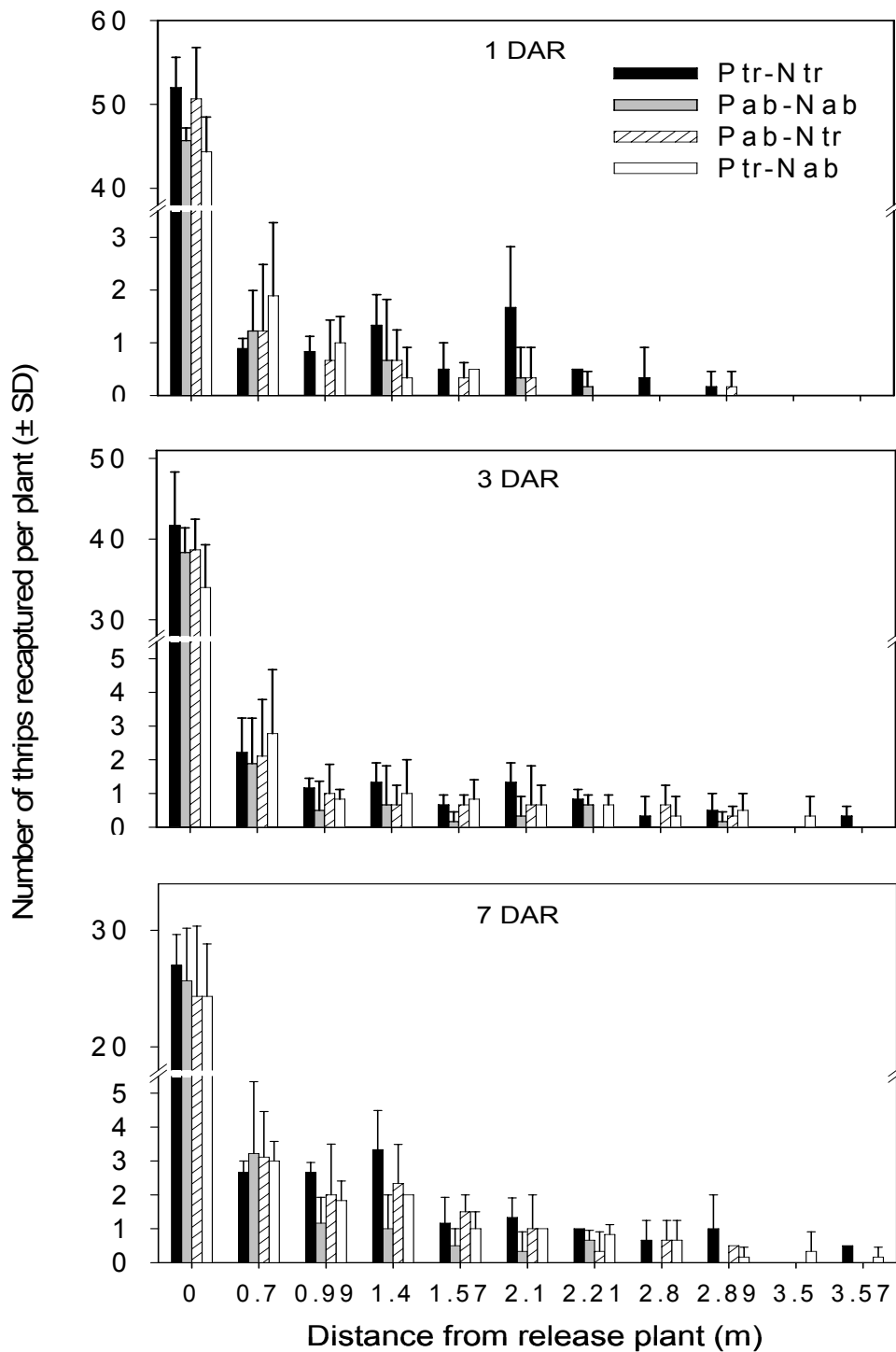


Figure 23. Dispersal of thrips *C. claratris* in the greenhouses clad with different UV transmitting and absorbing plastics and nets on 1, 3 and 7 day after release of thrips (DAR) on a plant at the one end of greenhouses. Data show the means of three replications. Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

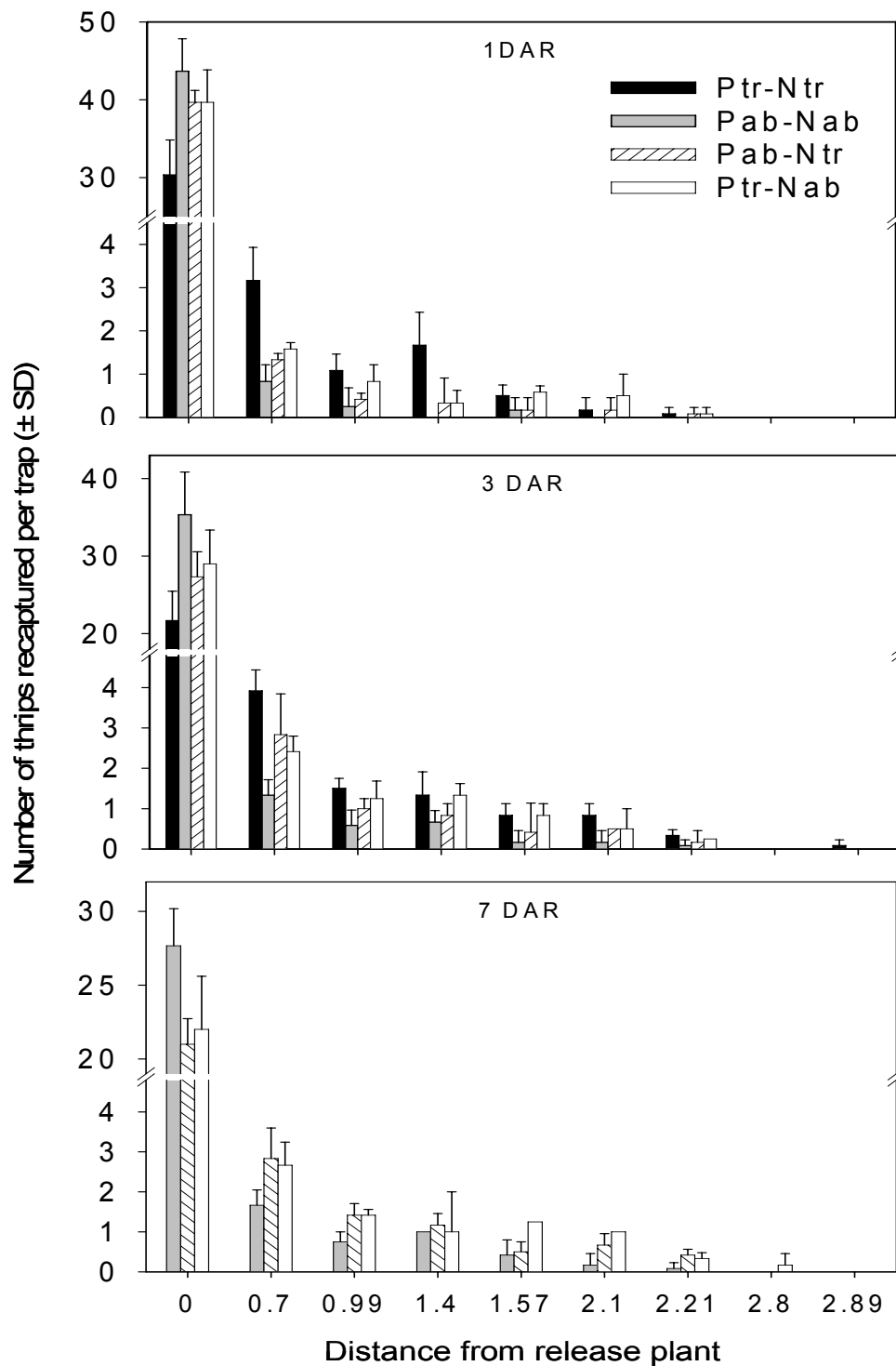


Figure 24. Dispersal of thrips *C. claratris* in the greenhouses clad with different UV transmitting and absorbing plastics and nets on 1, 3 and 7 day after release of thrips (DAR) on a plant in the middle of greenhouses. Data show the means of three replications. Ptr: UV transmitting plastic, Pab: absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Table 5. Parameters (\pm SE) of the diffusion regression model $\mathcal{N}(x) = a \exp(-bx)$ describing the relationship between the density of *C. claratris* and the dispersing distance at various time points after release of 100 thrips on a plant at the one-end of greenhouses.

Greenhouses	DAR	a	b	R^2	F	$P > F$
Ptr - Ntr		52.00 \pm 0.79	5.47 \pm 0.95	0.998	3,883	<0.0001
Pab – Nab	1	45.67 \pm 0.26	5.22 \pm 0.29	0.999	27,904	<0.0001
Pab- Ntr		50.67 \pm 0.29	5.15 \pm 0.29	0.999	25,924	<0.0001
Ptr - Nab		44.33 \pm 0.23	4.36 \pm 0.15	0.999	31,948	<0.0001
Ptr - Ntr		41.66 \pm 0.74	3.97 \pm 0.37	0.997	2,770	<0.0001
Pab – Nab	3	38.33 \pm 0.32	4.26 \pm 0.22	0.999	12,809	<0.0001
Pab- Ntr		38.66 \pm 0.44	4.00 \pm 0.25	0.999	6,713	<0.0001
Ptr - Nab		33.99 \pm 0.52	3.51 \pm 0.22	0.997	3,724	<0.0001
Ptr - Ntr		26.88 \pm 1.28	2.61 \pm 0.34	0.986	360	<0.0001
Pab – Nab	7	25.66 \pm 0.32	2.92 \pm 0.11	0.998	5,642	<0.0001
Pab- Ntr		24.25 \pm 0.84	2.54 \pm 0.23	0.987	698	<0.0001
Ptr - Nab		24.27 \pm 0.75	2.65 \pm 0.23	0.989	880	<0.0001

P and R^2 are the probability level and regression coefficient for the model, respectively

Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

Table 6. Parameters (\pm SE) of the diffusion regression model $N(x) = aexp(-bx)$ describing the relationship between the density of *C. claratris* and the dispersing distance at various time points after release of 100 thrips on a plant in the middle of greenhouses.

Greenhouses	DAR	a	b	R^2	F	$P > F$
Ptr - Ntr		30.32 \pm 0.52	3.14 \pm 0.19b	0.997	2,916	<0.0001
Pab – Nab	1	43.67 \pm 0.07	5.61 \pm 0.11a	0.999	335,637	<0.0001
Pab - Ntr		39.67 \pm 0.15	4.79 \pm 0.14a	0.999	65,387	<0.0001
Ptr – Nab		39.66 \pm 0.34	4.44 \pm 0.25ab	0.999	12,094	<0.0001
Ptr - Ntr		21.64 \pm 0.42	2.41 \pm 0.12b	0.997	2,182	<0.0001
Pab – Nab	3	35.33 \pm 0.26	4.55 \pm 0.24a	0.999	15,792	<0.0001
Pab - Ntr		27.33 \pm 0.29	3.19 \pm 0.12ab	0.999	7,469	<0.0001
Ptr – Nab		28.98 \pm 0.55	3.32 \pm 0.24ab	0.997	2,377	<0.0001
Ptr - Ntr		-	-	-	-	-
Pab – Nab	7	27.66 \pm 0.38	3.84 \pm 0.26a	0.998	4,648	<0.0001
Pab - Ntr		20.97 \pm 0.40	2.70 \pm 0.15b	0.997	2,283	<0.0001
Ptr – Nab		21.97 \pm 0.59	2.79 \pm 0.22b	0.994	1,108	<0.0001

P and R^2 are the probability level and regression coefficient for the model, respectively.

The decay rates of diffusion regression of thrips in different greenhouse types at the same investigated time periods followed the same letter are not significant different (LS-mean, Tukey's test, $P < 0.5$). Ptr: UV transmitting plastic, Pab: UV absorbing plastic, Ntr: UV transmitting net, Nab: UV absorbing net.

2.7.3 Discussion

The distribution of thrips decreased in relation to the increase in distance from the released plant. As a typical function of exponential decay model, the distribution of released thrips rapidly declined near the release point (Taylor 1980); most of the released thrips focused around the release plants. This dispersal pattern was recorded for thrips released in all greenhouse types regardless of different UV light regimes. However, the higher decay rates of the diffusion regression of thrips and the shorter mean and total distances moved by thrips under UV deficient conditions compared to higher UV intensity areas suggest that the dispersal of thrips is hindered by the poor UV light conditions. This effect was more pronounced under the extremely low UV conditions, i.e. in the greenhouse type Pab - Nab.

These results corroborated findings of Antignus et al. (2001) about the effect of UV deficient conditions on the dispersal pattern of whiteflies, *B. argentifolii*. The dispersal activity of this insect was significantly reduced under the UV absorbing covers (Pab) compared to that under the UV transmitting covers (Ptr). While more whiteflies were recaptured near to the release point under UV absorbing plastics they dispersed more randomly to further distance under transmitting roof material. Similarly, Mutwiwa et al. (2005) reported the negative impacts of UV deficient conditions on the dispersal ability of whiteflies, *T. vaporariorum*, in small growing tunnels fully covered with UV absorbing plastic. The spread of the aphids, *M. persicae*, in a sweet pepper crop stand was also limited inside a greenhouse covered with UV absorbing plastic (Chyzik et al. 2003). Winged aphids in this greenhouse were less active than those under the rich UV intensity. Additionally, the population growth of aphids was significantly impaired under the UV absorbing plastics. The mean flight duration of aphids was also decreased in the areas without UV (Kring 1972).

On the contrary, Costa and Robb (1999) reported no negative impacts of the UV deficient conditions on the flight ability and dispersal of whiteflies, *B. tabaci*, although this insect obviously showed no preference to the area with poor UV light of 380 nm and below in a choice experiment. Higher numbers of *B. tabaci* were found on traps at the farther distance (1.5 m) from the released point than at the closer distance (0.6 m) in a plastic tunnel made of this plastic film. Unfortunately, their attempt to study the dispersal ability of thrips, *F. occidentalis*, failed due to the low rate of recapture

under the UV poor conditions. Antignus et al. (2001) criticized the experimental design used by Costa and Robb (1999). According to Antignus et al. (2001), the narrow structure of plastic tunnels tested in this experiment (0.5 x 0.5 x 1.8 m) most likely disturbs the natural flight behaviour of whiteflies. The tendency of the whiteflies to fly upwards to contact the low tunnel ceiling may alter their distribution behaviour inside the tunnel. Another possible explanation for the discrepancy in the dispersal behaviour of *B. tabaci* in Costa and Robb's experiment compared to others was the inadequate filtration of the UV light between 300 to 350 nm (Antignus et al. 2001). While the UV absorbing plastic used by Antignus et al. (2001) filtered almost 100% of UV light in this range, film used by Costa and Robb (1999) allowed about 15% of that UV range passing through.

The UV may also drive the takeoff behaviour of flying insects. Aphids took off more readily in the presence of UV (Kring 1972). Similar effect was also recorded for the whiteflies, *T. vaporariorum* (Coombe 1982). They took off and/or walked faster under the short wavelengths (< 400 nm) than at the wavelengths above 400 nm (Coombe 1982). On the other hand, the takeoff of *F. occidentalis* did not exhibit spectral specificity (O'Leary and Kirk 2004). No significant difference was recorded in the takeoff of this thrips under light regimes with pure UVA, visible light without UVA, and a mixture of both UVA and visible light.

Recently, the dispersal pattern of western flower thrips, *F. occidentalis*, was comprehensively studied in a uniform array of chrysanthemum and cucumber plants in the greenhouses (Rhainds and Shipp 2003, Rhainds and Shipp 2004, Rhainds et al. 2005). The released *F. occidentalis* also most likely aggregated close to the central release points like *C. claratris* in the present study. The density of dispersing thrips was significantly decreased with increasing dispersal distances. However, females of *F. occidentalis* dispersed more actively than males in response to high densities of thrips and the increase of senescent inflorescences (Rhainds and Shipp 2003, Rhainds et al. 2005). Nevertheless, the only and relatively low density of released thrips in the here study did not allow to see such density - dispersal dependent effect in *C. claratris*.

In practical management terms, the weak dispersal ability of the thrips *C. claratris*, particularly under the UV deficient conditions, opens a 'window' period for applying

insecticides only to the early infested niches (hot spots), e.g. first infested plants along the sidewalls after thrips cross the net or any specific plant within the greenhouses presumably infested by workers, before they disperse to other plants. This targeted treatment method was also proposed by Rhainds and Shipp (2004) for control of thrips in the greenhouses. Moreover, for bio-control purposes such as the release of natural enemies, the limited pest dispersion from hot spots is also favourable and allows target orientated local releases. However, efficient monitoring system is necessary to detect even the first small pest colonies. Also for the very detrimental virus transmission, slowing down the dispersal of the vector can substantially limit the spread of the pathogen.

In conclusion, the present study significantly contributes to understand the dispersal behaviour of thrips within a protected greenhouse system after accidental immigration and/or infestation. Particularly, understanding the dispersal behaviour of thrips under the UV deficient conditions will forward the development of proper additional control methods for such greenhouses.

3 Effect of UV reflecting mulch film on the immigration of *Ceratothripoides claratris*

3.1 Introduction

Coloured plastic mulches with different optical characteristics (transmittance and reflectance of light) have been widely used in agricultural and horticultural practices for specific purposes, e.g. to improve the micro-environmental conditions (soil temperature) around the crop stand promoting for plant growth and productivity, to control weeds, and to prevent pathogen propagation and insect immigration (see Reitz et al. 2003, Heissner et al. 2005).

The effect of mulches is based on their properties to influence transmittance and reflectance of the incoming incident light. Black and transparent mulches absorb most of the incoming light, consequently heated up the soil underneath; whereas an opposite effect was recorded with white mulches, which strongly reflect most of the long wavelength light (e.g. Diaz-Perez and Batal 2002, Heissner et al. 2005, Johnson and Fennimore 2005). Regulation soil temperature provided optimum conditions for root system development and consequently promoted plant growth (Diaz-Perez and Batal 2002). Reflectance of selected wavelengths, e.g. increasing far-red/red ratios and blue light, can lead to an increase in the quantity of photosynthetic light, resulting in increasing plant growth and yield/total weight of the product (Antonious and Kasperbauer 2002, Kasperbauer and Loughrin 2004, Glenn and Puterka 2007). The heat built up under the black mulch and the absorbance of this mulch type to incident light were also well known in suppressing the growth of weeds (Ngouajio and Ernest 2004, Johnson and Fennimore 2005, Radics et al. 2006). Last but not least, the equalisation of the colour and brightness contrast between plants and the ground caused by mulches (Döring et al. 2004) confused herbivorous insects during the host plant selection and recognition process; and excessive reflection of incident light from mulches, especially the UV light portion repelled them from visiting and alighting on plants (Kring 1972, Terry 1997, Summers et al. 2004).

Thus far repellent effects to insects were most widely reported for metalized (aluminium) or silver mulches, which were attributed to the increased reflection of the short wavelengths in the range 350 to 390 nm (Terry 1997). In few other papers, repellent effects of mulches to insects were also reported for white and other colour mulches (green and yellow), which were said to reduce the contrast between crop and background (Döring et al. 2004). Several insect herbivores were repelled from immigration into crop stands by metalized and silver mulches, including thrips such as *F. occidentalis*, *F. fusca*, *F. tritici* and *T. tabaci* (Greenough et al. 1990, Stavisky et al. 2002, Riley and Pappu 2004); aphids such as *A. gossypii*, *M. persicae*, *Brevicoryne brassicae* and other aphids species (Wilson 1999, Brust 2000, Döring et al. 2004, Summers et al. 2004); and whiteflies such as *B. argentifolii* (Summers and Stapleton 2002a, Summers et al. 2004). Consequently, insect-borne virus infections were also reduced in accordance to the restriction of insect vectors. On the other hand, in many studies reflective mulches failed to protect crops (e.g. Csinszky et al. 1995, Van Toor et al. 2004). Apart from the light reflective properties of the mulch materials, the efficacy is dependent on many factors such as insect behaviour, weather as well as the crop morphology and its growing characteristics.

So far, most of the studies dealing with repelling effects of reflective mulches to invasive insects have been carried out in the open fields. In this study, reflective silver mulch was tested to reduce the immigration pressure and crop infestation of *C. claratris* in netted greenhouses, aiming to enhance the anti-thrips effect of netted greenhouses. Improving the repellent properties of the greenhouse environment may allow increasing the net's porosity for proper ventilation during hot periods.

3.2 Materials and method

The silver/black mulch (Ginegar Plastic Product Ltd, Kibbutz, Israel) was designed to cover the floor inside and/or outside the greenhouses. The greenhouses was clad with UV transmitting plastic film (PE-1A, RKWAG, Worms, Germany) and net (40 mesh, Econet M, Ludvig Svensson, Sweden). Greenhouses' inner floor was entirely covered with either mulch colour surface, e.g. silver or black. The floor outside the greenhouses was either covered with silver mulch in a width of 1 m from the net walls or left with bare ground. The combination of the inside and outside greenhouse floor

mulching resulted in four arrangements tested in this study, i.e. (i) a greenhouse with black mulch inside, (ii) a greenhouse with silver mulch inside, (iii) a greenhouse with black mulch inside and silver mulch around, and (iv) a greenhouse with silver mulch both inside and outside around the greenhouse (Figure 25, and figure 26).

The spectral reflectance properties of the plastic mulches were measured in the laboratory using a PerkinElmer lambda 900 UV/VIS/NIR spectrophotometer (PerkinElmer Life and Analytical Science, Boston, MA) (Figure 27). At the greenhouse site, the amount of incident UV from solar radiation was recorded using UVA and UVB sensors (Indium sensor, Neuenhagen, Germany) installed 50 cm upwards towards the sky; whereas these sensors were turned around at the same position facing downwards to measure the UV reflectance from the mulches both inside and outside greenhouses (Figure 26). Data from sensors were automatically transferred to and recorded in a low-power consumption data-logger for every 5 minutes (Institute of Horticulture and Biosystem Engineering, Leibniz University Hannover). Amount of reflected UV light was calculated as a proportion of the incident UV light.

Each greenhouse contained thirty three week old tomato plants. Thrips infestation and population growth was recorded weekly after planting for one month on ten randomly selected plants. The number of adults and larvae were counted separately and directly on plants.

Experiment was repeated three times, i.e. from February to March, from April to May, and from June to July, 2007.

Statistical analysis

Data were first subjected to UNIVARIATE test and HOVTEST = LEVENE option of SAS version 8 to check distribution of the population and homogeneity of the variance. Since the normal distribution assumption was violated, data were subjected to nonparametric test option in SAS. In case of a significant difference between greenhouses, the means of number of thrips on tomato plants in different greenhouses were compared using Tukey option in ANOVA test. The significant value was set at $P < 0.05$.

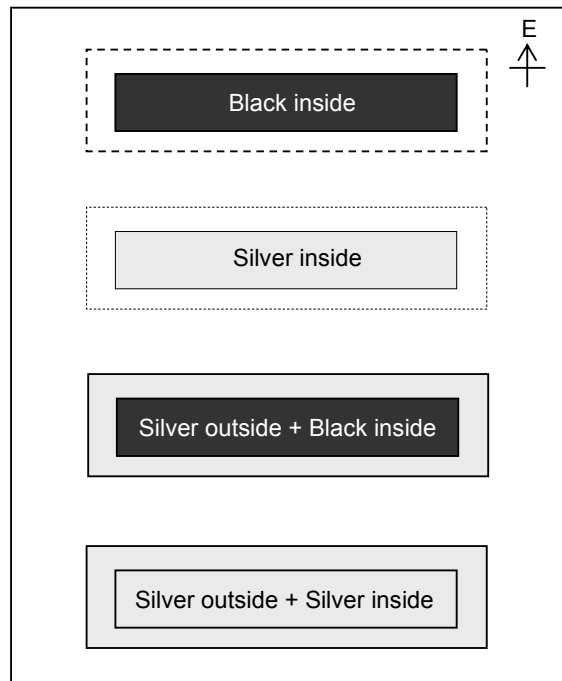


Figure 25. Experimental design to study the effect reflective silver plastic mulch on the immigration of *C. claratris* into greenhouses.



Figure 26. Experimental setup: Position of UV sensors (left), greenhouse and mulch arrangement from outside (middle), and inside (right).

3.3 Results

3.3.1 Light reflectance of the mulches

The silver mulch used in this experiment reflected up to 30% of the UV (from 250 to 400 nm) whereas only about 6% of the UV was reflected from the black mulch when reflectance was measured in the laboratory (PerkinElmer Lambda 900 UV/VIS/NIR spectrophotometer) (Figure 27). However, the percentage of UV reflectance was lower when measured with UV sensors facing downwards at 50 cm above the mulch at the greenhouse site. The highest reflectance proportion of UV was measured above the silver mulch inside the greenhouse, accounting for about 11% UVA (Figure 28) and 13% UVB (Figure 29). An almost consistent amount of UV light was reflected from the same silver mulch type arranged either inside or outside greenhouse regardless of a much higher incident UV intensity measured outside than inside greenhouses (Figure 30 and Figure 31). Consequently, the relative percentage of UV reflection from this mulch type outside greenhouse was accounted for only about 5%, which was as low as that from the black mulch arranged inside greenhouse.

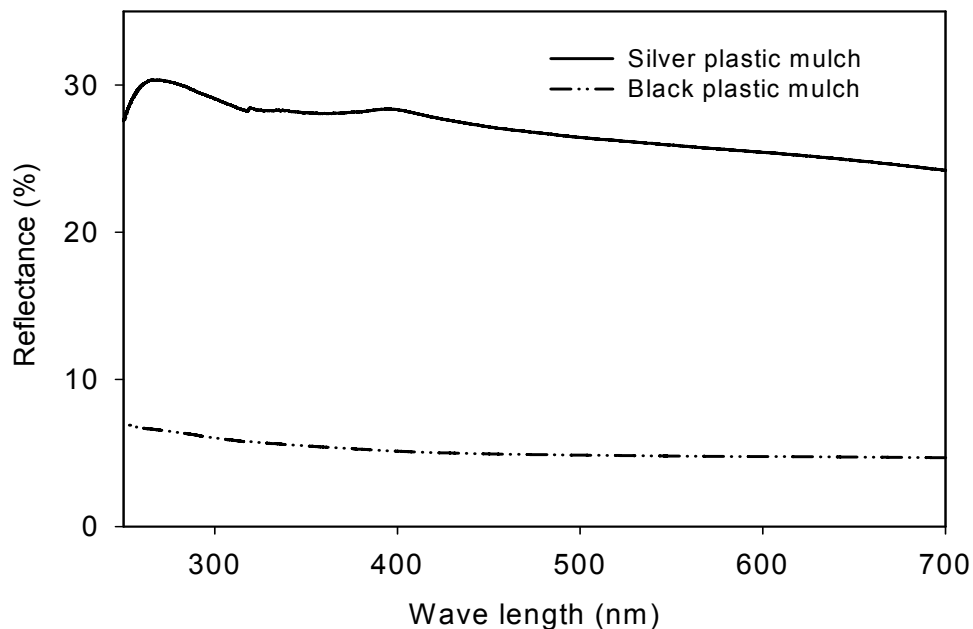


Figure 27. Spectral reflectance of reflective silver mulch from the silver and black sides (Ginegar Plastic Products Ltd., Israel) measured with a PerkinElmer Lambda 900 UV/VIS/NIR spectrophotometer.

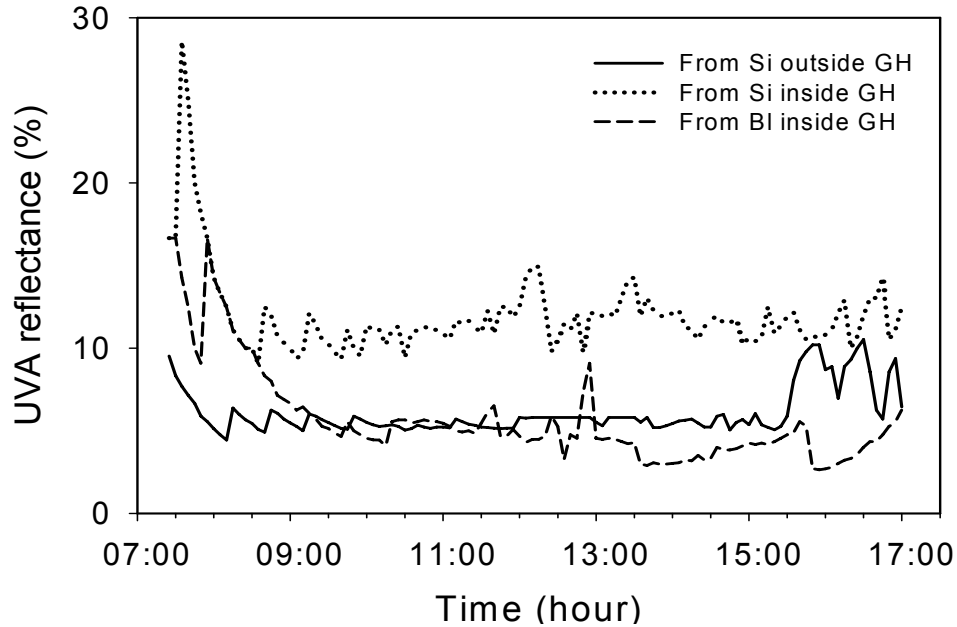


Figure 28. Percentage reflectance of UVA intensity in relation to the incident UV intensity at the same measuring sites from different mulch types outside and inside greenhouse (GH) (data from inside and outside greenhouses were taken on two different days and plotted as example). Si: silver mulch, BI: black mulch.

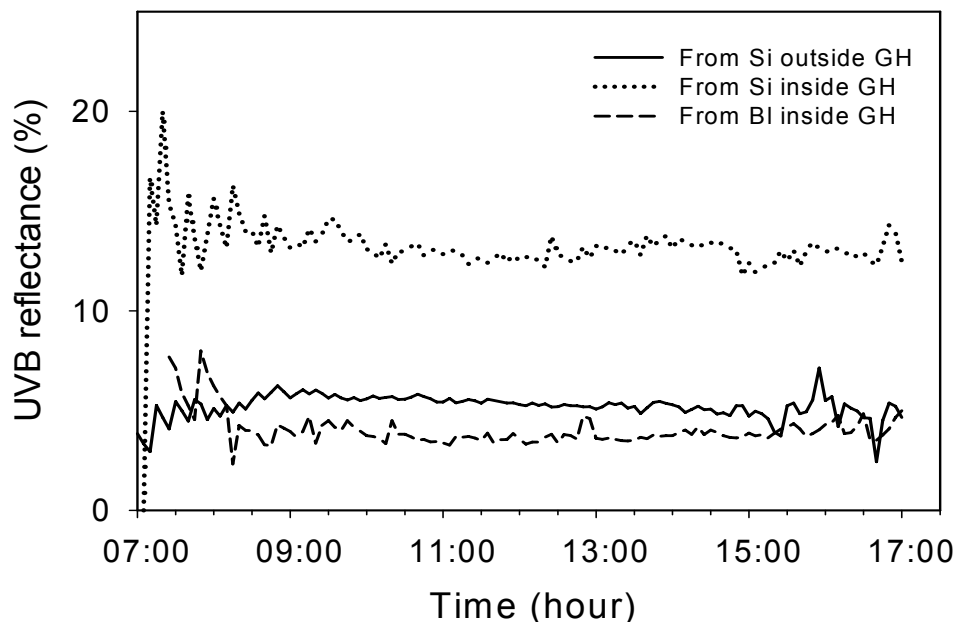


Figure 29. Percentage reflectance of UVB intensity in relation to the incident UV intensity at the same measuring sites from different mulch types outside and inside greenhouse (GH) (data from inside and outside greenhouses were taken on two different days and plotted as example). Si: silver mulch, BI: black mulch.

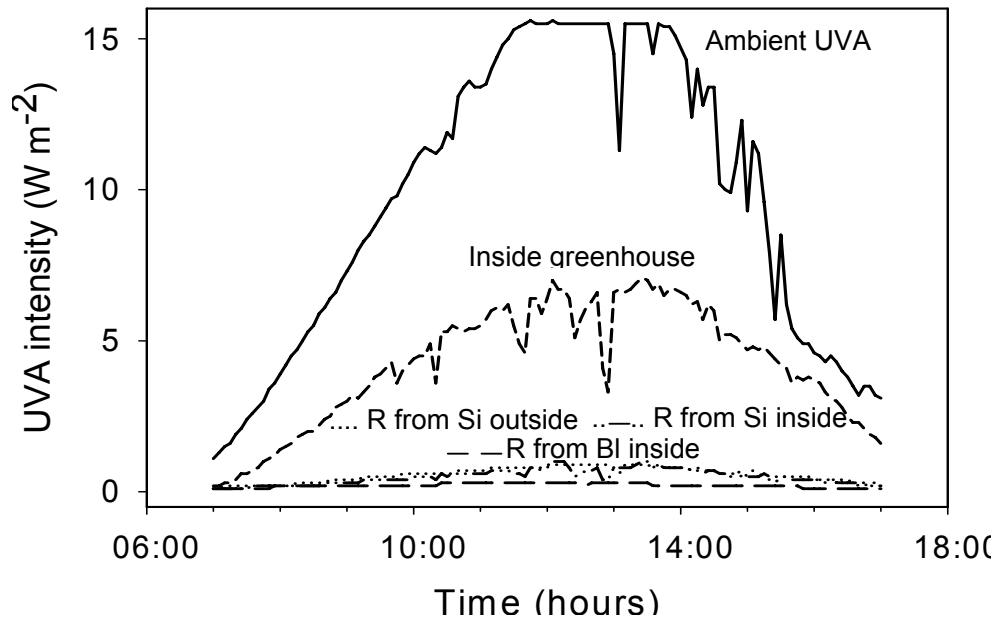


Figure 30. UVA intensity from the incoming light and the reflection (R) from different mulch types outside and inside greenhouse (data from inside and outside greenhouses were taken on two different days and plotted as example). Si: silver mulch, BI: black mulch.

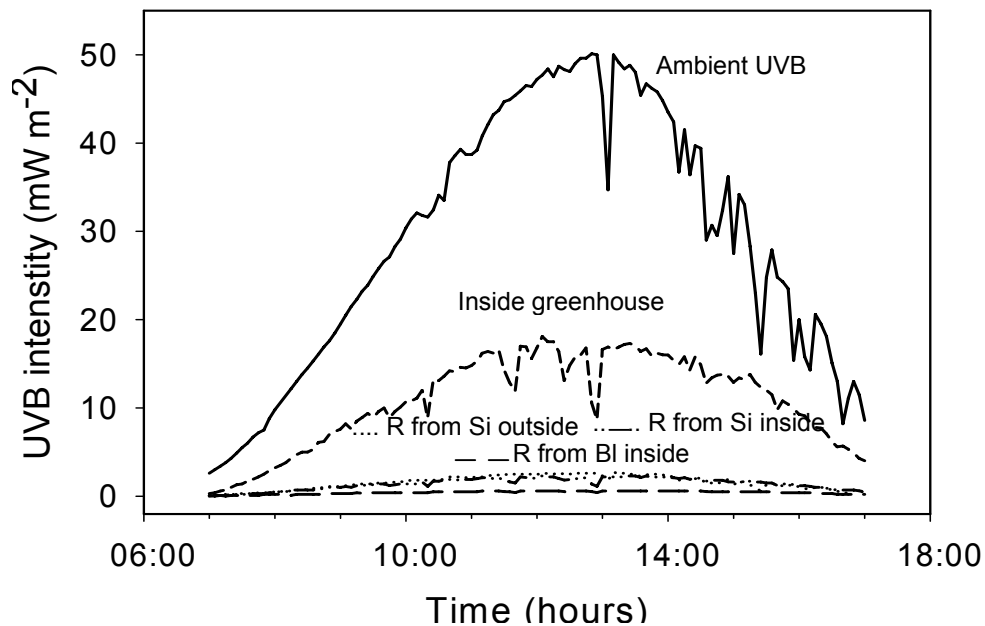


Figure 31. UVB intensity from the incoming light and the reflection (R) from different mulch types outside and inside greenhouse (data from inside and outside greenhouses were taken on two different days and plotted as example). Si: silver mulch, BI: black mulch.

3.3.2 Immigration and population development of *Ceratothripoides claratris* inside greenhouses with different mulching designs

Replication 1 (from February to March, 2007). All greenhouses equipped with silver mulch delayed the immigration and limited the population abundance of *C. claratris* (Figure 32). In the conventional greenhouse with only black mulch inside, all plants were already infested 3 weeks after planting, whereas infestation rates reached 50% not before 4 weeks after planting in greenhouses mulched with silver mulch either inside or outside. In the greenhouses with silver mulch, thrips infestation rate was at most retarded when this mulch type covered either the greenhouse floor inside or was arranged as floor surrounding outside, however the difference to the greenhouse covered both inside and outside with this silver mulch was not significant. Regarding the thrips densities per plant, abundances of all determined developmental stages were significantly lower in silver mulch equipped greenhouses than in the greenhouse with black mulch inside (e.g. $\chi^2 = 13.57$, $P = 0.0036$ for adult; $\chi^2 = 18.38$, $P = 0.0004$ for L1; and $\chi^2 = 19.82$, $P = 0.0002$ for L2; $df = 3$, at 5 weeks after planting) (Figure 32B, C, and D).

Replication 2 (from April to May, 2007). Again, the infestation rate of the plants in the greenhouse with silver mulch outside was slowest among the greenhouses tested. 50% of the sampled plants were infested with thrips in this greenhouse 3 weeks after planting compared to about 80% infested plants in other greenhouses (Figure 33A). Although a higher infestation rate was found in the greenhouse mulched with silver plastic inside than the outside mulch, the differences in thrips densities in these two greenhouses were not significant. Thrips abundance in these two greenhouses was significantly lower than that in the greenhouse with black mulch inside (e.g. $\chi^2 = 16.8$, $P = 0.0008$ for adult; $\chi^2 = 12.61$, $P = 0.0056$ for L1; and $\chi^2 = 15.66$, $P = 0.0013$ for L2; $df = 3$, at 4 weeks after planting) (Figure 33B, C, and D). Although the greenhouse with silver mulch both inside and outside also moderately limited the immigration of thrips, no significant difference was found between thrips population inside this greenhouse and that in the normal greenhouse with only black mulch inside.

Replication 3 (from June to July, 2007). Infestation rate was lowest in the greenhouse with silver mulch inside, however not significantly different to the other treatments. Concerning thrips number per plant, the first difference between treatments became obvious not before 4 weeks after transplanting (Figure 34B, C, and D). Significant difference were only found with L2 larvae between the greenhouse mulched with silver plastic inside and that with black mulch inside ($\chi^2 = 11.21$, $P = 0.01$; $\chi^2 = 12.28$, $P = 0.0065$, $df = 3$, for 3 and 4 weeks after planting, respectively) (Figure 34D).

3.3.3 Virus infection

Until 5 weeks after planting, almost no plant in the treatments with silver mulch inside or outside of the greenhouses was infected with virus except 10% of the plants in the greenhouse with this mulch type outside in the second replication (Table 7). The most severe virus infection was consistently recorded on the plants in the greenhouse with only black mulch inside, accounting for 20 – 30% infected plants. The infection in treatment with silver mulch both inside and outside varied from 0 to 20%.

Table 7. Incidence of virus infection (CaCV) (%) in tomato plants in greenhouses with different mulch arrangements in 2007 at 5 weeks after planting

Greenhouse	Virus incidence (%)		
	Replication 1 (Feb. - March)	Replication 2 (April - May)	Replication 3 (June - July)
Bl inside	30	30	20
Si inside	0	0	0
Si outside & Bl inside	0	10	0
Si inside & outside	0	10	20

(Note: Bl: Black mulch, Si: Silver mulch)

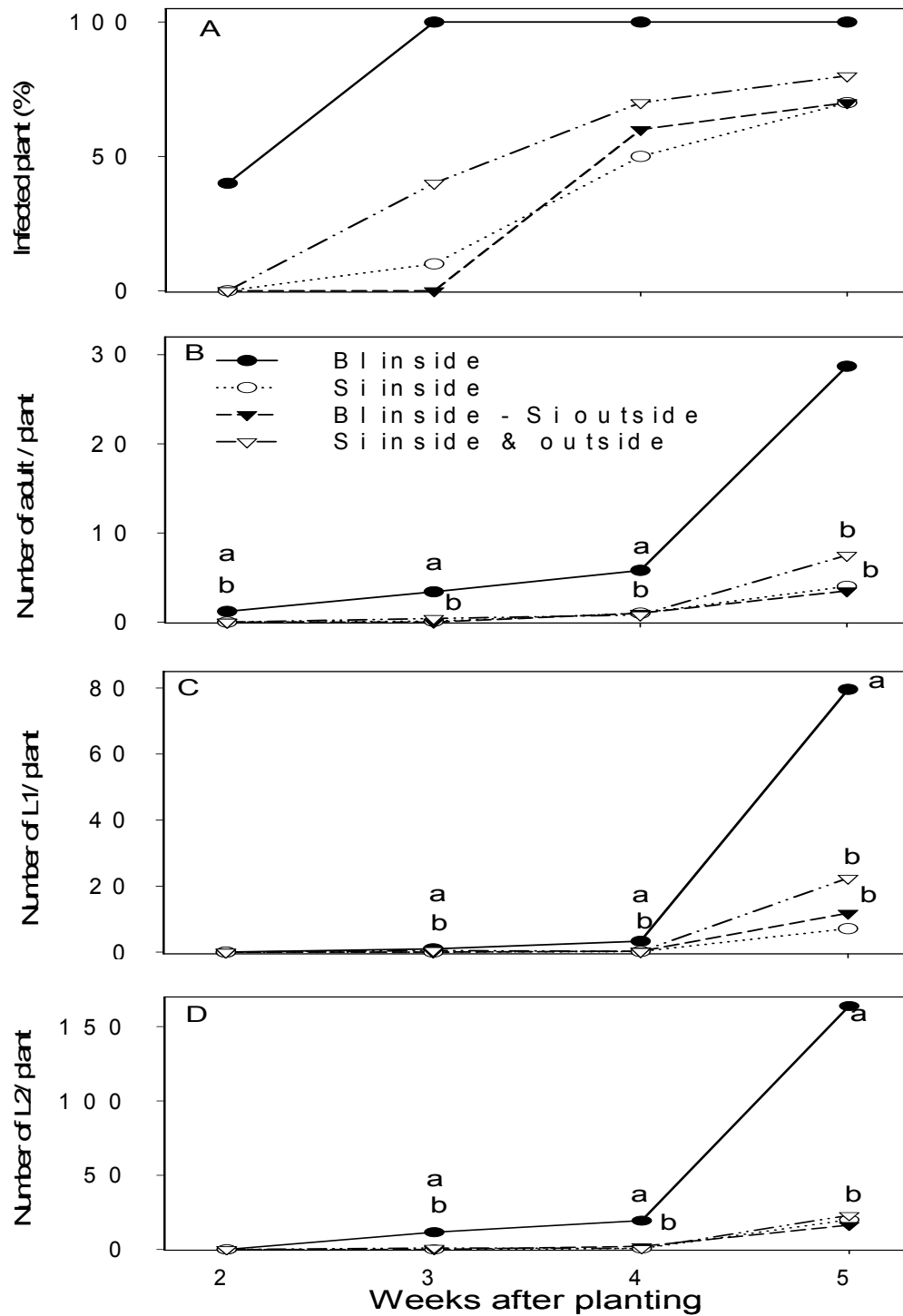


Figure 32. Plant infestation (A), population development of *C. claratris* adults (B), first larval stage (C) and second larval stage (D) on tomato inside different mulching greenhouses in replication 1. Mean number of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter is not significantly different (Tukey's test, $P < 0.05$). (Si: Silver mulch, Bl: black mulch).

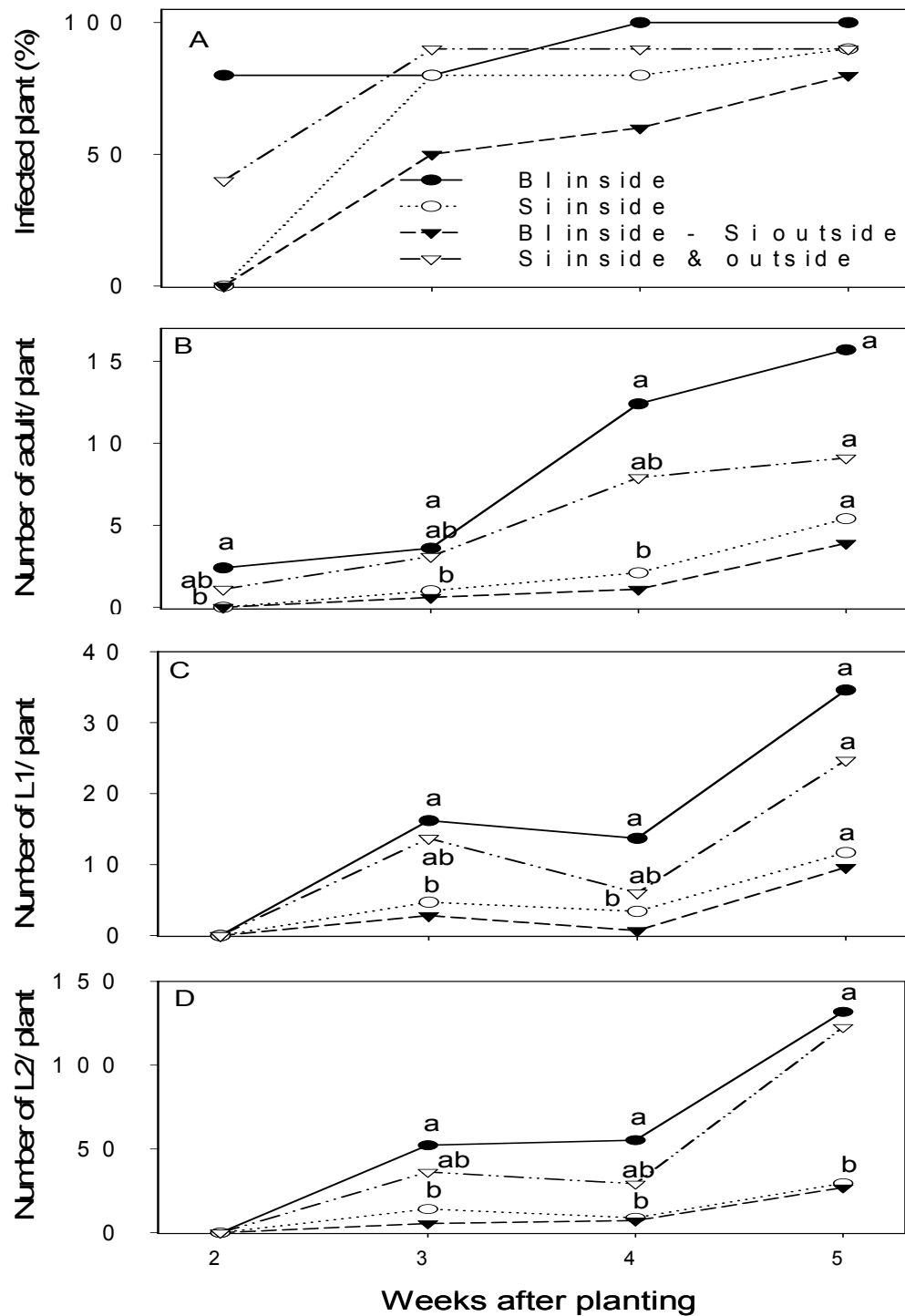


Figure 33. Plant infestation (A), population development of *C. claratris* adults (B), first larval stage (C) and second larval stage (D) on tomato inside different mulching greenhouses in replication 2. Mean number of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter is not significantly different (Tukey's test, $P < 0.05$). (Si: Silver mulch, Bl: black mulch).

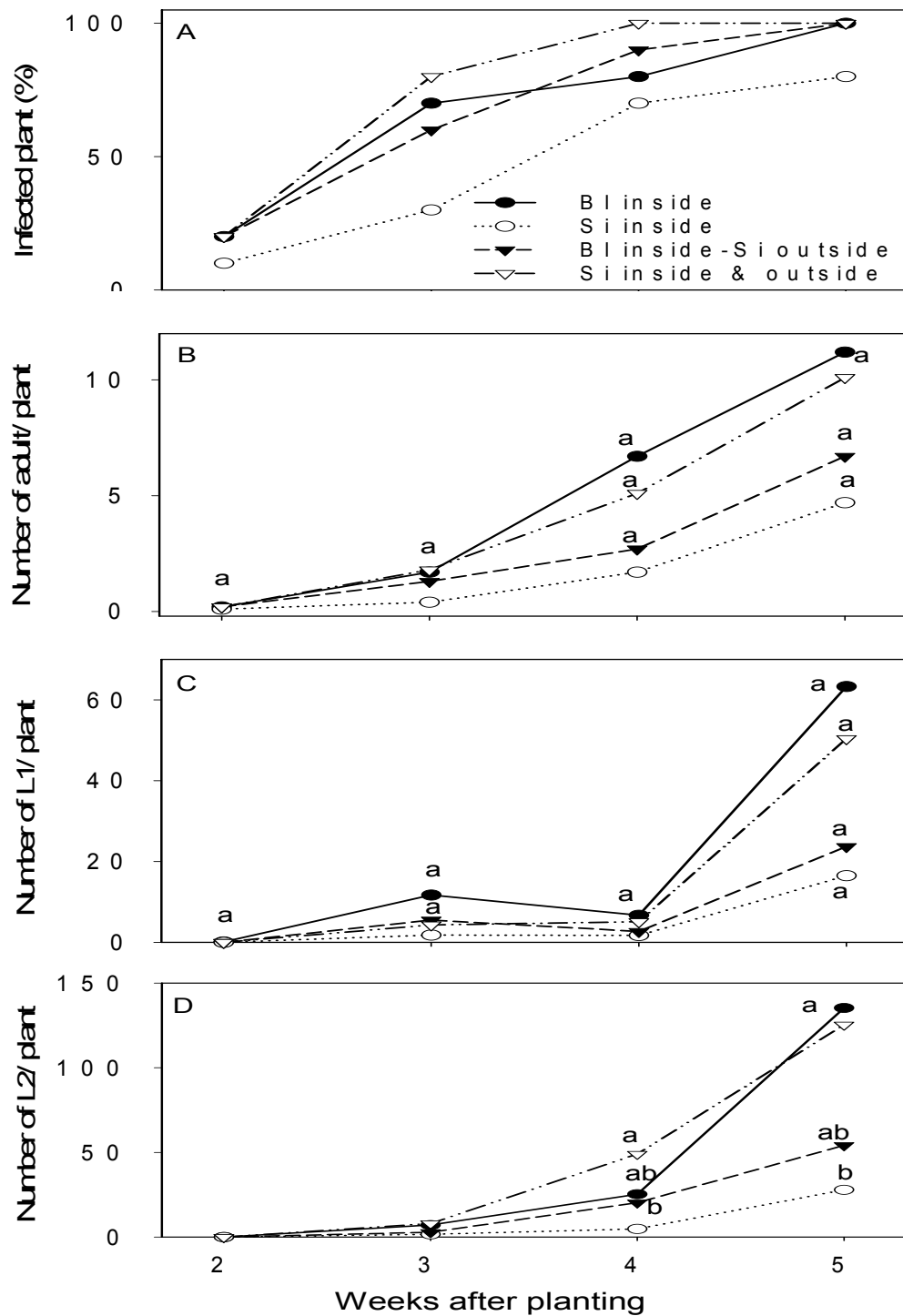


Figure 34. Plant infestation (A), population development of *C. claratris* adults (B), first larval stage (C) and second larval stage (D) on tomato inside different mulching greenhouses in replication 3. Mean number of each developmental stage of *C. claratris* per plant in each observation week followed by the same letter is not significantly different (Tukey's test, $P < 0.05$) (Si: Silver mulch, BI: Black mulch).

3.4 Discussion

Coating of the ground inside or around greenhouses with reflective silver mulch delayed the infestation with the thrips, *C. claratris*, and significantly limited its population development on tomato plants inside the greenhouses compared to conventional greenhouses equipped with black mulch inside. Subsequently, the infection with thrips vectored CaCV virus was also postponed in relation to thrips infestation pattern. However in absolute terms, substantially high thrips infestation rates were still recorded in these greenhouses, especially towards the later period in the experiments. Hence, the reduction in thrips immigration caused by the silver mulch is not sufficient to protect the tomato crop for a whole production period; however, the considerable delay in infestation opens capabilities to reduce pesticide treatments or to improve efficiency of biological control agents.

It is widely acceptance that the excessive reflectance of UV radiation from metalized, silver and other reflective mulches confuses the incoming insects and reduces the incident of their alighting on plants (e.g. see Kring 1972 and therein citations, Terry 1997, see Summers et al. 2004, Döring et al. 2004). Kring (1972) reviewed the flight behaviour of aphids in response to the enhanced light reflectance areas by reflective mulches and summarized that aphids may fly approaching these areas but fly along the edge border or over the areas, refuse to alight on plants and finally fly away. According to Terry (1997) and Van Toor et al. (2004), the reflectance of 30% of the UV spectrum, which we measured at the surface of the silver mulch used in this study in the lab, or only 11 and 13% for UVA and UVB, respectively, as measured at 50 cm above the greenhouse exposed mulch will not strongly deter insects from crop immigration. The comparatively low but distinct repellent effects of the silver mulches to *C. claratris* in this study may not only have resulted from the reflection of UV but also the visible light portion (range from 400 to 700 nm) of the incoming incident light (see Kring 1972, Terry 1997, Döring et al. 2004). The increased brightness (or increased reflectance of the long wavelengths in the incoming incident light) may affect the contrast in the brightness of reflected light between the target (plant) and the background (mulch) (Kring 1972, Döring et al. 2004). This effect was also assumed to trigger the reduction of the immigration of insects as recorded on white (Kring 1972, Döring et al. 2004), green and yellow mulches (Döring et al. 2004) and

straw mulches (Döring et al. 2004, Summers et al. 2004). More than 25% of visible light was also reflected from the silver mulch used in this study. A similar consistent reflection of the visible light portion to the UV portion of the incident light was also measured on other metalized mulches (Summers et al. 2004, Kigathi 2005) and silver mulches (Döring et al. 2004). However, in most of the cases the repellent effect of these mulch types to insects was only discussed in the aspect of reflection of UV portion. Hence, the effect of enhancement brightness from the ground against insect immigration still needs to be validated by further research.

The unequal light reflectance from different mulch arrangement areas (Terry 1997) also explained the decrease in thrips infestation when reflective silver mulch was used either inside or around the greenhouses compared to black mulch inside. However, the reason for the less efficient treatments with reflective silver mulch placed both inside and outside of the greenhouse compared to other arrangements (though not significantly different) remained unexplained.

Regardless of the low repellent effects of reflective silver mulch to thrips in this study, the results corroborate previous findings on deterrent effects of reflective mulches to various insect species on different crops. The effects varied from high to moderate depending on the reflectance properties of the mulches and plant morphology. Most of the studies reported high repellent effects of reflective mulches early in the crop season, which diminished with progressing crop growth. The full expansion of the leaves and plant canopy late in the cropping season limited the ratio of the exposed reflective mulch area to the attractive plant surface, thus limiting the repellent effect of the mulch to insect immigration. In most of the cases, like in our study, reflective mulches only delayed the colonization and limited the population growth of herbivorous insects, but did not completely prevent crop infestation. Significant less number of thrips, *F. occidentalis*, chose to fly to a tunnel with ground covered by metallic mulch compared to that covered with black mulch when given a choice (Kigathi 2005). The use of silver and metallic reflective mulches has also significantly reduced thrips populations of *F. occidentalis*, *F. fusca*, *F. tritici* and *T. tabaci* tomato fields compared to un-mulched fields with bare soil (Greenough et al. 1990, Stavisky et al. 2002, Riley and Pappu 2004). Similarly, population built up of several species of the genus *Frankliniella* spp. on sweet pepper was delayed in the early season

when using reflective mulch, but not later when the plants were full-grown (Reitz et al. 2003). Significantly less aphids and thrips were also found on vegetables grown on silver and metallic reflective mulches compared to bare soil (Greer and Dole 2003). Reflective plastic mulches have also significantly delayed the colonization of the whitefly *B. argentifolii* and the aphid *A. gossypii* in zucchini compared with white mulch treatments (Summers and Stapleton 2002a, Frank and Liburd 2005). The repellence to these two insect species caused by metalized mulch was as effective as synthetic insecticide treatment (Summers et al. 2004). Likewise, the leafhopper, *Dalbulus maidis*, on sweet corn was controlled better by reflective mulch than with an insecticide treatment (Summers and Stapleton 2002b). The colonization and landing of alate aphids on pumpkin, cantaloupe and lupin was significantly inhibited by UV reflective mulching compared to black mulch or bare soil field (Jones 1991, Brust 2000, Summers et al. 2004). Several aphid species including *B. brassicae*, *M. persicae* were strongly deterred from traps exposed on white and silver backgrounds compared to uncovered and other colour backgrounds (Wilson 1999, Döring et al. 2004). These authors described a significant negative correlation between UV reflectance and aphid abundance. Andino and Motsenbocker (2004) also reported a stronger repellent effect of silver reflective mulch to cucumber beetle in watermelon than bare soil cultivation. The only attempt to incorporate UV reflective mulch to a greenhouse, i.e. at the near entrances and vents of the greenhouse, also resulted in the reduction of thrips immigration into that greenhouse (McIntyre et al. 1996).

However in other studies, the slight repellent effects of reflective mulches observed early in the crop season, which disappeared with progressing crop development, were stated to be insufficient as the only pest control method. The early suppression effect of UV reflective mulch to the colonization of onion thrips, *T. tabaci*, up to 8 weeks after sowing, did not result in a successful control of thrips population later compared to bare soil field (Van Toor et al. 2004). Populations of aphids, thrips and whiteflies on tomato were also only significantly reduced on aluminium mulch in the early crop season compared to other colour mulches but not later (Csinszky et al. 1995). Similarly, New Zealand flower thrips on nectarine was insufficiently controlled using reflective mulch (McLaren and Fraser 2001). The high density of crops and plant morphology were attributed to the failure of the protective effect of the reflective

mulches, especially at the late crop season when leaves were fully developed and covered the mulches and thus shaded the reflective area of the mulches (Kring 1972, Van Toor et al. 2004).

Regarding insect vectored plant virus, most studies showed that reflective mulches did not completely prevent the colonization of insects to field crops; however, it was argued that the delay of insect immigration and the suppression of population abundance at the beginning of the crop season can be quite important to reduce the damage of insect borne virus infection and thus increase the crop yield significantly (Jones 1991, Csinszky et al. 1995, Brust 2000, Stavisky et al. 2002, Stapleton and Summers 2002, Summers and Stapleton 2002a,b, Summers et al. 2004, Riley and Pappu 2004). Silver and white mulches were also reported to prohibit the incidence of the development of tomato spotted wilt virus (TSWR) in tomato independently from vector infestation compared to black mulch. This effect was attributed to the reduction of root area temperature under the silver and white mulches, which resulted in a stronger development of the plant and thus more resistant to development of virus diseases (Diaz-Perez et al. 2007).

In conclusion, although the reflective silver mulch deposited inside or around netted greenhouses did not strongly prevent immigration of thrips in our study, the reduced infestation and abundance of thrips on tomato grown on this mulch type opens a promising potential for thrips management tactics in IPM aiming at reducing the use of insecticides. The efficiency of reflective mulches may be improved when mulches with higher reflectance properties of the UV range are used. In addition, white mulch was also many times reported with a similar repellent effect to insects as UV reflective mulches, hence testing this mulch type with netted greenhouse may provide other promising alternative control method for *C. claratris*.

4 General discussion

Protected cultivation systems gain more and more interest of the growers for growing high quality crops because of their promising perspectives in protecting plants from adverse environmental conditions, and optimising the use of resources such as inputs like water and nutrients, and land. Protected systems allow the highest possible production intensity of convenient and high value crops. Greenhouses also provide barriers to prevent the immigration of herbivorous insects, thus opening venues to reduce pesticide treatments and offering much better opportunities for Integrated Pest Management (IPM) based mainly on Biological or Biotechnical Control options. However, from a technical point of view in tropical regions protected cultivation systems encounter the risks of heat overload combined with high humidity as a main and permanent problem. Hence, efficient ventilation is a prerequisite, and in contrast to temperate regions with dominance of closed greenhouse construction made of glass or plastics, in the hot regions of the world greenhouses are preferentially constructed with sidewalls clad with porous net material or as partially opened tunnels. However, such netted greenhouses are vulnerable for the immigration of herbivorous insects, particularly small sized mobile insects such as thrips, which can penetrate large net holes. Thus, the physical barrier function of such constructions is often inefficient to protect the crop from pest damage and other control methods are still indispensable to manage such insects. For a sustainably horticultural practice, however, pest control tactics that are environmentally friendly such as biological agents, pest behaviour manipulation or bio-rational insecticides should be set as priority. A physical method that manipulates the optical orientation of insects can affect their flight and dispersal behaviour. Such attempts recently attended more and more interest of researchers and seem to have a promising potential as alternative pest control method in protected cultivation systems. This latter approach was the main objective of this study aiming to promote the IPM in protected cultivation systems in the tropics.

Insects with optical orientation, in particular those related to plants as food source, are known to have sensitive photoreceptors in their compound eyes for a few limited intercepts ("colours") of the wavelength spectrum of incident solar radiation, including UV, blue and green; among these, the UV irradiation ranging from 350 to 390 nm is

most important for insect orientation and host location. The alteration of the UV portion of the incident light was presumed to optically confuse herbivorous insects during recognition of the host and repel them from visiting and alighting on plants. Modification of the UV was attempted in greenhouses and tunnels using UV absorbing cladding materials for control of insect immigration. Plastic film and net materials impregnated with UV absorbing additives can filter the transmittance of UV. On the other hand, reflective metalized and silver mulches covering the field ground can intensively reflect ambient light with a disproportional high proportion of UV in the spectrum. Both filtration and excessive reflection of the UV were proved to affect the visual orientation of several herbivorous insects. Integration of these materials to conventional greenhouse covering materials may supplement the mere physical barrier function against insects and thus may allow using large porous net materials or partial opening vents for a better ventilation efficiency in the hot regions.

Based on these backgrounds, UV absorbing plastics and nets were compared with conventional UV transmitting materials covering greenhouses to study the impacts on immigration and flight behaviour of insects under the tropic conditions in central Thailand. A thrips species, *C. claratris*, recently described as a most devastating pest to greenhouse tomato in central Thailand because of its intensive propagation in hot microclimate and intensive plant damage both by direct destruction of leaf area ("feeding scars") and by transmission of the very detrimental tospovirus CaCV, was selected as a kind of indicator organism for all experiments. Impacts of UV light filtration on the immigration, population dynamics, dispersal as well as virus association and transmission of this thrips species were comprehensively investigated. These investigations were conducted following the initial promising results of using UV absorbing greenhouse covering materials to prevent the immigration of the same thrips species in the region presented by Kumar and Poehling (2006). In addition, possible repellent effects of a reflective metalized film (mulch), which had already been demonstrated in field crops, were studied by equipping small experimental greenhouses with different arrangement of a ground covering reflective mulch either inside and/or outside the greenhouse constructions in comparison to a conventional greenhouse ground cover (black mulch).

Several preceding studies have proven a distinct avoidance behaviour of insects, including thrips, whiteflies, and aphids, to areas ("rooms") where UV was fully or

partially excluded. This was mainly confirmed by choice experiments where the tested organism could select between environments (flight chambers) with different amounts of UV intensity (e.g. Kigathi 2005, Costa and Robb 1999, Costa et al. 2002, Mutwiwa et al. 2005, Doukas and Payne 2007a). A similar behaviour was obviously observed in thrips, *C. claratris*, in the here presented study (for detail see section 2.4).

The deterrent effect of UV exclusion was further proved in commercial growing tunnels even though the entrances or sidewalls were opened occasionally or during the whole cultivation period to enhance the ventilation (Antignus et al. 2001, Costa et al. 2002, Kumar and Poehling 2006). The initial infestation of the crop by the pest was significantly delayed; consequently the insect abundance was also reduced relatively to the conventional greenhouses, which received 'normal' high amount of UV intensity. As a consequent, virus infection rate also markedly decreased. Overall, in relation to a much slower and lower total infestation intensity with reduced feeding damage and limited virus infection, a much better long-term performance of the plants and thus higher production efficacy could be expected with very limited or even no additional use of insecticides.

The combination of UV absorbing net at the sidewalls and UV absorbing plastic covers like that of the here studied greenhouse designs can be a reasonable compromise for the conflicting issues: ventilation and pest control. Even 50 mesh nets can ensure a strong prevention of thrips immigration in greenhouses if clad with UV absorbing materials (for detail see section 2.5). Plants in this greenhouse type could be efficiently protected over the whole cultivated period if the doors were carefully kept close and all appropriate sanitation procedures were followed to avoid the artificial introduction of thrips, e.g. by workers, plant materials or infested labour tools. The integration of UV absorbing properties to both plastic covers and net sidewalls made the 50 mesh netted greenhouses as effective in control of thrips as 78 mesh or even tighter nets, which could exclude thrips by their physical properties. Such extremely tight nets, however, are incapable under the tropic conditions as extreme heat and humidity accumulation is generated inside the greenhouses, which strongly affects the plant performance (Harmanto et al. 2006, Mutwiwa 2007).

The insect repellent potential of greenhouses is related to the intensity of UV exclusion. This was obvious from the comparison of different net/roof plastic combinations, which highly recommends for the combination of roof film and sidewall net types with UV absorbing properties. However, the greenhouse with only UV absorbing additive in the plastic roof may also sufficiently protect the crop from thrips but only if the immigration pressure (population density) from outside is low and the proportion of viruliferous thrips is small.

Opening the front and rear end doors (1 x 2 m) of the greenhouses has significantly impaired the preventative effect to thrips in this study (for detail see section 2.5). Same experience was already described by Kumar and Poehling (2006). However, under these favourable barrier-free immigration conditions, the construction still exhibited a distinct protective effect if clad with both UV absorbing plastic and net. The infestation was then limited to plants at the opening entrances and thrips only slowly spread towards the centre of the greenhouse (for detail see section 2.5). Obviously, the dispersal of thrips under the UV blocking conditions was restrained compared to UV rich conditions. The spread limiting effect of UV poor conditions could be clearly proved with artificial selective releases of thrips at distinct release points (for detail see section 2.7). The results supported previous findings by Antignus et al. (2001), Mutwiwa et al. (2005), and Chyzik et al. (2003). Therefore, if the opening of greenhouses' entrances and vents are necessary to improve the ventilation, population built up will be very limited inside the greenhouse area and spraying of insecticides could focus on a few plants at those hot spots to prevent the slow dissemination of the pest.

The conventional greenhouses clad with UV transmitting plastic and 50 mesh net failed to protect tomato from thrips infestation. However, with a small and simple outer modification of this greenhouse design, by supplementing with an UV absorbing projecting roof construction upright to the sidewalls (out "collar"), the protective effect of this greenhouse type against the immigration of thrips could be significantly improved (for detail see section 2.6). The addition of such a projecting roof can repel the immigration of thrips nearly as effective as the UV absorbing roof covers or sidewalls of the greenhouses. Consequently, the thrips proof properties of the existing constructions can be upgraded with a small investment.

The repellent effect of reflective mulches, e.g. metalized and silver mulches, against the immigration of several insect species documented in opened field crops (e.g. Stavisky et al. 2002, Greer and Dole 2003, Reitz et al. 2003, Riley and Pappu 2004) inspired to test such measures to supplement barrier effects of netted greenhouses. However, none of the arrangements with the reflective mulch tested in this study, i.e. inside and/or outside of the greenhouses, showed a desired high efficiency in preventing thrips immigration in the long term, nevertheless a distinct delay and limitation in thrips abundance was recorded (for detail see section 3). The low reflectance of the UV portion from the mulch used could be attributed to such limited repellent effects. However, the considerable reduction in thrips infestation recorded in this study suggests that a combination of netted greenhouses as physical barriers with reflective mulches as optical repellents could be a promising attempt for thrips control. Yet, further studies with reflective mulch products that have higher reflectance properties and being more selective for the UV range are necessary to verify and improve the pest repelling properties.

In the here presented study, the manipulation of the UV radiation was studied exclusively for prevention of pest immigration into tropical greenhouses. However, possible impacts on beneficial arthropods, especially for parasitoids and predators, pollinators and antagonistic microorganisms such as entomopathogenic fungi, all with possible distribution in the same environment should not be neglected. In addition, the growth and performance of the crop itself and the product quality such as important internal compounds also need to be validated if UV manipulating systems will be implemented.

The observed UV effects on herbivorous insects are based on their specific visual di- or trichromatic system with sensitive receptors in the UV range of the light spectrum. However, this optical system is not unique to phytophagous insects but widely distributed in the insect kingdom. Hence, similar negative impacts seem to be likely for beneficial insects foraging in the same habitats as their host/prey. Like herbivorous insects, for instance many hymenopteran parasitoids also possess a di- or trichromatic colour vision system with sensitivity peaks for UV, blue and/or yellow green wavelengths (Peitsch et al. 1992). For instance, the parasitoid wasps such as *Encarsia formosa* and *Trybliographa rapae* have two visual sensitivity peaks for UV and yellow green wavelengths similar to their host insects, the whitefly,

T. vaporariorum and the cabbage root fly, *Delia radicum*, respectively (Mellor et al. 1997, Brown et al. 1998). Therefore, it seems likely that parasitoids are also affected by the modification of the light spectrum that alters pests' behaviour. Indeed, many parasitoid species were proved to prefer areas with higher UV intensities when given a choice to UV deficient environments, e.g. *Eretmocerus mundus* (a parasitoid of *B. tabaci*), *Aphidius colemani* (a parasitoid of *M. persicae*), *Diglyphus isaea* (a parasitoid of *Liriomyza bryoniae*) (Chiel et al. 2006), and *E. formosa* (a parasitoid of *T. vaporariorum*) (Doukas and Payne 2007b). Furthermore, some parasitoids lost their ability to locate and parasitize the host insects under the UV exclusion conditions, e.g. *E. mundus* (Chyzik et al. 2003), whereas many others were not affected for this specific behavioral traits, e.g. *A. colemani* and *D. isaea* (Chiel et al. 2006) and *A. matricariae* (Chyzik et al. 2003). Most researchers argued that the UV reduced conditions do more severely affect the orientation of pest insects compared to the host finding and parasitism efficiency of parasitoids. Chiel et al. (2006) discussed that parasitoids rely more on olfactory than optical cues in host finding, thus they can overcome the constraint of UV poor conditions. Doukas and Payne (2007b) proved that the light intensity (brightness) is more important for spatial orientation of *E. formosa* than the relative amount of UV radiation. However, the relative impact of UV manipulation for pest vs. parasitoids should be carefully considered if parasitoids are intended as biocontrol agents. On the other hand, many predators that are not airborne and forage more or less by random search in host patches are not reacting to UV as shown for *Chrysoperla carnea* or *Macrolophus pygmaeus* (Wulf 2007, Horscht 2007).

Studies dealing with possible side effects of reflective mulches on non-target organism are scarce. Reitz et al. (2003) showed that the enhancement of UV reflection by UV reflective mulches affected the natural abundance of the predatory bug *Orius insidiosus*, an important antagonist of *Frankliniella* spp. However, the UV enhancement did not impair the prey ability of the bugs, which were artificially released at the mulched areas. On the other hand, the total abundance and species diversity of natural enemies in field zucchini mulched with reflective plastic film did not differ from the bare ground field (Frank and Liburd 2005). However, effects of reflective mulches on the host finding and parasitisation/predatory abilities of

parasitoids and predators need much more investigations before more universal conclusion can be drawn.

Bumblebees, important pollinators of commercial crops, e.g. tomato in the greenhouses, also possess a trichromatic colour vision system. The colour vision and specific UV reflecting pattern from flowers are a prerequisite for the bees to detect flowers and consequently for pollination (Bellingham et al. 1997, Gronquist et al. 2001, Möller 2002). The elimination of UV radiation, therefore, will considerably change the characteristics of UV reflecting pattern of the flowers. However, these authors also proved that released bumblebee can quickly adapt to the poor UV conditions and further search for flowers successfully. Under UV poor conditions only the initial searching time was extended and the initial landing of bees on the flowers was interfered.

In conclusion, UV poor greenhouses will not benefit from natural immigration of pollinators or natural enemies such as parasitoids (Morandin et al. 2002). In terms of released pollinators or natural enemies, however, the greater risk in using UV absorbing cladding materials is probably not a loss of efficiency of introduced beneficials inside the greenhouse but the emigration of released organisms via opening vents. According to the UV gradient, the outside area should be more attractive especially if flowers (for pollinators) or hosts (for parasitoids) are frequent adjacent to the greenhouses.

The reason why availability of UV light plays such an important role in different insects in terms of flight initiation, flight direction, orientation in space, selection of host plants and specific flowers and even parts of flowers is still speculative in many aspects. It can be assumed that a missing stimulus for the UV receptors in the compound eyes under UV deficient conditions severely influences the ability of insects for differentiation (impression) of colours. In di- or trichromatic visual systems, like that of insects and vertebrates, colours are "mixed impressions", composed by the nervous system from interpolation of stimuli ratios of the different receptors. Non-stimulation of one receptor type results in a loss of the ability to differentiate colours, a kind of colour blindness, and brightness impressions alone merge the objects with surroundings. If this is the case, the long range navigation and colour based short distance orientation are supposed to strongly be affected. However, the restricted

dispersal of insects under these conditions suggests also an involvement of changing flight activity in general. Probably, the insects were not motivated to take off to start the dispersion flight under the UV deficient conditions and/or the flight duration after takeoff is reduced. But more investigations are needed to validate such speculations with trustful evidence.

Apart from arthropods (insect pests or beneficials) the development and efficiency of several microorganisms and nematodes can also be affected by UV manipulation, which can be an important aspect in biocontrol if beneficial are considered. UV radiation, with more pronounced effect of UVB compared to UVA, can influence microorganism and nematodes in many ways: Sporulation of fungal conidia and conidial viability as well as germination of entomopathogenic and antagonistic fungi can be affected (Fargues et al. 1997, Inglis et al. 2001, Costa et al. 2001, Paul et al. 2005); the stability of *Bacillus thuringiensis* (Falcol 1971, Sanchis et al. 1999, Tamez-Guerra et al. 2000, Ruan et al. 2004) and Baculovirus (Ignoffo et al. 1997, Shapiro and Domek 2002, Petrik et al. 2003) and the efficacy of entomopathogenic nematodes (Fujiie and Yokoyama 1998, Shapiro-Ilan et al. 2006) are reported to be diminished. Thus, the elimination of UV may promote these biocontrol agents and support and stabilize such beneficials after release.

The importance of ambient solar UV for plants is not comprehensively understood (Gitz and Liu-Gitz 2003) and still remains several open questions. Most of the so far studies were carried out to understand the effect of enhanced levels of UV, particularly UVB, on plants' performance and the change of internal metabolic compounds. Only a few studies made the effect of lowered UV to a subject of discussion. The exclusion of UV was stated to cause no or very little effect on the growth, yield, maturing time, and fresh or dry weights of plants, e.g. tomato, cucumber, pepper (Antignus 2007) and some dwarf shrub species (Phoenix et al. 2002). Even in some cases, the exclusion of UV improved both quantity and quality of production (eggplant) (Kittas et al. 2006), increased dry weight (lettuce) (Tsormpatsidis et al. 2008), promoted root length (southern beech tree) and plant height (the sedges) (Zaller et al. 2002) or number of tillers (subarctic grass species) (Zaller et al. 2002).

On the other hand, it was reported that elimination of UV, particularly UVB (290 - 320 nm), decreased the synthesis of UV absorbing pigments and compounds, i.e. anthocyanins, flavonoids and phenolic compounds (Gwynn-Jones and Johanson 1996, Santos et al. 2004, Tsormpatsidis et al. 2008). The reduction in biosynthesis of anthocyanins and pigmentation is supposed to diminish the coloration of flowers and fruits. Hence, the application of UV exclusion may not be recommended for those crops that quality is determined by pigmentation (Antignus 2007). However, it was also reported that the chlorophyll and carotenoid contents were not impaired under the UV eliminated conditions (Gwynn-Jones and Johanson 1996, Tsormpatsidis et al. 2008).

The production of flavonoids compounds was also assumed as a defence reaction of plants against UVB damage (Wilson et al. 2001, Gitz and Liu-Gitz 2003, Flint et al. 2004, Ulm and Nagy 2005) or against insects and diseases attacks (Flint et al. 2004, Ulm and Nagy 2005). The UVB induced flavonoids compounds were also sometimes mentioned as a signal for plants to regulate transpiration rates and water use efficiency in reaction to drought stress (Alexieva et al. 2001, Gitz and Liu-Gitz 2003). A synthesis of systemic proteinase inhibitor triggered by exposure to UVA and UVB was also reported as a defence of tomato in response to wounding (Stratmann et al. 2000, Ulm and Nagy 2005). Loss of defence properties and plant performance would be a severe drawback of artificial UV poor environments. However, other authors argued that under a trade off viewpoint the repellent effect of UV deficient areas to insects' immigration outweighed losses in induced plant resistance caused by a lack of flavonoid and other defence related metabolic compounds (Mazza et al. 2002, Rousseaux et al. 2004). It must be considered that most of the above mentioned UV effects on plants were achieved under laboratory conditions and artificially increased UV treatment of plants. There is still a big gap of knowledge about the importance of such effects, especially under production (field) conditions with relatively moderate manipulation in plant UV exposure.

In summary, the use of UV absorbing plastics and nets as well as reflective mulches appeared promising as cladding materials or outer ground cover around greenhouses, respectively, especially for netted greenhouses in the tropics. Such constructions can very efficiently prevent thrips immigration and internal spread and on the other hand allow good ventilation properties by using net material of

sufficient porosity or even by opening of vents. The results of this study strongly improve IPM measures for protected cultivation systems in the tropics. Presumably, the accidental introduction of pests into such a protected systems via vents or by labourers or by already infested plant material in the nursery can not completely avoid; the infestation of thrips and possible subsequent virus infection will be restricted to limited areas and/or plants (hot spots) and can then be easily controlled by a frequent monitoring and target orientated spraying of insecticides to infested niches only. Possible negative effects of UV deficient conditions on the behaviour of beneficial arthropods or the plant quality or defence potential of plants have to be considered. However, regarding the recent state of the art, it can be concluded that the high control potential of the optical barriers outweighs such adverse effects. Hence, the implementation of these UV absorbing material types for covering tropic greenhouses could be recommended although yet many aspects need to be understood in detail. Future studies should evaluate more in detail the performance of the insects under UV reduced conditions, e.g. complete life tables, behaviour of males and females, fecundity of the females, the ability of males to search for females, fertilization rates as well as host finding ability of newly emerging adults. Studies should also focus more on the multi-trophic relationships and interactions of plant, pests and natural enemies. The performance and efficacy of beneficial microorganisms and entomopathogenic nematode under the UV eliminated conditions also need to be verified. Understanding the effect of UV exclusion on the growth, yield and quality of the crop and production is also necessary.

Although the applications of tested reflective mulch did not yield really convincing results in repelling insects in this study, which is far less effective than greenhouse cladding UV absorbing materials, the delay and reduction in thrips abundance by the mulch treatments suggest a further promising tool for an IPM strategy, especially when combinations of UV absorbing cladding materials and reflective mulches are used for new greenhouse arrangements. Further experiments are necessary to examine more mulch materials with higher and more selective UV reflectance properties.

The effect of UV manipulation on the behaviour of thrips, *C. claratris*, in this study opens promising control method for others economically invasive insect species

damaging greenhouse crops in the tropics, e.g. whiteflies and aphids. Studies with multiple mixed pest systems may complement the here presented studies.

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Declaration by candidate

I, Nguyen Thi Hanh Nguyen, declare that this thesis, entitled “Control of thrips (*Ceratothripoides claratris* (Shumsher)) by using UV absorbing films, nets and reflective mulches” is an original work conducted by myself and has not been submitted for a degree in any other University.

Hannover, September 2008

Nguyen Thi Hanh Nguyen