THE 1998 BRIGHTON CONFERENCE

Pests & Diseases
Volume 2

Session 5B

UNDERSTANDING THRIPS AS PESTS AND APPROACHES TO THEIR CONTROL

Pest thrips in perspective - T Lewis

grass-dwellers affecting seed production (*Chirothrips* spp.; *Anaphothrips obscurus*) increased in prominence in Scandinavia and North America. Also in North America, species of *Thrips, Frankliniella, Scirtothrips* and *Hercinothrips* on fruit and vegetables received much attention as agro-businesses were expanded during mid-century, and this impetus has been maintained as conventional chemical control methods have gradually failed. Likewise, in southern Africa in the 1930 s. *Scirtothrips* on citrus attracted attention as this region raised production for export and in the 1950's *Selenothrips* on cocoa and cashew in the Caribbean were studied in depth. The discovery in 1927 that thrips could transmit a "spotted wilt" to plants and that it had a viral etiology widened the coverage to include thrips as plant virus disease vectors, a topic which is still evolving and expanding. In the last decade the focus of interest has again moved with the enormous increase worldwide in airborne transport of fresh flowers, fruit and vegetables, the establishment of mass plant propagation enterprises especially for greenhouse ornamentals in western Europe and North America, plus the increase in intensive vegetable production in east and south Asia. These developments have particularly facilitated the recent rapid spread of many species, especially *Frankliniella occidentalis* and *Thrips palmi*, with serious consequences to some of the most productive and lucrative agricultural and horticultural areas in the world.

Alongside changing geographical and crop interests there has been an evolution of control measures. Early in the century they consisted mainly of simple cultivations, stubble burning, regard to planting and harvesting times, and the use of crude chemicals and natural plant extracts sprayed at high volumes. These were gradually superseded by sophisticated synthetic insecticides applied at low volume by increasingly, refined equipment. Some 40 compounds are now available for use against thrips. As many pest species responded to these pesticides by developing resistance, there has been intense interest in finding ways of encouraging beneficial organisms, including fungal and nematode pathogens and a wide range of mites and insects, not least predatory thrips, either alone or more commonly as components of integrated pest management (IPM) systems. The aim has been to recombine some of the older cultural approaches with modern chemicals compatible with biological control agents and tolerant cultivars, to produce more effective and environmentally-benign control.

THE SCALE OF THRIPS DAMAGE

The status of thrips as pests differs greatly between crops and geographical areas. A few crops are attacked by the same species in widely separated parts of the world. *Thrips tabaci* is a cosmopolitan pest of onions grown between sea level and 2000 m and *Hercinothrips femoralis* may occur on bananas almost wherever they are grown. By contrast, some corps grown in different regions are infested by different species in each place. In Southern Africa *Scirtothrips aurantii* is the most harmful thrips on citrus, in California *S. citri* fills this niche, and in Florida, *Frankliniella bispinosa* and *Heliothrips haemorrhoidalis*, among others, attack citrus flowers and fruit. In East Africa *Caliothrips impurus* and *C. sudanensis* attack cotton whereas *Frankliniella fusca* and *F. tritici* cause comparable damage in North America.

Direct feeding damage to leaves, flowers and fruit caused by the ingestion of sap is most harmful in dry climates and seasons when heavily attacked plants lose moisture rapidly. In these conditions, infestation can seriously deplete yields and sometimes render crops

uneconomic. Indeed, there is evidence that some crops under water stress not only develop symptoms more rapidly than adequately watered ones, but provide thrips with more nutritious food and therefore encourage heavier infestations. On the other hand, many crops can outgrow damage to seedlings and young plants, especially in cooler climates, and eventual yield losses are small or undetectable. The overall impact of infestation of a crop thus depends on many factors: the size of the thrips populations, the plant growth stage, its vulnerability to direct feeding and oviposition damage or to virus infection, the duration of the infestation, and the suitability of weather for population growth. Generally, the more important the visual appearance of a crop, the more likely is the grower to suffer substantial economic losses. Many orchard and field grown fruits become scarred and distorted lowering their value or making them unmarketable. High-value greenhouse crops, vegetables, fruit and especially ornamentals, are particularly vulnerable because of the high capital investment and production costs involved, and the requirements for totally unblemished produce. The scale of damage can be appreciated from three viewpoints: the area of crops affected, decline in yields, and loss of revenue.

In field and plantation crops, initial infestations usually by airborne immigrants can spread rapidly to cover extensive areas. There are records of 20,000 ha of sugar cane in Taiwan being seriously damaged annually by *Fulmekiola serratus* and sometimes huge tracts of cassava in the Andean foothills of Colombia are almost totally defoliated by *Corynothrips stenopterus*. More limited infestations, but nevertheless extensive and often first affecting the edges of plantings, can spread through large arable fields (e.g. cabbages, cereals, cotton, onions, soyabean, tobacco) and plantations (e.g. citrus, coffee, tea, stonefruit) to reach densities of tens, hundreds or even thousands of millions of individuals per hectare.

Before modern control methods were available, in some years up to 80% of the citrus crop in California were spoilt by *Scirtothrips* spp. Even now in California, yield increases in navel oranges ranging from 8-25% can be obtained by controlling *Scirtothrips citri*. Considerable yield losses caused by *Thrips tabaci* to onions still occur widely despite the numerous studies made for decades on this problem; in 1988 and 1989 losses of 34% and 43% respectively were recorded in Canada.

Typically grain losses in wheat, barley and rye have been assessed as 2-10% in Europe and slightly more in North America but the economic relevance of such records depends on whether they are determined using grain numbers or weight, because damaged heads usually bear heavier individual grains. Recent evidence from wheat infested with *Haplothrips tritici* in Spain indicates that the more larvae per ear, the lighter in weight is a given volume of grain. Reported losses in grass seed crops are usually greater than in cereals, often reaching 30%. In New Zealand each 1% increase infestation of *Chirothrips pallidicornis* in cocksfoot seed crops corresponds to a 0.76% yield loss.

The ultimate measure of crop loss is revenue to the grower which is determined by production costs and market forces at time of sale. As marketing becomes more sophisticated and international, high quality produce is at a premium. As illustrations, damage to sweet peppers in Florida caused by *Frankliniella occidentalis* and *Thrips palmi* in 1993 exceeded $10 M; scarring of citrus fruit in California by *Scirtothrips citri* contributes to a loss of circa $2 per carton of fruit; *Frankliniella occidentalis* can cause up to 90% loss of summer replanted

cucumbers in UK glasshouses worth up to £50,000 ($75,000) ha^{-1} each year; resowing UK sugar beet fields after early spring attack by *Thrips angsticeps*, as occurred widely in 1996, costs about £145 ($218) ha^{-1}. Examples of yield losses caused around the world are given in Table 1.

Table 1. Examples of % loss of yield in field crops due to direct damage by thrips.

Crop	Species	% loss	Location
Alfalfa	*Thrips tabaci*	33	Iowa
Carrot seed	*Frankliniella* spp.	50	California
Cassava	*Corynothrips stenopterus*	8-15	Columbia
Cowpea	*Megalurothrips sjostedti*	30-90	Senegal
Onion	*Thrips tabaci*	34-43	Canada
Peas	*Kakothrips pisivorus*	20	Holland
Red clover	*Haplothrips niger*	10	Romania
Rice	*Haplothrips ganglebaueri*	50-63	W. Bengal
Rye	*Limothrips* spp. *Haplothrips aculeatus*	3-13	Sweden
Tea	*Scirtothrips* spp. *Heliothrips haemorrhoidalis*	100	Kenya
Tobacco	*Scirtothrips aurantii*	75	S. Africa

Infection of plants with tomato spotted wilt virus transmitted by thrips may likewise be costly to growers. In a survey of greenhouse ornamental plants in Pennsylvania during 1989-1990, plants infested with the *Impatiens* strain of TSWV with a retail value of £450,000 ($675,000) were destroyed under the direction of the States Department of Agriculture to prevent further spread of the virus and vectors. Similarly, in Denmark, plant health inspectors visit exporting greenhouse nurseries unannounced approximately every three weeks. If *Frankliniella occidentalis* is found on plants, or on sticky traps, the growers export licence is temporarily suspended until the thrips are controlled.

ECONOMICS OF PESTICIDE USAGE

Despite extensive reporting on the damage inflicted by pest species there are very few published assessments of the costs and financial returns of applying pesticides. The effect of infestations on produce range from the occasional total loss of high value glasshouse ornamental crops on which there is no tolerance of even minor cosmetic injury, to detectable effects on yield in field crops grown for processing, even when the non-harvestable foliage may have been heavily infested during early growth.

In the Netherlands aerial spraying of flax infested with *Thrips angusticeps* allegedly increased profits by \$146 ha^{-1}. Barley growers in North Dakota can supposedly increase profits by \$5 ha^{-1} by spraying against *Limothrips denticornis*. Savings on control costs in field crops can often by made by applying insecticides with other routine treatments such as herbicides. For example, when demeton was added to applications of 2-4D herbicide on cereals in Bulgaria, adult *Haplothrips tritici* were controlled, but it is doubtful whether the costs of spraying against the thrips alone would have been worthwhile. Control directed specifically at thrips attacking peanuts in the U.S. is rarely profitable. Similarly, in California there was little return from spraying cotton seedlings but in Louisiana, control of infestation during the four weeks after seedling emergence eventually produced yield increases of 10%.

In California citrus, the total cost of pesticides for thrips control alone reached almost \$12m in 1990, equivalent to \$116 ha^{-1} excluding application costs. Such expenditure is considered justifiable by growers to prevent scarring particularly of small fruit which may advance maturation and cause water loss. Unblemished fruit attracts a \$2 bonus per carton, so prevention of this damage is a major factor affecting profitability. Nevertheless, the maintenance of such high, largely cosmetic standards results in extremely low economic thresholds in the month before petal fall, and the intensive spray programmes that ensue are gradually becoming counterproductive.

Late pre-harvest applications to fresh edible crops must be balanced against the dangers of exceeding maximum residue levels (MRL s) and the resulting unmarketability of produce. Acceptable residue levels are usually strict; for example in New Zealand the MRL for fluvalinate used to control *Thrips obscuratus* on peaches is nil at harvest; in France there is a 7-day withholding period for peaches contaminated with 0.1 ppm of this compound, regulations which could each lead to rejection of consignments.

Pesticide contamination is less of a problem in non-edible crops, such as pyrethrum, in which the economic injury level caused by *Thrips nigrophilosus* appears to be very low justifying insecticidal control virtually as soon as even one individual adult or larva is detected on the leaves. Infestations reduce the number of flowers per plant rather than affecting the pyrethrum content of seeds but overall yield loss of up to 43% can occur. Average monetary losses for three cultivars were calculated to be \$284 ha^{-1} in a crop potentially capable of producing returns exceeding \$1000 ha^{-1}. Control of this pest with dimethoate costs only \$34 ha^{-1} so it would clearly pay to spray.

Another drawback to some pesticides is their phytotoxicity to many glasshouse ornamentals. For example, very few cultivars of chrysanthemums can tolerate dimethoate or formothion

sprays; malathion sprays or dicofol smokes should not be used on open blooms. Many other compounds are harmful to just one or a few cultivars.

The overall impression of chemical control of thrips in the majority of field and glasshouse crops grown for food or fibre, and in plantation crops and forestry, is that as a sole approach to control it is becoming either less effective or less acceptable environmentally or to the public. The future for these valuable materials lies in their timely but restricted use as part of IPM systems.

REFERENCE

This paper provides an overview of the importance of thrips as pests. More discussion of individual areas can be found in:

Lewis T (ed) (1997),*Thrips as Crop Pests*. CAB International, Wallingford. 740 pp.

Thrips and tospoviruses: Present and future strategies for management

D E Ullman, C A Casey, A E Whitfield, L R Campbell
Department of Entomology, University of California at Davis, One Shields Ave., Davis, California 95616 USA

K L Robb
University of California Cooperative Extension San Diego County, 5555 Overland Ave., Building 4, San Diego, California 92123 USA

R B Medeiros, T L German
Department of Plant Pathology, Russell Laboratories, 1630 Linden Drive, Plant Pathology, Madison, Wisconsin 53706 USA

J L Sherwood
Department of Plant Pathology, University of Georgia, Julian H. Miller Plant Sciences Building, Athens, Georgia 30602-7274 USA

ABSTRACT

The tospoviruses are group of emerging plant viruses transmitted by several species of thrips. The two most studied viruses in the genus *Tospovirus* are tomato spotted wilt virus (TSWV) and impatiens necrotic spot virus (INSV). The western flower thrips, *Frankliniella occidentalis*, is thought to be the most important vector in many locations worldwide. Thrips are only able to transmit tospoviruses if they acquire virus during larval feeding, thus, only reproductive plant hosts are important epidemiologically. Currently, it is difficult to identify these plants or detect infective thrips before symptoms and crop injury occur. We have developed a monitoring system that uses petunia indicator plants and directional yellow sticky traps to detect infective thrips and their sources. Using this system, management strategies can be targeted to areas in which they will best suppress virus spread. In addition, we have developed a tissue immunoblotting assay (TBIA) that allow rapid diagnosis of infected plants in the field. Use of our monitoring system and the TBIA reduced virus incidence in a field flower production area from 70% to 1%. This low level of virus incidence has been maintained for two years. Investigations aimed at understanding the cellular and molecular mechanisms mediating virus acquisition by thrips revealed specific interactions between a putative receptor in the western flower thrips and TSWV membrane glycoproteins. We propose that characterization of the events mediating thrips acquisition of TSWV will lead to significant new management strategies that can ultimately be integrated with other methods of thrips control.

INTRODUCTION

In spite of tremendous advances in crop production technology and integrated pest management, essentially no effective methods are available to help growers control epidemics

of tospoviruses, such as impatiens necrotic spot virus (INSV) and tomato spotted wilt virus (TSWV). These viruses are transmitted by at least 8 species of thrips, among which *Frankliniella occidentalis*, the western flower thrips, is considered the most important in many parts of the world. The relationship between the thrips vectors and the viruses is extremely specific. Therefore, other thrips species, such as *Heliothrips haemorrhoidalis*, the greenhouse thrips, do not transmit tospoviruses. Combating tospovirus epidemics is difficult because the thrips vectors are abundant, have many plant hosts and are frequently resistant to available insecticides. These problems are compounded because the viruses also have a large host range that includes many food, fiber and ornamental (virtually all important bedding plant, flower, and pot crops with the exception of roses, poinsettias, and zonal geraniums) crops.

From peanut production to growing of cut flowers, growers rank thrips and associated tospovirus spread as one of their most important unsolved problems. In spite of the serious damage caused throughout the United States and elsewhere, and the high level of grower concern, techniques for control of thrips-transmitted tospoviruses are virtually nonexistent. Control of thrips vectors, particularly western flower thrips, with insecticides is ineffective due to high rates of pesticide resistance in most populations worldwide and because relatively small numbers of thrips can result in high rates of virus spread (Yudin *et al.*, 1990). Additionally, routine pesticide use disrupts integrated pest management or low input sustainable practices. Successful management will require short term and long term solutions. In this article, we address our short and long term research efforts towards managing the spread of tospoviruses. In particular, we highlight our development of a new monitoring system in which yellow sticky traps and tospovirus indicator plants are combined to detect infective thrips (those that can infect plants), a new direct tissue blot immunoassay for plant diagnosis and our new findings regarding identification and characterization of the initial interaction between TSWV and western flower thrips that leads to virus acquisition.

In preliminary field tests, we demonstrated that our system for monitoring gives growers important information for making informed mangement decisions and directing methods of suppression where they will do the most good. We propose that by understanding the components of the virus-vector interaction, it will then be possible to design methods for blocking viral attachment, subsequently preventing virus acquisition. An intriguing long term outcome of this more basic research would be a management strategy in which plants are bioengineered to express analogs to the binding domains of particular viral proteins that could block the cellular receptor for TSWV. Thrips developing on such plants could not acquire TSWV. For the many crops, i.e. peanuts, lettuce, chrysanthemum, where secondary spread due to thrips acquisition within the crop causes epidemics, such a solution would dramatically reduce TSWV incidence. The potential for this strategy has been demonstrated with insect viruses (Hammock *et al.*, 1993; McCutchen *et al.*, 1991). In this benchmark research, a baculovirus was engineered with the gene for an insect specific toxin from the venom of a North African scorpion. During baculovirus infection of the insect midgut, the toxin is expressed, causing rapid paralysis and ultimately death of the insect. Such novel approaches have potential to reduce pesticide use and are compatible with other methods for controlling thrips and spread of TSWV.

Growers need to understand how thrips develop and transmit tospoviruses to successfully use the information from monitoring to control tospoviruses or to appropriately apply new

technologies to management in their particular situations. Perhaps the most critical point to understand is that individual thrips can only infect a plant if they acquired the virus as an immature. Infective adult thrips can transmit the virus to healthy plants by feeding for as little as 15 minutes, and will retain the ability to transmit the virus throughout their adult life. A thrips that did not feed on an infected plant while immature cannot acquire or transmit the virus as an adult, even if it feeds on infected plants as an adult. Immature thrips do not have wings and if undisturbed, don't generally move off the plant on which they were born until they pupate. As a consequence, only those plants that host the virus and support thrips reproduction produce infectious thrips and are important to virus spread. The challenge in developing a monitoring system is to devise a strategy to find these plants so they can be targeted for removal or for thrips control when appropriate. Those that are virus hosts, but do not support thrips reproduction are considered "dead-end" hosts for tospoviruses, because they do not produce infective thrips. Hence, these plants do not contribute to continued virus spread. Directing management strategies at "dead-end" hosts will not help reduce the spread of the virus.

Early detection of plants that are producing infective thrips and assessment of thrips numbers is essential if methods for suppressing virus spread are to be deployed successfully. Monitoring for thrips and tospoviruses has traditionally consisted of using sticky traps and plant samples to detect thrips and visual assessments of plants to detect virus symptoms. Common symptoms of tospovirus infection include stunting, leaf distortion, mosaic mottling on the foliage, vein clearing, ringspots, dark purple-brown sunken lesions, stem necrosis, wilting on one side of the plant, or irregular line patterns on the foliage. Some plants will show just one type of symptom while others may have several. Accurate virus detection can be difficult, because, symptoms expressed by some plants can be easily confused with those caused by fungi, bacteria, or nutritional disorders. Because diagnosis is so difficult using visual observations alone, it is important to confirm your diagnosis with a laboratory test.

METHODS AND MATERIALS

The laboratory test most commonly used to detect tospovirus infection is enzyme-linked-immunosorbant assay (ELISA). This test is based on a reaction between the virus and specific antibodies. The ELISA test requires grinding or smashing of the plant material to obtain sap and can be time consuming. In our field studies, a tissue blot immunoassay (TBIA) developed in the Ullman laboratory was used. The TBIA is similar to the ELISA method in that antibodies are used for detection. The TBIA is easier to perform than ELISA because no plant grinding or smashing is necessary and virtually no special equipment is needed. The assay also has potential to be more portable than ELISA and to provide faster results. In our work, use of the TBIA was critical in monitoring virus incidence during the growing season and at harvest. TBIA was conducted by cutting suspicious plant tissue with a razor blade and pressing it onto a nitrocellulose membrane. The membrane was then treated with an antibody against the non-structural protein encoded by the S RNA of TSWV or antibody against INSV, followed by a sequence of solutions (Whitfield *et al.*, unpublished). At the end of the assay infected plants leave a distinctive purple mark and healthy plants either leave no mark or a green mark where plant sap stained the membrane. The current test can be used for INSV or TSWV.

In our effort to develop a monitoring system, we have deployed petunia indicator plants as a rapid means for locating sources of infective thrips. These plants show distinctive local lesions when infective thrips feed on them. The lesions appear as small brown to black spots on the leaves and look very different than the whitish feeding scars left by noninfective thrips. Local lesions result from a hypersensitive response which is the strategy the petunia uses as protection from the virus. In a hypersensitive response, the tissue around the virus entry site dies rapidly preventing the virus from spreading and causing a system wide infection in the plant. Local lesions are apparent on petunias about 3-7 days after feeding by an infective thrips. If indicator plants are used routinely at standard locations inside and outside production areas, they can provide growers with invaluable information about where infective thrips are located and/or where they enter a production area. Control efforts, whether they include pesticides, exclusion strategies or removal of weeds, can then be directed to those areas where they will do most good.

Petunia indicator plants also give growers the advantage of knowing when infective thrips are on the move in an area, even if the crop is not yet showing symptoms. This is important because, many tospovirus sensitive plants, such as chrysanthemums, may be infected at any time in production, but symptoms are not visible until the plant sets buds. Relying on symptoms to indicate virus presence or on feeding damage to indicate thrips presence generally does not allow the grower to respond soon enough to limit virus spread. The rapid appearance of local lesions on petunias allows for a timely response in deploying thrips control strategies. Although lesions may not be immediately obvious to the untrained eye, growers and scouts can easily learn to recognize them.

Our research has focused on the use of selected petunia cultivars as indicators of tospovirus transmission by thrips. These are 'Blue Carpet', 'Cascade Blue', 'Summer Madness', 'Burgundy Madness', 'Red Cloud', and 'Super Magic Coral'. Other plants, such as fava beans, have been evaluated as indicator plants, but the most reliable cultivar of fava beans, 'Toto', is no longer available. Petunias are an excellent choice as an indicator, because the plants do not support thrips development and seldom become systemically infected. As a result, the plants do not serve as a source of the virus or additional thrips.

With regard to cellular and molecular investigations of thrips/tospovirus interactions, we have used gel overlay assays and immunolabeling at the light and electron microscopy levels to document that the membrane glycoproteins (GPs) of TSWV selectively bind a 50 kDa protein present in extracts from whole insects and dissected midguts of the TSWV vector, the western flower thrips (Bandla *et al.*, 1998). The 50 kDa protein was shown to be abundant in larvae, the developmental stage known to acquire TSWV, but absent or present in low quantities in adults, which are refractory to acquisition of the virus. Anti-idiotypic antibodies that mimic the TSWV GPs specifically bound this protein in western blots and labeled midgut membranes of larval western flower thrips. The 50 kDa protein was not detected in a nonvector thrips species, aphids or leafhoppers, nor did any of the other viral proteins tested (the nucleocapsid, the nonstructural protien encoded by the S RNA) bind thrips vector proteins. Based on our earlier documentation of events in virus acquisition by the western flower thrips (Ullman *et al.* 1995b) and these recent findings (Bandla *et al.*, 1998), we hypothesize that one or both of the TSWV GPs serve as viral attachment proteins that interact with one or more cellular receptors in the western flower thrips midgut to mediate virus acquisition and that the 50 kDa thrips protein we identified serves as one such cellular receptor or a component thereof. This

hypothesis is consistent with mechanisms of virus acquisition described for mosquito transmitted membrane-bound viruses of vertebrates in which the fidelity of virus acquisition relies on the interaction between viral attachment proteins and corresponding cellular receptors in the vector (Houk *et al.*, 1990). The possibility that a similar mechanism exists for insect acquisition of membrane-bound phytopathogenic viruses, such as TSWV, has not previously been tested, nor are the specific mechanisms underlying virus acquisition for any of the circulatively transmitted plant viruses fully understood. Thus, the TSWV-western flower thrips system provides an excellent model for molecular characterization of acquisition of a membrane-bound plant-infecting virus by its insect vector.

RESULTS AND DISCUSSION

We have demonstrated the efficacy of monitoring for infective thrips using petunia indicator plants in conjunction with directional sticky traps. In trials conducted in field-grown flowers, monitoring stations were placed in the field and at the edges of the field. Each station contained directional sticky traps (north, south, east, and west facing traps) and a plant stand for the petunias. It is important that the petunias be at or slightly above the crop canopy, and that they be placed on a blue surface to increase their attractiveness to the thrips. The plants are placed in self-watering containers so that they don't dry out in the field. Sticky traps and plants showing lesions must be replaced once a week. See Robb *et al.* (1998) for an example of a monitoring station. In our studies, observations were made in the field and all petunias were removed weekly, held in the laboratory for a few days and examined again for lesions.

For this monitoring system to work, it is essential that the petunias are grown in an area isolated from thrips and tospovirus sensitive plants. Otherwise, growers won't know whether the lesions they observe on the petunia indicator originated in the petunia propagation area or the production area being monitored. Plants can be used while they are still relatively small (3 1/2" pot). Flowers should be removed from the plant before placing them at the monitoring station. This is important because the thrips are more attracted to the flowers than the foliage and the petals do not express local lesions.

The information gained from using the four directional traps at each monitoring station has provided valuable insight about the direction from which thrips enter the field. In our trials, the greatest numbers of thrips were consistently caught on the north facing sticky traps and the first lesions were detected on petunia at trapping stations at the north end of the field. This result directed our attention to the fields north of the production area where a large block of TSWV infected, thrips infested malva (a noxious weed) was discovered. The grower quickly focused on removing the malva from his field and from the surrounding areas to the north.

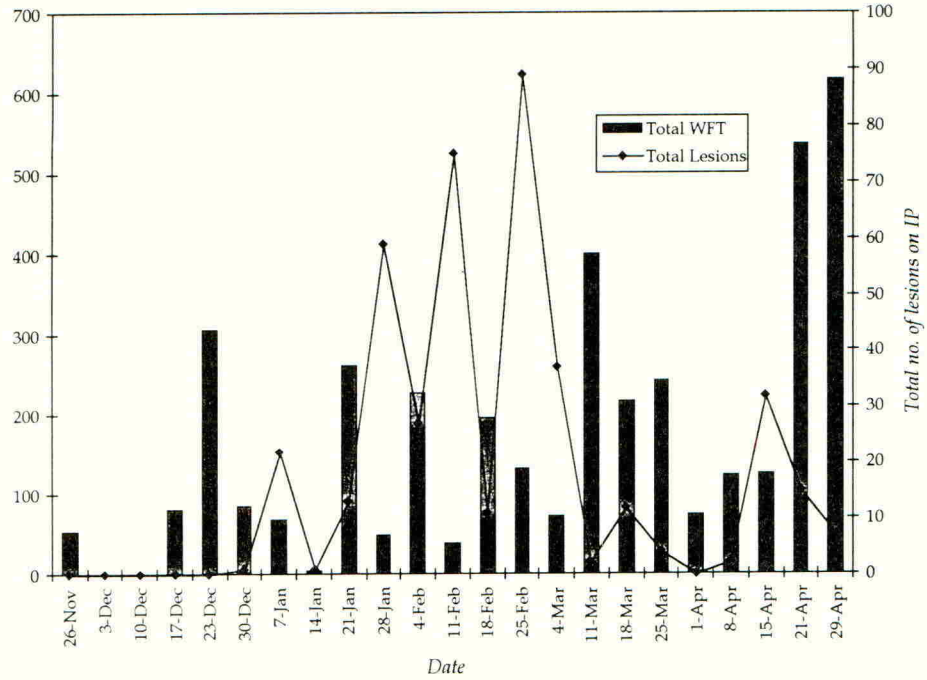


Figure 1. Relationship between western flower thrips populations and lesions detected on petunia indicator plants over time in field grown flowers.

Directing this control effort to a specific area made it feasible and the result was a dramatic decrease in spread of TSWV to the grower's flower production area.

"Why use indicator plants at all?" "Why not just spray the crop regularly or when thrips are found on sticky traps?" These questions are often asked by growers before trying our monitoring system. Although many growers have tried routine spraying, they often find that they still have problems with INSV or TSWV. Sticky trap counts alone do not necessarily reflect the number of infective thrips present, nor do they reveal their source. Figures 1 and 2 show that there is no relationship between the average number of western flower thrips collected on sticky traps and the average number of lesions found on petunias. This is because only the infective thrips in the population can cause lesions on the petunia and these are the only thrips important to virus spread. Since one insect can infect several plants, it is not surprising that low levels of infective thrips can reflect a high level of virus. In our trial, peak lesion numbers occurred in an area where the western flower thirps populations were relatively low (see Block 6 on Figure 2). Conversely, the peak numbers of western flower thrips on sticky traps were observed where there were relatively few lesions observed (see Blocks 3 and 5 on Figure 2).

Use of the petunia indicator plant/directional trap system alerts the grower to the presence of infective thrips and helps locate their source. In our experience with the system, removal of these sources resulted in greatly reduced virus incidence. For example, in our trials with field grown flowers, the number of infected plants dropped from 70% to less than 1% the first year the monitoring system was tested. The direct blotting immunoassay provided an essential tool for diagnosing plants with TSWV and INSV. We used this assay in the field and demonstrated its efficacy as a tool for indexing ornamental plants, bulbs and tubers for tospoviruses. We compared TBIA and ELISA and found no significant difference in their accuracy. An example of a TBIA for TSWV can be found in Robb *et al.* (1998). We are currently expanding our research to determine the optimal number of trapping stations and the best strategies for indicator plant placement in different types of greenhouses and crops.

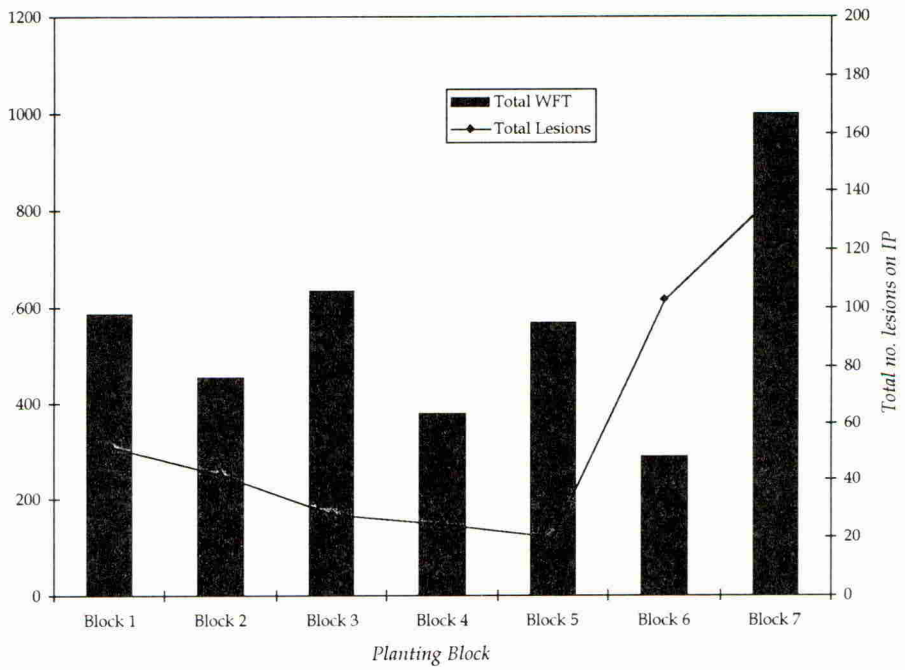


Figure 2. Relationship between total western flower thrips and total lesions detected on petunia indicator plants (the line) in each of the seven production blocks monitored.

Our results from investigations of thrips/TSWV interactions showed that TSWV membrane glycoproteins were selectively bound to thrips proteins from extracts of whole insects and dissected midguts. A single band in the region of 50 kDa was detected in all gel overlay assays. Assays using separated proteins from whole thrips and isolated virus as the overlay, revealed a single band at 50 kDa from western flower thrips larvae and adults when probed by monoclonal antibodies to GP1 and/or GP2. In all the replications of this assay (more than 10),

a difference in the intensity of the bands from larvae and adults was observed, with only a faint band detected from adults. Bands at or near 50 kDa were absent in lanes containing preparations of all the non-vector insect species tested (*Schizaphis graminum* , *Circulifer tenellus* and *Heliothrips haemorrhoidalis*). Furthermore, no bands were detected from separated proteins of any of the insects assayed when polyclonal antibodies against TSWV nucleocapsid or the nonstructural protein encoded by the small RNA were used as probes. Nor did any of the antibodies react with separated insect proteins in the absence of virus protein overlays. Similar results were obtained in assays using blots of separated proteins from dissected thrips midguts and isolated virus or gel purified TSWV GPs as the overlay. A single band at or near 50kDa was detected from separated larval and adult western flower thrips proteins. Although similar quantities of thrips midgut protein were loaded per lane from larval and adult samples, the band detected from larval midgut preparations was very intense, while only a faint band could be observed from adult preparations. As in experiments using isolated TSWV as the overlay, no bands were observed from aphid extracts, indicating that gel purified TSWV GPs did not bind aphid proteins.

The anti-idiotype antibodies against the murine monoclonal antibodies to GP1 and GP2 provide reagents that mimic TSWV GPs and can be detected by a tagged anti-mouse antibody. In western blots with insect proteins, both anti-idiotype antibodies labeled a single band at 50 kDa in wells containing extracts of westerm flower thrips, but did not label bands at this molecular weight from the thrips nonvector species, *H. haemorrhoidalis*. As observed in gel overlay assays, the band in lanes containing larval preparations was intense while the band in lanes containing adults preparations was faint, although the same amount of thrips protein was loaded onto each lane. Immunolabeling experiments revealed that the anti-idotype antibodies bound specifically to the plasmalemma of the epithelial cells of dissected larval midguts, an expected location for cellular receptors

These results, in combination with previous electron microscope findings (Ullman, *et al.*, 1993, 1995a) support the hypothesis that the TSWV GPs serve as viral attachment proteins that interact with one or more cellular receptors in the thrips midgut. This conclusion is supported by several pieces of evidence. First, TSWV GPS selectively bind separated western flower thrips proteins that form a single band at or near 50 kDa, but do not bind separated proteins from nonvector insects, including another species in the family Thripidae. The consistent difference in band intensity in lanes containing larval versus adult extracts indicates that abundance of the putative cellular receptor(s) is greater in the larvae, the developmental stage known to acquire the virus, than in adults, which are refractory to the virus. This latter finding is consistent with previous hypotheses that receptor abundance is an important determinant of vector competence with membrane bound viruses (Hardy *et al.*, 1983; Houk *et al.*, 1983), as well as governing endocytosis of *Bacillus thuringiensis* d-endotoxins (van Rie *et al.*, 1989). Proteins that serve as virus receptors generally serve some other fundamental function in the host (Rossman 1994). Therefore, finding a potential cellular receptor for TSWV that is present in larvae and adults of western flower thrips is quite plausible, even though the adult does not acquire the virus. Given the importance of pH and proteases in mediating endocytosis of membrane bound viruses, we also expect that the physiology of the thrips midgut will be important in determining whether interactions between viral proteins and cellular receptors lead to virus acquisition. By analogy to other membrane bound viruses, it is likely that TSWV endocytosis will be mediated by receptor abundance in combination with other factors that may vary between larvae and adults, i.e. processing of viral proteins

governed by conditions in the midgut or formation of transient structures, such as a peritrophic membrane. This hypothesis is consistent with biological data showing that efficiency of virus acquisition by larvae is dramatically reduced as development proceeds, with the highest level of acquisition occuring during the first few hours of the first instar larvae (van de Wettering *et al.*, 1996). Finally, the midgut plasmalemma, the expected location of a cellular receptor in thrips, was specifically labeled when thrips midguts were reacted with the anti-idiotype antibodies.

Our findings strongly support the role of TSWV GPs as viral attachment proteins and the presence of one or more putative cellular receptors in the thrips midgut. This conclusion is consistent with the most commonly accepted models of virus entry for membrane bound viruses, including other members of the family *Bunyaviridae* (i.e. LACV), human immunodeficiency virus (HIV) and certain baculoviruses. These investigations and many others, reviewed in White (1990), indicate that viral glycoproteins serve as attachment proteins and virus fusion to host cells results from a conformational change in the GPs that is driven by receptor binding or acidic pH. The tospoviruses infect their vectors, much like insect pathogenic viruses or mosquito transmitted animal viruses. Although no cellular receptors have been identified for insect vectors of plant viruses or vectors of animal-infecting bunyaviruses, several receptors have been characterized for insect pathogenic viruses and other mosquito transmitted animal viruses , i.e. polyhedron-derived baculovirus (Horton & Burand 1993), the *Bacillus thuringiensis crylA*(b) and *crylA*(c) d-endotoxins (Knight *et al.*, 1994; Vadlamundi 1995) and western equine encephalomyelitis (WEE) virus (Houk *et al.*, 1990). These studies localized cellular receptors to the brush border membrane of the host cells and provide additional support for our contention that a putative cellular receptor is localized at the midgut plasmalemma of thrips. Experiments are underway to further support this proposal by demonstration of saturable attachment of virus to host cells, virus competition for limited receptor sites, and characterization of the binding motif in the GPs.

We propose that characterization of the events mediating thrips acquisition of TSWV will lead to significant new management strategies that can ultimately be integrated with other methods of thrips control.

ACKNOWLEDGEMENTS

This research has been generously supported by the American Floral Endowment, the Carlsbad Agricultural Improvement Fund, donations from Mellano and Company, the University of California Integrated Pest Management Project and the Binational Agricultural Research and Development Fund (BARD #US-2495-94). We are grateful to Dr. John Cho, University of Hawaii for providing antibodies to TSWV.

REFERENCES

Bandla, M D; Campbell, L R ; Ullman, D. E.; Sherwood, J L (1998). Interaction of tomato spotted wilt tospovirus (TSWV) glycoproteins with a thrips midgut protein, a potential cellular receptor for TSWV. *Phytopathology* **88**, 98-104.

Bandla, M D; Westcot, D M; Ullman, D E; German, T L; Sherwood, J L (1994). Use of monoclonal antibody to the nonstructural protein encoded by the small RNA of the tomato spotted wilt tospovirus to identify viruliferous thrips. *Phytopathology* **84**, 1427-1431.

Hammock, B D; McCutchen, B F; Beetham, J; Choudary, P V; Fowler, E; Ichinose, R; Ward, V K; Vickers, J M; Bonning, B C; Harshman, L F; Grant, D; Uematsu, T; Maeda, S (1993). Development of recombinant viral insecticides by expression of an insect-specific toxin and insect-specific enzyme in nuclear polyhedrosis viruses. *Archives of Insect Biochemistry and Physiology* **22**, 315-344.

Hardy, J L; Houk, E J; Kramer, L D; Reeves, W C (1983). Intrinsic factors affecting vector competence of mosquitoes for arboviruses. *Ann. Rev. Entomol.* **28**, 229-262.

Horton, H M; Burand, J P (1993). Saturable attachment sites for polyhedron-derived baculovirus on insect cells and evidence for entry via direct membrane fusion. *J. Virology* **67**, 1860-1868.

Houk, E J; Arcus, Y M; Hardy, J L; Kramer, L D (1990). Binding of western equine encephalomyelitis virus to brush border membrane fragments isolated from mesenteronal epithelial cell of mosquitoes. *Virus Research* **17**, 105-118.

Knight, P J K; Knowles, B H; Ellar, D J (1995). Molecular cloning of an aminopeptidase N that serves as a receptor for *Bacillus thuringiensis* CryIA(c) toxin. *J. Biol. Chem.* **270**, 17765-17770.

McCutchen, B F; Choudary, P V; Crenshaw, R; Maddox, D; Kamita, S G; Palekar, N; Volrath, S; Fowler, E; Hammock, B D; Maeda, S (1991). Development of a recombinant baculovirus expressing an insect-selective neurotoxin: potential for pest control. *Bio/Technology* **9**, 848-852.

Robb, K; Casey, C; Whitfield, A; Campbell, L; Ullman, D (1998). Fight impatiens necrotic spot virus and tomato spotted wilt virus using a new monitoring system to make informed decisions. *Grower Talks* (February Issue).

Rossmann, M G (1994). Viral cell recognition and entry. *Protein Science* **3**, 1712-1725.

Ullman, D E; German, T L; Sherwood, J L; Westcot, D M; Cantone, F A (1993). *Tospovirus* replication in insect vector cells: Immunocytochemical evidence that the nonstructural protein encoded by the S RNA of tomato spotted wilt tospovirus is present in thrips vector cells. *Phytopathology* **83**, 456-463.

Ullman, D E; German, T L; Sherwood, J S; Westcot, D M (1995a). Thrips transmission of Tospoviruses: Future possibilities for management. In: Parker, B. L., Skinner, M. and Lewis, T. S. (ed.) *Thrips Biology and Management*. New York, Plenum Press.

Ullman, D E; Westcot, D M; Chenault, K D; Sherwood, J L; German, T L; Bandla, M D; Cantone, F A; Duer, H L (1995b). Compartmentalization, intracellular transport and autophagy of tomato spotted wilt tospovirus proteins in infected thrips cells. *Phytopathology* **85**, 644-654.

Vadlamudi, R K (1995). Cloning and expression of a receptor for an insecticidal toxin of *Bacillus thuringiensis*. *The Journal of Biological Chemistry* **270**, 5490-5494.

Van de Wettering, F; Goldbach, R; Peters, D (1996). Tomato spotted wilt tospovirus ingestion by first larval instar of *Frankliniella occidentalis* is a prerequisite for transmission. *Phytopathology* **86**, 900-905.

van Rie, J; Jansens, S; Hofte, H; Degheele, D; van Mellaert, H (1989). Specificity of Bacillus thuringiensis delta-endotoxins. *European Journal of Biochemistry* 186, 239-247.

White, J M (1990). Viral and cellular membrane fusion proteins. *Annu. Rev. Phys.* **52**, 675-697.

Yudin, L S; Tabashnik, B E; Cho, J J; Mitchell, W C (1990). Disease prediction and economic models for managing tomato spotted wilt virus disease in lettuce. *Plant Disease* **74**, 211-216.

Is there a natural enemy good enough for biological control of thrips?

J C van Lenteren, A J M Loomans
Laboratory of Entomology, Wageningen Agricultural University, P.O. Box 8031, 6700 EH Wageningen, the Netherlands

ABSTRACT

Thrips species have become pests in many cultivated crops throughout Europe and elsewhere in the world during the past decades. *Thrips tabaci* Lind. was the most prevalent thrips pest in Europe, but since its accidental introduction in 1983, western flower thrips (*Frankliniella occidentalis* (Pergande)) has become the number one pest in European greenhouses. Today, Europe is faced with introductions of other serious thrips pests. To control thrips pest, growers are forced to intensively apply chemical pesticides, thus upsetting commercially successful greenhouse IPM programmes. Chemical control of thrips often proves to be difficult. Although a large variety of predators (anthocorids, mirids, thrips and mites), entomopathogenic fungi, thrips attacking nematodes and parasitoids are known, control practices are still often based on chemical applications. Predatory mites and anthocorid predators have provided adequate control of thrips in greenhouse crops like sweet pepper and cucumber worldwide, while performance in floriculture was less satisfactory until recently. Pathogenic fungi might be useful as additional control agents. Parasitoids, though the only specific natural enemies of thrips, have not shown much potential for control to date.

INTRODUCTION

During the last decades thrips have become pests in many cultivated crops throughout Europe and elsewhere in the world. Until the early eighties *Thrips tabaci* was the most prevalent thrips pest in Europe, but caused problems only occasionally. Since its accidental introduction in 1983, western flower thrips (*Frankliniella occidentalis*) has become the number one key pest in European greenhouses and, under Mediterranean conditions, also caused problems in the field. *Echinothrips americanus* Morgan is now spreading through Europe and there is a risk of introducing *Thrips palmi* Karny. *F. occidentalis* started its expansion in Europe, *T. palmi* did so in the Far East and the Pacific (Loomans & Vierbergen, 1997). Currently, *T. palmi* is an important pest throughout large parts of tropical and subtropical vegetable and flower producing areas. The exchange of horticultural products all over the world makes this quarantine pest a serious threat to Europe as well. Interceptions from vegetables and cut flowers imported from the Caribbean and Asia have increased in numbers over recent years. Apart from greenhouse crops in temperate areas, it is an important potential problem for the horticultural industry in the Mediterranean Region. In 1996 *E. americanus*, found occasionally on bedding plants since 1993, became a pest in sweet pepper in The Netherlands. In 1997 it occurred on more than 70 sweet pepper holdings and is now hampering IPM in this crop (Vierbergen, 1998).

At the end of the 1980s, *F. occidentalis* was first recorded in the Mediterranean Region and established itself rapidly, both in the greenhouse and in the open (Loomans *et al.*, 1995). *T. palmi* has not become established in Europe yet, but is considered as an EPPO and EU quarantine

organism (A1 status: EPPO/CABI, 1997, see Loomans & Vierbergen, 1997) with a zero tolerance level and a high priority given to prevention of entry and establishment. In 1992, infestations in The Netherlands on *Ficus* spp. urged the Dutch Plant Protection Service to start a drastic eradication programme and since then EU import inspections have intensified. Nevertheless, because of various characteristics of the pest as well as the ever increasing pressure of international trade, it is very likely that *T. palmi* will obtain a foothold in Europe eventually.

To control thrips pest outbreaks caused by native or inadvertedly introduced exotic species, growers are forced to apply chemical pesticides intensively, thus upsetting commercially successful greenhouse IPM programmes (van Lenteren, 1995). The short development time of thrips and the excessive pesticide treatments to which they are subjected contribute to the rapid development of resistance to insecticides. When *F. occidentalis* arrived in Europe, it was already resistant to many insecticides and the same problem will be experienced when *T. palmi* establishes.

Chemical control of thrips often proves to be difficult, because (1) a large proportion of the juvenile stages escapes treatment (eggs and pupae are concealed during most of their development), (2) resistance to a range of commonly used insecticides, and (3) limited availability of active ingredients for control (several insecticides cause phytotoxic effects). Application of organo-phosphates may even enhance outbreaks of thrips pests, because they destroy natural enemies and leave the pest unharmed. Although a large variety of predators (anthocorids, mirids, predatory thrips and mites), entomopathogenic fungi, nematodes and parasitoids of thrips are known, proper stock taking and in depth pre-introductory evaluation of natural enemies has not occurred yet (van Lenteren & Woets, 1988). As a result, control practices are still often based on chemical applications.

The development of biological control programmes for exotic thrips species is cumbersome and has been more complicated than finding effective natural enemies for pests like exotic leafminers and whiteflies (van Lenteren *et al.*, 1996). When biological control of thrips was initiated in The Netherlands, researchers had to accept that natural enemies became commercially available that had not been tested thoroughly, and that biocontrol results were not as reliable and predictable as that of leafminers and whiteflies. Such developments are particularly risky in early phases of the development of Integrated Pest Management programmes where growers have just started to trust new forms of pest control.

At the start of the 1990s, several European research groups initiated a collaborative project to develop an effective and economic method for the biological control of thrips. In this project (1) the literature on thrips pests and the control capacity of already studied natural enemies was evaluated, (2) field surveys were performed for native natural enemies (predators and parasitoids) in Europe, and for parasitoids outside Europe, (3) rearing methods were developed for thrips and their natural enemies, and (4) new natural enemies were evaluated under laboratory, greenhouse and field conditions. An extensive review of points 1 and 2 was presented by Loomans *et al.* (1995). A summary of the results of point 4 and other recent work on biological control of thrips is given in this paper.

THRIPS PESTS

The order of Thysanoptera includes over 5,200 species of thrips (Lewis, 1997). They are small slender insects (1-2.5 mm). Most crop damaging Thysanoptera, some 100 species,

belong to the family Thripidae of the suborder Terebrantia. Initially, only *Thrips tabaci* caused problems in Europe. *T. tabaci* is extremely polyphagous, and known to infest more than 300 plant species including greenhouse and field vegetables, ornamentals and cotton. Currently, *F. occidentalis* and *T. palmi* are considered much more important pests than *T. tabaci*. *F. occidentalis* is a species of nearctic origin, first reported by Pergande in California at the end of the 19th Century. It now occurs worldwide, and was first found in Europe in 1983. *F. occidentalis* is strongly polyphagous, known to attack more than 250 plant species belonging to more than 60 families, including many vegetables, ornamentals and fruit trees. *T. palmi* was first collected in Indonesia in 1921, was accidentally introduced to Japan in 1978 and later to the United States. *T. palmi* mainly attacks cucurbits and solanaceous plants. In addition to the above-mentioned genera *Thrips* and *Frankliniella*, species belonging to other genera, e.g. *Caliothrips*, *Scirtothrips* and *Megalurothrips* may also cause serious pests.

Thrips species cause direct damage (leaf necrosis, growth deformation, gall formation, and damage to young leaves, buds, flowers, fruits, bulbs and rhizomes). When thrips densities are low, injury to vegetables can often be tolerated, but in ornamentals such injury leads to cosmetic damage. At higher thrips densities, large leaf surfaces will show necrosis (and plant assimilation decreases), fruit and flowers may show scars or growth deformations, and may even drop prematurely. Several thrips species also vector plant viruses (Loomans *et al.*, 1995; Wijkamp *et al.*, 1995) and virus symptoms vary widely among host plant species. These viruses are acquired only during the larval stages that feed on the diseased plants. After acquiring the virus, adults can transmit the virus for the remainder of their lives.

PREVENTION AND CONTROL OF THRIPS

As thrips control is very difficult, importance should be placed on methods that prevent immigration of thrips into the greenhouse. Prevention methods include (a) extermination of thrips at the end of the production season by cleaning the greenhouse so as to hinder survival to the next cropping period, (b) purchase of clean plants, (c) strict hygienic measures applied to personnel and visitors, and (d) mechanical exclusion of thrips through screening of ventilation (and all other) openings with fine mesh screens. Also, weeds that may host thrips and that can serve as reservoir of viruses, should be removed from the greenhouse and its surroundings. Another way to prevent thrips and virus problems is the use of host plants that are resistant to thrips, and recent studies into host-plant resistance with tomatoes, sweet pepper, cucumber and chrysanthemum have led to some success. The first cucumber lines resistant to *F. occidentalis* are now available for breeding companies to develop commercial cultivars (O.M.B. de Ponti, pers. com.).

Chemical control of thrips is still the most frequently used method for pest suppression. But a combination of spray application problems (good coverage, penetration into plant parts where thrips feed, and the requirement to treat both the plant and the soil), the high frequency of sprays needed, the incompatibility of many pesticides with concurrent use of natural enemies of other pests in IPM programmes, phytotoxicity and the general occurrence of resistance make chemical control an unattractive option. Therefore, an intensive search for natural enemies was initiated about 10 years ago, and biological control of thrips is now a realistic option for several crops.

NATURAL ENEMIES OF THRIPS

The major groups of natural enemies, consisting of predators, parasitoids and pathogens, will be summarized below. Recent, detailed information about thrips predators can be found in Riudavets (1995) and Sabelis & van Rijn (1997), about thrips parasitoids in Loomans and van Lenteren (1995) and Loomans *et al.* (1997), and about fungal pathogens of thrips in Butt and Brownbridge (1997). A thorough analysis of the relative importance of predators, parasitoids and pathogens under natural conditions in the field is not available yet.

Predators

Many arthropods are known to be predators of thrips, including Anthocoridae, Miridae, Syrphidae, Cecidomyidae, Chrysopidae, Sphecidae, Araneida, Pseudoscorpiones and Thysanoptera (Table 1). Most predators of thrips are generalists. They do not restrict their predatory activities to thrips, but also eat many other plant-inhabiting arthropods, including beneficial species, and some species of predators even use food of plant origin. The best studied families of predators are the Anthocoridae and Phytoseiidae.

Heteroptera: Hemiptera: Anthocoridae. The Anthocoridae, including the genera *Orius* and *Anthocoris* are known as active general insect predators, and are effectively used in biological control programmes (van Lenteren, 1997; van Lenteren *et al.*, 1997). For control of *F. occidentalis*, several *Orius* species have been evaluated (Tommasini & Maini, 1995). Field surveys in Europe resulted in collection of the following *Orius* species: *O. albidipennis* Reuter, *O. laevigatus* (Fieber), *O. majusculus* (Reuter), *O. minutus* (L.), and *O. niger* (Wolff). *O. insidiosus* (Say), a nearctic species, proved to be a good biological control candidate in North America (Riudavets, 1995). It was initially used with success in Europe for control of *F. occidentalis*. After more efficient European species were found, the use of *O. insidiosus* in Europe decreased, but it is still used in North America. Another North American species, *O. tristicolor* (White), has been used successfully in Canada for control of thrips.

In Europe, *O. laevigatus* appeared to adapt well to protected environments. It can survive without thrips prey, and strains collected in southern Italy do not show diapause, which means that they can also be used in winter. They can be mass-produced on *Ephestia kuehniella* (Zeller) eggs. Mass production is still quite expensive when compared to several other natural enemies (van Lenteren *et al.*, 1997). *O. laevigatus* is now the most commonly used biocontrol agent for *F. occidentalis* on vegetable crops in Europe. IPM strategies for *T. palmi* are currently being developed for solanaceous and cucurbit crops, based on the releases of *O. sauteri* (Poppius) in Japan (Yano, 1996) or predatory mites (*Amblyseius cucumeris* (Oudemans)) in Florida. *O. sauteri* showed a very good suppressive effect of *T. palmi* on eggplant, sweet pepper and cucumber in experimental greenhouses in Japan (E. Yano, pers. com.). *O. sauteri* is expected to be registered as a biocontrol agent in Japan in 1998. Like other *Orius* species, it can be mass reared on *E. kuehniella*. Biological control measures, which have proven to be effective to keep populations of *F. occidentalis* and *T. tabaci* down in Europe, using predatory bugs (*Orius*) and predatory mites (*Amblyseius, Hypoaspis*), will likely be able to control *T. palmi* as well once established, and are currently under investigation (Loomans & Vierbergen, 1997). Because *Orius* spp. are generalist predators, intraguild predation will be a general phenomenon in greenhouses where *Orius* spp. are released, and the potential negative effect of *Orius* releases on other natural enemies merits pre-introductory evaluation of this phenomenon (Sabelis & van Rijn, 1997). Other heteropteran predators are listed in table 1. Of these, none is currently widely used for control of thrips.

Table 1. Natural enemies of thrips.

	Prey/host*	Generalist/Specialist	Type of cropping system	Mass production
Predators				
Heteroptera				
Orius spp.	Fo Tt Tp	generalist	protected, field	possible, expensive
Anthocoris nemorum	Fo	generalist	protected	possible, expensive
Geocorus spp.	Fo	generalist	field	not yet developed
Nabis spp.	Fo Tt	generalist	field	not yet developed
Dicyphus spp.	Fo Tt	generalist	protected, field	not yet developed
Macrolophus spp.	Fo	generalist	protected	possible, expensive
Thysanoptera				
Aeolothrips spp.	Fo Tt	generalist	field	not yet developed
Franklinothrips spp.	Tp Hh Pd	generalist	protected	possible, expensive
Acari				
Amblyseius/ Neoseiulus spp.	Fo Tt Tp	generalist	protected	possible, cheap
Hypoaspis spp.	Fo Tt	generalist	protected, field	possible, reasonable
Various predators				
Neuroptera, Diptera	Fo Tt Tp	generalist	protected, field	availabe for some
Parasitoids				
Ceranisus spp.	Fo Tt Tp	specialist	protected, field	difficult
Thripobius semiluteus	Hh	specialist	protected, field	possible, expensive
Pathogens				
Thripinema sp.	Fo	unknown	protected	not yet developed
Verticillium lecanii	Fo Tt Tp	generalist	protected	possible, cheap

*Fo = *F. occidentalis*, Tt = *T. tabaci*, Tp = *T. palmi*, Hh= *Heliothrips haemorrhoidalis*, Pd = *Parthenothrips dracaenae*

Acari: Phytoseiidae. Phytoseiidae have several predatory genera, including predators of thrips in the genus *Amblyseius*. *Amblyseius* spp. can easily be mass produced on the storage mite *Tyrophagus putrescentiae* (Schrank). *A. (=Neoseiulus) cucumeris* (Oudemans) is a cosmopolitan species and preys on several thrips species, as well as on phytophagous mites. There have been successes and failures in introductions in greenhouses in different countries. The success rate has been lower on cucumber than on peppers (where *Amblyseius* can use pollen as an alternative food source), and it has been lower for the control of *F. occidentalis* than for *T. tabaci*. Very high numbers of predators need to be released to obtain control. Addition of pollen to cucumber leads to improved control of thrips. *A. barkeri (= mckenziei)* (Hughes) is also cosmopolitan. It preys, among others, on thrips and spider mites. It is a very good predator of *T. tabaci* in cucumber. *A. degenerans* Berlese is preferentially used during conditions where other *Amblyseius* species enter diapause. The most recent addition to predators of this group is *A. limonicus* Garmon and McGregor, which originates from New Zealand and performs much better on vegetables in greenhouses than *A. cucumeris*. Further, this species does not enter diapause at short day length (Van Houten *et al.*, 1995). It is expected that this species will replace *A. cucumeris* soon.

Recently, soil inhabiting mesostigmatic predatory mites of the genus *Hypoaspis* (cosmopolitan) have been used for the control of thrips stages that live in the soil. At this moment *H. aculeifer* Canestrini and *H. miles* (Berlese) are the advised species for additional thrips control, but are not capable of complete control. Various other predators of thrips are listed in table 1, and are discussed by Riudavets (1995) and Sabelis & van Rijn (1997).

Parasitoids

Thrips parasitoids all belong to the superfamily Chalcidoidea. Most of them are solitary endoparasitoids of larvae (Eulophidae) or eggs (Mymaridae, Trichogrammatidae) of thrips. All thrips parasitoids are specific to a single subfamily, a few genera or even a few species. Loomans & van Lenteren (1995) and Loomans *et al.* (1997) reviewed the biology of thrips parasitoids and concluded that information on parasitoids is presently very incomplete. The Eulophidae are best studied, and are all minute (0.5 to 1.1 mm), solitary endoparasitoids of thrips larvae, although sometimes the prepupae and/or pupae may be attacked. Currently, 27 species are described, parasitizing over 70 species of Thysanoptera (Loomans & van Lenteren, 1995), many of which are known as pests. Species within the genus *Ceranisus* seem to be the most promising group for biological control. In the field, natural parasitism may reach levels of 50% or more, but this is not necessarily a good indicator of capacity to control thrips under production conditions. Natural control in most cases seems insufficient in itself to prevent damage to a crop, reaching, as it usually does, a maximum late in the growing season, after the peak of pest outbreaks. Except for commercial use of *Thripobius semiluteus* Boucek, attempts to control thrips pests by parasitoid releases, either in a classical or in a seasonal inoculative way, have failed (Loomans & van Lenteren, 1995).

Attempts to control thrips pests by seasonal inoculation or inundation of thirps parasitoids in temperate greenhouse ecosystems, a common strategy with predators, have been relatively few. In experimental releases of *C. menes* (Walker) and *C. americensis* (Girault) to control *F. occidentalis* infestations in sweet pepper, cucumber and roses in Dutch greenhouses, parasitoids spread readily and established themselves throughout the crops but did not reduce thrips numbers. Releases of the egg parasitoid *T. semiluteus* in avocado orchards in California for the control of *Heliothrips haemorrhoidalis* (Bouché), were more successful and they established in many orchards (McMurtry & Badii, 1991). Thrips populations declined when the rate of parasitism reached 50 to 60 per cent. *T. semiluteus* is now produced commercially for thrips control in avocado and citrus orchards and ornamentals, but the very high numbers needed, and being unable to mass produce both thrips and parasitoids efficiently is a serious constraint for their economic exploitation.

Pathogens

Steinernema spp. and *Heterorhabditis* spp. kill thrips, but percentage kill has varied widely among trials. As these nematodes only kill the soil inhabiting stages, frequent applications will be needed for effective control. Other entomophilic nematodes, *Thripinema* spp., have been found inhabiting abdomens of female *F. occidentalis* in the USA (Greene & Parrella, 1995), and appeared to be the most common natural enemy of this thrips in flower bud samples (Heinz *et al.*, 1996), but whether they can be commercially exploited to significantly and timely reduce thrips populations is unclear yet. They might, however, be effectively transmitted within a thrips population on the plant and thus be more useful than the purely soil inhabiting nematodes.

More than 15 species of entomopathogenic fungi have been found to infest thrips species. They are summarized by Butt and Brownbridge (1997). Scientific studies on the potential of fungi for thrips control are, however, limited. Presently, the best studied species which is commercially produced, *Verticillium lecanii* (Zimmerman) can be used for additional control of thrips populations, but not as the major control agent. The main limitation for application of fungi is the high humidity needed for infection of thrips.

BIOLOGICAL CONTROL OF THRIPS UNDER PRACTICAL CONDITIONS

Orius and *Amblyseius* spp. are successfully used commercially for biological control of thrips in vegetables (mainly sweet pepper and cucumber). *Orius* spp. are introduced as a single seasonal release (in pollen producing crops like sweet pepper) or twice (in crops without pollen). *Amblyseius* spp. have to be introduced in the form of inundative releases either regularly into crops without pollen (e.g. every two weeks in cucumber) or in "slow release systems" in which food for the predatory mites is provided. In pollen producing crops one release of *Amblyseius* is sufficient. Usually very high numbers *of A. cucumeris* have to be released. *A. degenerans* is a more efficient predator of thrips, but its mass production is difficult. Ramakers & Voet (1996) developed on open rearing system where *A. degenerans* is reared on potten pollen-bearing *Ricinus communis* plants. These banker plants can be put in the greenhouse to establish early colonies of the predator in a crop that does not yet have pollen, or even in plant propagation houses where biological control is becoming increasingly popular. In winter, it is important to release non-diapausing species or strains of *Orius* and *Amblyseius*. Releases of the predatory mites *Hypoaspis* and the fungus *Verticillium* have some additional control effect on thrips.

In ornamentals, thrips are considered the most problematic pest to control. In roses, several cultivars are very sensitive to thrips and frequent chemical control is applied. Other cultivars need very few chemical applications or none at all. Chemical control against thrips makes biological control of other pests very difficult as most thrips pesticides have a long lasting negative effect on natural enemies. Biological control of thrips can be started very early by release of the soil inhabiting predatory mite *H. miles* which kills soil visiting thrips stages. For thrips control on the plant, *A. cucumeris* is advised. Results with *Orius* and *A. degenerans* are not satisfactory. *Orius* is able to significantly reduce thrips in chrysanthemum. Usually thrips are not a serious problem in poinsettia and a quite high thrips density can be tolerated before control is needed.

CONCLUSIONS

Although far from all options for biological control of thrips have been tested, several natural enemies have been found that are good enough to control thrips, but only under specific conditions. Biological control of thrips in vegetables is much more common than in ornamentals. Most attention has been paid to phytoseid predators, but recently studies on *Orius* have strongly increased. Phytoseiidae were studied initially more because of ease of mass rearing than because of control efficiency. Because of the problems to effectively control *F. occidentalis* with Phytoseiidae, Anthocoridae are currently receiving much attention. The predacious mites and anthocorid predators have provided sufficient control of thrips in greenhouse crops like sweet pepper and cucumber worldwide, while performance in floriculture was less satisfactory until recently. Pathogenic fungi have been used as additional control agents. Predatory thrips and parasitoids, though the only very specific natural enemies of thrips, have not shown much potential for control to date.

REFERENCES

Butt T M; Brownbridge M (1997). Fungal pathogens of thrips. In: *Thrips as Crop Pests*, ed. T Lewis, pp. 399-433. CAB International, Wallingford, Oxon.

Greene I D; Parrella M P (1995). Two new natural enemies of western flower thrips in California, In: *Thrips Biology and Management*, eds B L Parker M Skinner & T Lewis, pp. 277-283. NATO ASI Series, Series-A: Life Sciences, Vol. 276.

Heinz K M; Heinz L M; Parrella M P (1996). Natural enemies of western flower thrips indigenous to California ornamentals. *IOBC/WPRS Bulletin* **19 (1)**, 51-54.

Houten Y van; Rijn P C J van; Tanigoshi L K; Stratum P van; Bruin J (1995). Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomologia Experimentalis et Applicata* **74**, 225-234.

Lenteren J C van (1995). Integrated Pest Management in Protected Crops. In: *Integrated Pest Management,* ed. D. Dent, pp. 311-343, Chapman and Hall.

Lenteren J C van (1997). Benefits and risks of introducing exotic macro-biological control agents into Europe. *Bulletin OEPP/EPPO* **27**, 15-27.

Lenteren J C van; Roermund H J W van; Sütterlin S (1996). Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*): how does it work? *Biological Control* **6**, 1-10.

Lenteren J C van; Roskam M M; Timmer R (1997). Commercial mass production and pricing of organisms for biological control of pests in Europe. *Biological Control* **10**, 143-149.

Lenteren J C van; Woets J (1988). Biological and integrated pest control in greenhouses. *Annual Review of Entomology* **33**, 239-269.

Lewis T ed. (1997). *Thrips as Crop Pests*. CAB International, Wallingford, Oxon. 740 pp.

Loomans A J M; Lenteren J C van (1995). Biological control of thrips pests: a review on thrips parasitoids. *Wageningen Agricultural University Papers* **95-1**, 88-201.

Loomans A J M; Lenteren J C van; Tommasini M G; Maini S; Riudavets J eds (1995). Biological Control of Thrips Pests. *Wageningen Agricultural University Papers* **95-1**.

Loomans A J M; Murai T; Greene I D (1997). Interactions with hymenopterous parasitoids and parasitic nematodes. In: *Thrips as Crop Pests*, ed. T Lewis, pp. 355-397. CAB International, Wallingford, Oxon.

Loomans A J M; Vierbergen G (1997). *Thrips palmi*: a next thrips pest in line to be introduced into Europe? *IOBC/WPRS Bulletin* **20(4)**, 162-168.

McMurtry J A; Badii M H (1991). Greenhouse thrips, *Heliothrips haemorrhoidalis*, in California avocado orchards: biological control studies. In: *Towards Understanding Thysanoptera*, eds B L Parker; M Skinner & T. Lewis, T, pp. 393-398. Proceedings International Conference on Thrips, Burlington, Vermont USA, February 21-23, 1989.

Ramakers P M J; Voet S J P (1996). Introduction of *Amblyseius degenerans* for thrips control in sweet peppers with potted castor beans as banker plants. *IOBC/WPRS Bulletin* **19(1),** 127-130.

Riudavets J (1995). Predators of *Frankliniella occidentalis* (Perg.) and *Thrips tabaci* Lind.: a review. *Wageningen Agricultural University Papers* **95-1**, 43-87.

Sabelis M W; Rijn P C J van (1997). Predation by insects and mites. In: *Thrips as Crop Pests*, ed. T Lewis, pp. 259-353. CAB International, Wallingford, Oxon.

Tommasini M G; Maini S (1995). *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe. *Wageningen Agricultural University Papers* **95-1**, 1-42.

Vierbergen G (1998). *Echinothrips americanus* Morgan, a new thrips in Dutch greenhouses (Thysanoptera: Thripidae). *Proceedings Experimental and Applied Entomology, N.E.V., Amsterdam* **9**, 155-160.

Wijkamp I; Almarza N; Peters D (1995). Median latent period and transmission of Tospoviruses vectored by thrips. In: *Thrips Biology and Management*, eds B L Parker M Skinner & T Lewis, pp. 153-156. NATO ASI Series, Series-A: Life Sciences, Vol. 276.

Yano E (1996). Biology of *Orius sauteri* (Poppius) and its potential as a biocontrol agent for *Thrips palmi* Karny. *IOBC/WPRS Bulletin* **19(1)**, 203-206.

The commercial development of an *Amblyseius cucumeris* controlled release method for the control of *Frankliniella occidentalis* in protected crops

C Sampson[1]
Novartis BCM (Bunting Biological Control Ltd), Aldham, Colchester, Essex, CO6 3PN, UK

ABSTRACT

When *Frankliniella occidentalis* (western flower thrips) arrived in the UK it established rapidly in a range of protected salad and flower crops causing economic damage. *Amblyseius cucumeris* was identified as a potential biological control agent and provided a partial solution but the release method of repeatedly sprinkling the predators over crops was time consuming, wasteful and resulted in variable control. To improve predator establishment Bunting Biological Control Ltd developed the controlled release system (CRS). This consisted of sachets containing breeding colonies of *A. cucumeris* with prey mites, *Tyrophagus* sp., formulated in a bran-based culture. Experiments to determine the optimum packaging type, predator:prey ratio, number of predators per sachet, strain type and nutritional content demonstrated that it was possible to achieve sustained release of predators onto crops in the absence of pests for at least two months. The sachets were tested on crops and their use resulted in earlier establishment of *A. cucumeris* with 13 times more predators on cucumber leaves and three times more on pepper leaves.

INTRODUCTION

Western flower thrips, *Frankliniella occidentalis*, was first identified in California, USA (Pergande, 1895) from where it spread throughout the world by trade (Vierbergen, 1995). Once in the UK (Anonymous, 1986) the pest established in protected cucumber, pepper and flower crops causing significant crop loss. Few chemicals were approved to control *F. occidentalis* on protected salads, and those available were either phytotoxic, disruptive to established biological control programmes or ineffective due to pesticide resistance (Mollema *et al.*, 1990). Growers therefore required a biological control option.

Amblyseius cucumeris had been identified as a possible candidate (Ramakers and van Lieburg, 1982) and initial use on commercial nurseries provided promising but variable control (Ramakers *et al.*, 1989; Bennison *et al.*, 1990). *A. cucumeris* feed on first instar thrips larvae (Gillespie and Ramey, 1991) and control relies on complete cover of a crop with the predator before thrips establish. Repeated sprinkling of the predator over crops proved labour intensive, wasteful and messy as much of the bran fell on the floor or got stuck on leaves. Establishment on crops was relatively slow and an improved method of release was needed.

At Bunting Biological Control Ltd, Dr Don Griffiths conceived the idea of releasing self-contained breeding colonies of predators onto plants providing a sustained release of predators from a single introduction, even in the absence of pests. Initial experiments were carried out

[1] Current address: Horticulture Research International, Stockbridge House, Cawood, N. Yorkshire, YO8 3TZ

between 1987 and 1989 using colonies of approximately 300 predators placed in eppendorf tubes together with prey mites (*Tyrophagus* sp.) to feed on, bran as a substrate to move on and a specially formulated prey food (Wall, unpublished data). These were replaced by breathable paper sachets, which were cheaper to produce, easier to transport and could be hung on plants. This prototype was launched at the end of 1989 for the 1990 season and was the first commercially available biological control product that did not rely on an existing pest population for establishment on a crop.

This paper reports on experiments carried out between 1990 and 1993 to refine the system in order to determine optimum packaging, predator:prey ratio and volume of prey food. A diapause resistant strain of *A. cucumeris* (Morewood & Gilkeson, 1991; Houten *et al.*, 1995) was compared to the strain in production in order to improve early season establishment. Following these trials a new formulation was launched in the 1994 season. The performance of the prototype sachets and that of the improved formulation was tested on commercial cucumber and pepper crops in 1990 and 1994 as compared to the sprinkle release system for the control of *F. occidentalis*.

MATERIALS AND METHODS

Development of the sachets

Packaging type

A. cucumeris cultures were placed into breathable paper, impervious paper or cardboard sachets. Eight sachets of each paper type were placed into separate sealed containers at 50%, 70% and 90% r.h. (23°C) maintained by potassium hydroxide solutions. The sachets were mounted on blue sticky traps and the numbers of emerging *A. cucumeris* were counted. After 42 days the number of live predators left in each sachet were counted. The same formulations were tested on a commercially grown, mature cucumber crop. For each treatment there were four replicates of ten plants. The numbers of *A. cucumeris* were counted on a leaf of the same size from each plant every 3 to 6 days up to 34 days from release. Daily minimum and maximum r.h. were recorded.

Predator:Prey ratio

A. cucumeris cultures containing low, medium and high predator:prey ratios were applied to a commercial cucumber crop at one per plant. For each treatment there were four replicates of 30 plants. The numbers of *A. cucumeris* were counted on a leaf of the same size from each plant at one, four, eight and 18 days after release. After 18 days the sachets were removed from the plants and the number of predators in each counted.

Sachet contents

A. cucumeris cultures containing 1000 or 300 predators per sachet, a medium predator:prey ratio and low, medium or high volumes of prey food were mounted on blue sticky traps (24°C,

65-70% r.h.). Ten sachets were monitored for each treatment and the numbers of *A. cucumeris* and *Tyrophagus* spp. emerging from each were counted weekly until emergence had stopped.

A. cucumeris strain

Three groups of 10 *A. cucumeris* females and three males of a diapause resistant strain and the strain in production were placed separately in plastic arenas containing wet oasis, with *Tyrophagus* sp. as a food source. These were placed in conditions known to induce diapause (10L:14D and 22°C:17°C). The numbers of eggs laid per day were recorded for ten days.

Results were analysed using analysis of variance.

Establishment of *A. cucumeris* on protected cucumber and pepper crops

The performance of 1990 (300 predators per sachet; low predator:prey ratio; breathable paper) and 1994 (1000 predators per sachet; medium predator:prey ratio; impervious paper) formulations were compared to the sprinkle system of *A. cucumeris* release formerly used by growers (Table 1). Treatments were replicated four times and the number of *A. cucumeris* on one top and one middle and one lower leaf were counted from each of 30 randomly selected plants per plot. Mean daily maximum and minimum temperatures and humidities were recorded.

Table 1. Release rates and methods tested on commercial crops.

Treatment	Crop	Start date	Location	Release strategy
1.1	Cucumber	Feb 1990	Humberside UK	500,000/ha on leaves/2 wks
1.2		Feb 1990	Humberside UK	1 sachet (300) /3 plants/4 wks
1.3		Jan 1994	Humberside UK	1 sachet (1000) /3 plants x1
2.1	Pepper	Feb 1990	Westland NL	750,000/ha on leaves x1
2.2		Feb 1990	Westland NL	1 sachet (400) /12 stems x1
2.3		Feb 1994	Westland NL	500,000/ha on leaves x1
2.4		Feb 1994	Westland NL	1 sachet (1000) /12 stems x1

RESULTS

Development of the sachets

Packaging type

The effect of paper type on the emergence of *A. cucumeris* from sachets at 50%, 70% and 90% r.h. are shown in Figures 1, 2 and 3. At 50% r.h., all the predators had emerged from the breathable paper and cardboard sachets within 10 to 14 days of release. Prey mite and predator mortality was observed in both sachet types with the greatest mortality in the cardboard sachets. Significantly more predators emerged from the impervious sachets ($F=13.7^{***}$; 2,21 d.f.) and the release pattern was steadier with predators emerging up to 42 days from release.

No live mites were found in any of the sachets at 42 days. At 70% r.h. there was no significant difference between the emergence of predators from the different paper types for the first 21 days. After that period the impervious sachets produced significantly more predators than the other two type (F=11.3**; 2,21 d.f). Less than 100 predators were found in each sachet type after 42 days. At 90% r.h., there was no significant difference between the numbers of predators or emergence pattern from all three types of sachet. Between 115 and 250 predators remained in the sachets after 42 days.

The average numbers of *A. cucumeris* on cucumber leaves, which had emerged from the same formulations, is shown in Figure 4. There were no significant differences between the numbers of predators per leaf between the different sachet types for the first 17 days. After 17 days the numbers of mites emerging from the two breathable formulations levelled off and declined, whereas the numbers of predators continued to increase from the impervious sachets with eight times more predators on leaves at the end of the experiment. Mean daily maximum and minimum temperature and humidity ranged between 17-27°C and 46-92% r.h. through the trial period.

Predator:Prey ratio

Figure 5 shows the number of predators that had emerged onto cucumber leaves from sachets containing different predator:prey ratios. There was a trend towards a higher productivity of *A. cucumeris* from the sachets with a higher predator:prey ratio. After 18 days there were significantly more *A. cucumeris* per leaf from sachets with medium and high predator:prey ratios than from the low ratio (F=7.7*; 2,6 d.f.). The average number of predators per sachet after 18 days were 889 (low), 1017 (medium) and 2339 (high) respectively. Large numbers of prey mites emerged in the first three days from the sachets with a high predator:prey ratio and the subsequent pattern of *A. cucumeris* emergence was in flushes.

Sachet contents

The total number of predators and prey emerging from sachets with different volumes of prey food and different numbers of predators is shown in table 2. There was a trend towards increased predator emergence with increased volumes of prey food, but the pattern of emergence varied. With 1000 predators per sachet but low volumes of prey food, half of the predators emerged within two weeks of release and few remained after five weeks. A medium volume of prey food provided the most stable pattern of emergence with approximately 500 predators emerging per two-week period throughout the trial. High volumes of prey food resulted in delayed emergence of predators with the greatest numbers 21 to 35 days after release. 300 predators with a low volume of prey food was the least productive formulation but when a high volume of prey food was added the total emergence was similar to that from the sachets containing 1000 predators with a medium volume of prey food. However, the pattern of emergence from the 300/high prey food sachets was less stable with emergence starting slowly, peaking at 21 days but declined rapidly afterwards, no live predators remained at 49 days in this formulation.

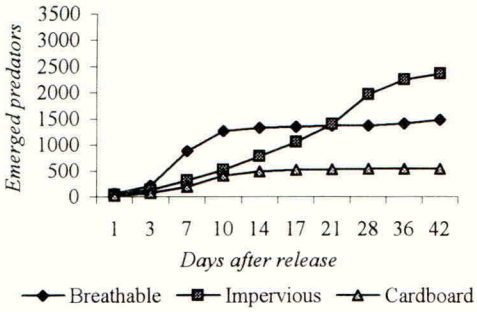


Figure 1. Cumulative number of *A. cucumeris* emerging from sachets made from different paper types at 50% RH.

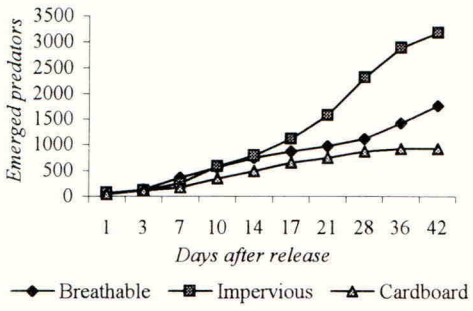


Figure 2. Cumulative number of *A. cucumeris* emerging from sachets made from different paper types at 70% RH.

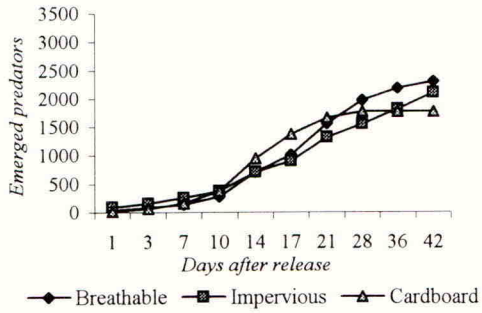


Figure 3. Cumulative number of *A. cucumeris* emerging from sachets made from different paper types at 90% RH.

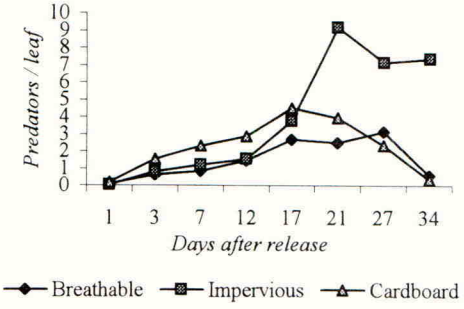


Figure 4. Average number of *A. cucumeris* per cucumber leaf emerged from different sachet types.

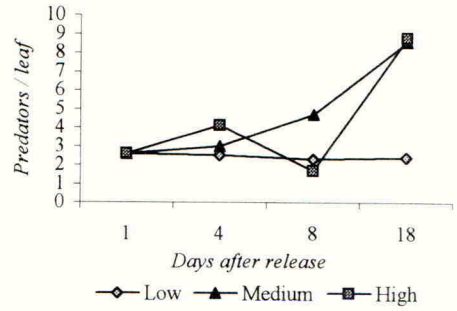


Figure 5. Number of *A. cucumeris* per cucumber leaf emerged from sachets with different predator:prey ratios.

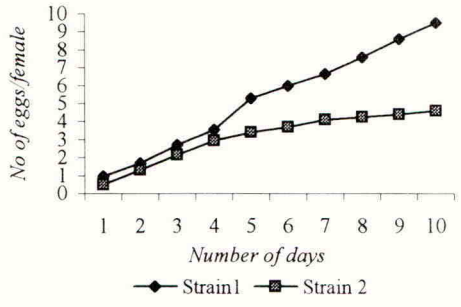


Figure 6. Cumulative number of eggs laid by diapause-resistant (strain 1) and diapause-susceptible (strain 2) *A. cucumeris* in short daylength conditions.

Table 2. The total number of *A. cucumeris* and *Tyrophagus* sp. emerging from sachets with different volumes of prey food and different numbers of predators over 42 days.

Initial No. of predators/sachet	1000	1000	1000	300	300
Volume of prey food	Low	Medium	High	Low	High
	Total number of mites emerged from different formulations				
Mean No. of *A. cucumeris*	1,796	2,285	2,714	583	2,178
Mean No. of *Tyrophagus* sp.	6,009	17,766	20,274	335	4,146

A. cucumeris strain

Figure 6 shows the mean number of eggs laid per day by diapause resistant and diapause susceptible strains of *A. cucumeris*. The diapause resistant strain laid twice as many eggs per female after 10 days in short daylength conditions.

Establishment of *A. cucumeris* on crops

The mean number of motile *A. cucumeris* recorded on cucumber and pepper leaves following different treatments and the number of live *A. cucumeris* remaining in sachets on the different assessment dates is shown in table 3.

The prototype controlled release system improved establishment and distribution of *A. cucumeris* by five times on cucumbers and by one and a half times on peppers as compared to the sprinkle method of release. The 1994 formulation improved establishment further with 13 times more predators on cucumbers and three times more on peppers. Average temperatures ranged between 20°C and 22°C in the monitored crops. Relative humidity averaged between 65% and 85% but regularly fell below 60% at night time and occasionally fell as low as 40% r.h..

Table 3: The mean number of *A. cucumeris* per leaf following different release strategies with the mean number of predators remaining per sachet shown in parentheses.

Treatment	Crop	Mean number of *A. cucumeris* per leaf				
		2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
1.1	Cucumber	0	0	0.1	0.2	0.03
1.2		0.02	0.3	0.5	2.0	2.3
		(478)	(999)	(317)	(856)	(-)
1.3		1.0	4.7	2.5	2.1	2.5
		(555)	(1480)	(1127)	(1892)	(-)
2.1	Pepper	0.4	1.9	1.8	1.3	1.3
2.2		2.4	3.3	1.4	1.3	1.9
		(256)	-	-	-	(5)
2.3		0.3	1.1	1.2	1.7	1.6
2.4		0.6	4.3	6.0	4.1	2.5
		(858)	(2000)	(1080)	(1092)	(822)

(-) No data

DISCUSSION

A. cucumeris controlled release system

Modifications to the sachet and its contents were shown to affect the productivity and release pattern of the sachets. The exact pattern and timing of emergence was dependent on a number of factors with the relationship between external relative humidity and the moisture retaining properties of the sachet being critical. In the crop environment the sachets performed in a similar way to those at 70% r.h. in the laboratory experiments. Sachets made of impervious paper were the longest lasting and the most productive as they retained a moister microclimate inside the sachets when external humidities fell below 70%. At 40-50 % r.h. there was prey mite mortality in sachets that did not retain moisture and the predators moved rapidly out of sachets. As r.h. increased the sachets produced more predators and the difference between paper types became less important but the impervious paper type always performed equal to or better than the other types and offered protection from adverse conditions. None of the packaging tested offered complete protection from drying out and growers should avoid placing the sachets directly over heating pipes in a young crop or leaving them unopened in direct sunlight.

The productivity of the sachets was further improved by increasing the available prey inside, either directly or by increasing the volume of food for the prey. However, with too much prey the emergence pattern became less stable. When there were large numbers of prey mites they became overcrowded and moved out onto crops, which occasionally caused crop damage. In addition, the prey food was quickly used up and the longevity of the sachet compromised. Where initial predator numbers were low but prey numbers high, the predators stayed inside the sachets until they built up and emergence was delayed. Increasing predator numbers provided an initial release of predators onto the crop and when combined with medium levels of prey this formulation provided the most stable release pattern. Under ideal conditions these sachets remained productive in crops for over 12 weeks.

Establishment of *A. cucumeris* on protected cucumber and pepper crops

Introducing breeding colonies of *A. cucumeris* enabled growers to improve establishment and distribution of the predator over crops whilst thrips numbers were low. This was essential in preventing early season build-up of thrips. The use of a diapause resistant strain also improved performance at this critical time of year. The greatest benefits of the system were observed on cucumber crops, which do not have pollen. On cucumbers, *A. cucumeris* establishment largely relied on release from the culture packs, which needed to be replaced periodically to provide full season protection. On peppers, *A. cucumeris* feed on pollen and can reproduce on the crop once the flowers are open and a single release was normally sufficient to provide full season protection.

The improved distribution and establishment of *A. cucumeris* on cucumber and pepper crops corresponded directly with improved control of *F. occidentalis* as has been reported elsewhere (Bennison and Jacobson, 1991; Jacobson, 1995). Over 95% of UK and Dutch cucumber and pepper growers now use the system or similar systems developed subsequently by competitors. The system is also used successfully in a variety of flower crops.

ACKNOWLEDGEMENTS

Thanks go to Dr Don Griffiths for the concept, to Dr Clive Wall for developing the prototype sachets, to Margaret Kay and Vanessa King for technical assistance, to Dr Richard GreatRex and Dr Clive Stinson for rearing *A. cucumeris* and producing the specifications, to Frits Veenman (Brinkman BV) for assisting in field trials and to numerous growers in the UK and The Netherlands for their willingness to try new ideas. The 1990 commercial cucumber trial was carried out by Rob Jacobson (ADAS, Leeds).

REFERENCES

Anonymous, Asia Pacific Plant Protection Commission (1986). Pest and disease records. *United Kingdom. Quarterly Newsletter*, **29 (3)**, 37.

Bennison J A; Hockland S; Jacobson R (1990). Recent developments with integrated control of thrips on cucumber in the United Kingdom. *International organisation for biological and integrated control of noxious animals and plants, Glasshouse pests* **XIII/5**, 19-25.

Bennison J A; Jacobson R (1991). Integrated control of *Frankliniella occidentalis* (Pergande) in UK cucumber crops–evaluation of a controlled release system of introducing *Amblyseius cucumeris*. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **56/2a**, 251-258.

Gillespie D R; Ramey C A (1988). Life history and cold storage of *Amblyseius cucumeris* (Acarina: Phytoseidae). *Journal of the Entomological. Society British Columbia* **85**, 71-76.

Houten Y M van; Rijn P C J van; Tanigoshi L K; Stratum P van; Bruin J (1995). Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomologia Experimentalis et Applicata* **74**, 225-234.

Jacobson R J (1995). Integrated management of cucumbers – prevention of establishment of *Frankliniella occidentalis* (Pergande). *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **60/3a**, 857-863.

Mollema C; Steenhuis G van; Rijn S P (1990). Development of a method to test resistance to western flower thrips (*Frankliniella occidentalis)* in cucumber. *Obnoxious Animals/ West Palaearctic Regional Section Bulletin* (IOBC/WPRS) **13(6)**, 113-116.

Morewood W D; Gilkeson L A (1991). Diapause induction in the thrips predator *Amblyseius cucumeris* (*Acarina:Phytoseiidae*) under greenhouse conditions. *Entomophaga* **36 (2)**, 253-263.

Pergande T (1895). Observations on certain Thripidae. *Insect Life, Wash.* **7**, 390-395.

Ramakers P M J; van Lieburg M J (1982). Start of commercial production and introduction of *Amblyseius mckenzie* (Acarina: Phytoseidae) for the control of *Thrips tabaci.* (Thysanoptera: Thripidae) in glasshouses. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **47/2**, 541-545.

Ramakers P M J; Dissevelt M; Peeters K (1989). Large scale introductions of phytoseiid predators to control thrips in cucumber. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit. Gent* **54/3a**, 923-229.

Vierbergen G (1995). International movement, detection and quarantine of Thysanoptera pests. In: *Thrips Biology and Management* eds Parker B L; Skinner M; Lewis T, 119-132.

Novel strategies for improving biological control of western flower thrips on protected ornamentals - attraction of western flower thrips to verbena plants

E M Pow, A M Hooper, M C Luszniak, J A Pickett & L J Wadhams
Dept. of Biological & Ecological Chemistry, IACR-Rothamsted, Harpenden, AL5 2JQ, UK

J A Bennison
ADAS Boxworth, Boxworth, Cambridge, CB3 8NN, UK

ABSTRACT

'Lure' plants, attractive to pests, can be used as part of a 'push-pull' strategy, within Integrated Pest Management (IPM) programmes in glasshouses, to concentrate pests in areas where supplementary biological control agents could then be applied. In olfactometer tests, flower volatiles from three verbena cultivars, Sissinghurst Pink, Pink Parfait and Tapien Pink, were attractive to western flower thrips (WFT), *Frankliniella occidentalis*. Volatiles were collected from flowers by air entrainment and the main components identified by GC-MS and microcell nuclear magnetic resonance spectroscopy. Tapien Pink and Pink Parfait both produced one enantiomer of linalool oxide pyran, which were diastereoisomic to each other. Sissinghurst Pink flowers produced both of the above. The linalool oxide pyran produced by Pink Parfait flowers was attractive to WFT in the olfactometer, but the pyran produced by Tapien Pink flowers and the mixture of pyrans produced by Sissinghurst Pink flowers was not attractive. Additional components identified include 4,8-dimethyl-1,3,7-nonatriene and 4,8,12-trimethyl-1,3,7,11-tridecatetraene. The former is of particular interest as it has been implicated as a plant distress signal for the attraction of parasitoids and predators to herbivore-damaged plants.

INTRODUCTION

Biological control agents are being increasingly used for the control of pests in a variety of pot and bedding crops grown under glass in the UK. However, at some times of year and on some crops, this control can break down and improvement of biological control strategies is therefore being sought.

The use of semiochemicals to manipulate the behaviour of pest and/or beneficial insects has shown considerable potential in several agricultural studies. In 'push-pull' strategies, pests are repelled from the crop using non-host volatiles or antifeedants, and attracted into a trap crop area where control measures can be concentrated (Pyke *et al.*, 1987; Miller & Cowles, 1990; Smart *et al.*, 1994; Pickett *et al.*, 1997). This type of strategy would fit directly into current IPM strategies within glasshouses, reducing application times and costs for biological control agents.

The western flower thrips (WFT), *Frankliniella occidentalis*, is an important pest on ornamentals and is amongst the pest species which most frequently require pesticide applications for their control, thus interrupting IPM programmes. *Verbena x hybrida* plants

are known to be very attractive to WFT (Bennison *et al.*, 1998a) and are therefore potential trap plants which can be used as a 'pull' component. We have investigated the attractiveness of these plants to WFT and identified the volatiles produced by verbena flowers.

METHODS AND MATERIALS

Laboratory bioassays

Frankliniella occidentalis was reared on potted chrysanthemum plants (cv. Yuba) at 20°C and 16:8 LD. *Verbena x hybrida* plants were grown under greenhouse conditions. Responses of adult female WFT to plant volatiles were tested in a modified Pettersson star olfactometer (Pettersson, 1970), with a weak air stream directed towards the centre from each of four side arms. The response to flowers of the cultivars Tapien Pink, Pink Parfait and Sissinghurst Pink was tested. Following preliminary bioassays with a range of concentrations, responses to 100 µg of linalool oxide enantiomers and mixtures of linalool oxide enantiomers were also tested. 4,8-Dimethyl-1,3,7-nonatriene was tested over the range 100 ng to 100 µg.

Test or control materials were placed in glass tubes attached to each arm of the olfactometer. The test material was placed in one of the arms, with the other three arms serving as controls. Where fresh plant material was tested, damp filter paper was put into all four arms to equalise humidities. Test chemicals were applied to filter paper and an equal volume of solvent on filter paper was used in the control arms. Control bioassays with no test stimulus in any arm were also carried out to check that there was no bias to one particular area. The arena of the olfactometer was divided into five zones, one for each arm and the central region as the fifth zone. Thrips were tested individually, and in each replicate the length of time the insect spent in, and the number of entries into each arm was recorded. Each replicate of a bioassay was run for 10 min and the olfactometer was rotated 90° every 2.5 min. There was a minimum of six replicates per test and the results were analysed using a paired *t*-test.

Air entrainment of plants and identification of chemicals

Volatiles were collected from cut flowering stems of verbena (cv. Tapien Pink, Pink Parfait and Sissinghurst Pink) using a dynamic head space (air entrainment) technique (Blight, 1990). Groups of flowers were contained in water in conical flasks placed in glass culture vessels (5 litre). Volatiles were drawn from the vessels, using purified air (1 litre min^{-1}), onto a tube containing 50 mg of the adsorbent Porapak Q (Waters Assoc. Inc, U.S.A) and eluted from the Porapak Q with distilled ether every 2-3 days.

Samples of volatiles were analysed on either a 50 m x 0.32 mm internal diameter (id) methyl silicone bonded–phase (HP-1) fused silica capillary column or a 30 m x 0.32 mm id HP-WAX column fitted in a Hewlett Packard 5890 gas chromatograph (GC), equipped with a cold on-column injector and a flame ionisation detector (FID). For both columns, the carrier gas was hydrogen and the oven temperature was maintained at 40°C for 2 min and then programmed at 10°C min^{-1} to 220°C. For identification of components, a capillary GC column (50 m x 0.32 mm id HP-1) was directly coupled to a VG Autospec mass spectrometer (MS) and integrated data system. Ionisation was by electron impact at 70eV,

230°C. The GC was maintained at 30°C for 5 min and then programmed at 5° min^{-1} to 180°C. Tentative identifications by GC-MS were confirmed by comparison with authentic samples and then by peak enhancement on GC.

Microcell ^{1}H NMR spectroscopy was used to identify the major volatile produced by Tapien Pink and Pink Parfait as diastereoisomers of linalool oxide pyran. Enantioselective synthesis using Sharpless asymmetric dihydroxylation methodology and chiral GC coinjection with natural material enabled verification of the absolute stereochemistry (Hooper *et al.*, 1998).

RESULTS

The volatiles from flowers of all three cultivars of verbena were attractive to WFT (Figure 1).

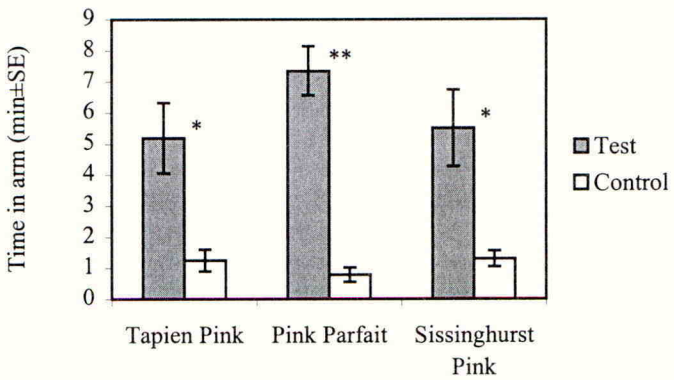
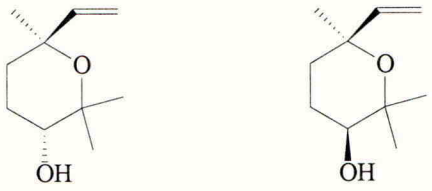


Figure 1. Responses of adult female WFT to volatiles from verbena (cvs Tapien Pink, Pink Parfait and Sissinghurst Pink) flowers in the olfactometer. * = $P < 0.05$, ** = $P < 0.01$

The main component of the volatiles produced by all three varieties of verbena were identified as linalool oxide pyrans. However, Tapien Pink produced one diastereoisomer (**I**), Pink Parfait another (**II**), and Sissinghurst Pink a mixture of the two in a 55:45 (**I**:**II**) ratio:



I **II**

The two diastereoisomers of linalool oxide were synthesised enantioselectively (Hooper *et al.*, 1998) and then tested in the olfactometer. Compound **II** was attractive to WFT but compound **I** was not (Figure 2). Compounds **I** and **II** were then presented together, in a 55:45 ratio as produced by the Sissinghurst Pink flowers. The total amount of linalool oxide presented was kept the same as in the previous two bioassays. Again, no attraction was seen (Figure 2).

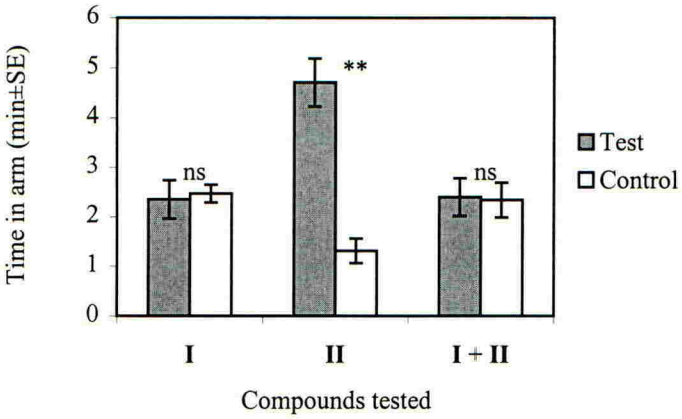


Figure 2. Responses of adult female WFT to linalool oxides in the olfactometer. 100 µg of linalool oxides **I** and **II** were tested separately, and then a 55:45 ratio of **I**:**II** giving a total amount of amount of 100 µg. ns = not significant at $P = 0.05$, ** = $P < 0.01$

To test whether compound **I**, which was not attractive to the WFT, affected the response to the attractive compound **II**, the compounds were again presented together in a 55:45 ratio but with compound **II** at a concentration which was previously attractive to WFT (100 µg), together with the appropriate amount of compound **II** (122 µg) to maintain the correct ratio produced by the Sissinghurst Pink flowers. Compound **II** was also presented alone (100 µg) to check the responsiveness of the WFT on that day. The thrips again spent significantly more time in the arm with compound **II** alone (mean time ± SE spent in test arm 4.20 ± 0.67 min, mean time ± SE spent in control arms 1.45 ± 0.25 min, $P<0.05$), but the addition of compound **I** reduced the attraction to compound **II** and increased the variability in response (mean time ± SE spent in test arm 3.58 ± 1.04 min, mean time ± SE spent in control arms 1.81 ± 0.29 min, not significant at $P=0.05$).

A number of other compounds were found to be produced by the verbenas cultivars including 4,8-dimethyl-1,3,7-nonatriene and 4,8,12-trimethyl-1,3,7,11-tridecatetraene. The former was also tested over a range of concentrations in the olfactometer but no attraction was seen at any concentration.

DISCUSSION

Kirk (1985) proposed that flower-dwelling thrips are likely to use flower volatiles for in-flight orientation, and a number of studies have shown that general flower volatiles such as *p*-anisaldehyde are attractive to WFT (Brødsgaard, 1990; Tuelon *et al.*, 1993; Frey *et al.*, 1994).

Linalool oxides, both furans, and pyrans, have been identified as components of the odours of a number of flower species from a range of families (eg; Pichersky *et al.*, 1994; Borg-Karlson *et al.*, 1996), and therefore may be another group of commonly produced flower volatiles which are attractive to WFT. However, the lack of attraction to the linalool oxide pyran produced by the Tapien Pink flowers, and its inhibitory effect on the attraction to the linalool oxide pyran produced by the Pink Parfait flowers, together with the lack of response to 4,8-dimethyl-1,3,7-nonatriene, suggests that, for a behavioural response, specific mixtures of volatile components may be required.

4,8-Dimethyl-1,3,7-nonatriene is frequently produced by plants in response to insect feeding and such herbivore-induced volatiles have been shown to be attractive to predators and parasitoids (see Dicke, 1994 for review). The response of the anthocorid bug, *Anthocoris nemorum*, which predates on WFT (Bennison *et al.*, 1998b) to verbena volatiles is now being investigated. This predator is known to be attracted to herbivore-induced volatiles in other herbivore-plant combinations (Scutareanu *et al.*, 1997).

ACKNOWLEDGEMENTS

Funding for this work was provided by the Ministry of Agriculture, Fisheries and Food, UK. IACR-Rothamsted is grant-aided by the Biotechnology and Biological Sciences Research Council, UK.

REFERENCES

Bennison J A; Pow E M; Wadhams L J; Maulden K A & Wardlow L R (1998a). Improving biological control of western flower thrips, *Frankliniella occidentalis,* on greenhouse ornamentals. *6th International Symposium on Thysanoptera, Antalya, Turkey.* In press.

Bennison J A; Maulden K A; Pow E M; Wadhams L J & Wardlow L R (1998b). Novel strategies for improving biological control of western flower thrips on protected ornamentals - potential new biological control agents. This volume.

Blight M M (1990). Techniques for isolation and characterization of volatile semiochemicals of phytophagous insects. In: *Chromatography and isolation of insect hormones and pheromones*, eds A R McCaffery & I D Wilson, pp. 281-288. Plenum Press: New York.

Borg-Karlson A K; Unelius C R; Valterova I & Nilsson L A (1996) Floral fragrance chemistry in the early flowering shrub *Daphne mezereum*. *Phytochemistry* **41**, 1477-1483.

Brødsgaard H F (1990). The effect of anisaldehyde as a scent attractant for *Frankliniella occidentalis* (Thysanoptera: Thripidae) and the response mechanism involved. *SROP/WPRS Bull.* **XIII/5**, 36-38.

Dicke M (1994). Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. *Journal of Plant Physiology* **143**, 465-472.

Frey J E; Cortada R V & Helbling H (1994). The potential of flower odours for use in population monitoring of western flower thrips, *Frankliniella occidentalis* Perg. (Thysanoptera: Thripidae). *Biocontrol Science and Technology* **4**, 177-186.

Hooper A M; Pow E M; Wadhams L J; Luszniak M C; Pickett J A & Bennison J A (1998). Verbena flower volatiles attractive to western flower thrips. *Pesticide Science*. In press.

Kirk W D J (1985). Effect of some floral scents on host finding by thrips (Insecta: Thysanoptera). *Journal of Chemical Ecology* **11**, 35-43.

Miller J R & Cowles R S (1990). Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology* **16**, 3197-3212.

Pichersky E; Raguso R A; Lewinsohn E & Croteau R (1994). Floral scent production in *Clarkia* (Onagraceae). I. Localisation and developmental modulation of monoterpene emission and linalool synthase activity. *Plant Physiology* **106**, 1533-1540.

Pettersson J (1970). An aphid sex attractant. 1. Biological studies. *Entomologica Scandinavica* **1**, 63-73.

Pickett J A; Wadhams L J & Woodcock C M (1997). Developing sustainable pest control from chemical ecology. *Agriculture, Ecosystems and Environment* **64**, 149-156.

Pyke B; Rice M; Sabine B & Zalucki M (1987). The push-pull strategy - behavioural control of *Heliothis*. *The Australian Cotton Grower* **May-July**, 7-9.

Smart L E; Blight M M; Pickett J A & Pye B J (1994). Development of field strategies incorporating semiochemicals for the control of the pea and bean weevil, *Sitona lineatus* L. *Crop Protection* **13**, 127-135.

Scutareanu P; Drukker B; Bruin J; Posthumus M A & Sabelis M W (1997). Volatiles from *Psylla*-infested pear trees and their possible involvement in attraction of Anthocorid predators. *Journal of Chemical Ecology* **223**, 2241-2260.

Tuelon A J; Penman D R & Ramakers M J (1993). Volatile chemicals for thrips (Thysanoptera: Thripidae) host-finding and applications for thrips pest management. *Journal of Economic Entomology* **86**, 1405-1415.