

Know-how for Horticulture™

Continued development of management strategies for western flower thrips and tomato spotted wilt virus in vegetables

Dr. Roger Jones Department of Agriculture Western Australia

Project Number: VG00065

VG00065

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Know-how for Horticulture™

FINAL REPORT

HORTICULTURE AUSTRALIA PROJECT VG00065

CONTINUED DEVELOPMENT OF MANAGEMENT STRATEGIES FOR WESTERN FLOWER THRIPS AND TOMATO SPOTTED WILT VIRUS IN VEGETABLES

Dr R.A.C. Jones, *et al.* Department of Agriculture, Western Australia





April 2004

HORTICULTURE AUSTRALIA PROJECT VG00065

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This is the final report for Project VG00065 'Continued development of management strategies for western flower thrips and tomato spotted wilt virus'.

April 2004

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MEDIA SUMMARY

TSWV causes serious losses in yield and quality of in vegetables growing in seedling nurseries, protected cropping or field systems worldwide. These losses and the resulting financial damage can be limited by controlling epidemics with measures that minimise the virus infection source or suppress virus spread.

Worldwide, western flower thrips (WFT) is an important pest of vegetables in its own right causing direct feeding damage which seriously decreases yield and quality in some vegetable crops, especially cucumbers but also to a lesser extent capsicum and lettuce. However for vegetables, the damage WFT causes through transmitting tomato spotted wilt virus (TSWV) is far more serious leading to large yield reductions, impairing quality and causing abandonment of crops. The vegetable crops most at risk from severe damage by TSWV and other tospoviruses are capsicum, chillies, lettuce, tomato, eggplant and others like celery are also affected. Reliability of production, amount of production and the ability to produce a product of a quality acceptable to the market are all seriously impaired. TSWV is also transmitted by onion or tomato thrips and in many regions these are the main vectors.

To reduce yield and quality losses in vegetable crops highly effective integrated disease management strategies for TSWV were developed for different scenarios (seedling nurseries, protected cropping, field crops). The results of field experiments and trials and field observations contributed to the different control measures included within each strategy.

Impact of granular and drench applied systemic insecticides in suppressing TSWV spread

Two preliminary field experiments were done over two consecutive years to help assess the use of the granular insecticide acephate, the wettable granular insecticide thiamethoxam and drenching soil with the flowable insecticide imidacloprid, to control thrips vectors and TSWV spread in capsicums. There were low overall final levels of TSWV spread in both field experiments (15% and 4% TSWV). However, both experiments indicated an early effect of suppression of TSWV spread from insecticide treating the tomato 'infector' source plants, which subsequently resulted in delayed spread of TSWV to capsicum plants.

Subsequently, three field experiments were done to determine whether drenching plants with the systemically active neonicotinoid insecticides thiamethoxam and imidacloprid is effective in suppressing spread of TSWV by thrips vectors in lettuce. Separate treatments to TSWV 'infector' tomato (source) and healthy lettuce (recipient) plants provided information on the relative importance of targeting control at virus acquisition by nymphs verses virus transmission to healthy plants by adults. Drench application was either to seedlings just before transplanting or to soil around plants. The vectors found were WFT, tomato and onion thrips, but tomato and onion thrips predominated.

Overall ratios of external to internal TSWV spread in plots without insecticide varied from 1:2.3 to 1:2.8 between field experiments.

In the three field experiments, applying thiamethoxam as a soil drench to young source plants and recipient seedling transplants together suppressed TSWV incidence by 86%, while such application to young source or recipient seedlings alone diminished it by 67-70%. When thiamethoxam was applied either as a soil drench to old source plants and at the same time as a seedling drench to recipient plants or as a seedling drench to recipient plants alone, incidence was suppressed by 65-73% and 54-73% respectively. Its application as a soil drench to old source plants alone diminished incidence by only 13% or not significantly. When imidacloprid was applied either as a soil drench to old source plants and at the same time as a seedling drench or as a seedling drench alone, it suppressed TSWV incidence by 90-92% and 80% respectively. Although adult vector thrips and nympal thrips numbers were low, diminished numbers of adult vector thrips and/or nymphal thrips were sometimes recorded due to insecticide application.

The key outcome was that drenching healthy seedlings with neonicotinyl insecticides just before transplanting can be an effective chemical control measure to include in integrated disease management strategies that suppress TSWV epidemics in short-lived crops like lettuce.

Roles of TSWV source plants and thrips vectors

Patterns of spread of TSWV were examined in lettuce and capsicum plantings in Western Australia into which thrips vectors spread the virus from external virus sources. After an initial trial which paved the way for the work, these plantings were: 1) eight separate field trials into which TSWV 'infector' plants of tomato were introduced alongside or near to plantings of lettuce or capsicum, and 2) three commercial lettuce plantings into which spread from nearby external infection sources was occurring naturally. The vector thrips species were WFT, onion and tomato thrips, at least two of which were always present. Spatial data for plants with TSWV infection collected at different stages in the growing period were assessed by plotting gradients of infection, and using Spatial Analysis by Distance IndicEs (SADIE) and maps of spatial pattern.

Despite the persistent nature of TSWV transmission by thrips vectors, in both lettuce and pepper plantings there was a steep decline in TSWV incidence with distance from external infection sources that were alongside them. The extent of clustering of infected plants increased over time and was greatest closest to the source. In lettuce, the relationship between percentage infection and assessment date was more typical of monocyclic than polycyclic spread. Significant clustering of infected plants distant from TSWV sources confirmed that limited secondary spread was occurring within both crops in addition to the primary introductions that predominated. Spread to lettuce was greater downwind than upwind of the virus source, with magnitude and proximity of source determining the amount of spread. When 15 m wide fallow or non-host (cabbage) barriers separated TSWV sources from lettuce plantings, spread was slower and there

was much less clustering with the latter. In commercial plantings, spread was favoured by TSWV movement within successive side-by-side plantings.

The spatial data from the diverse scenarios examined enabled recommendations to be made over 'safe' planting distances between external infection sources of different magnitudes and susceptible crops that were short-lived (eg. lettuce) or long-lived (eg. capsicum). They also helped validate the inclusion of isolation and 'safe' planting distances, planting upwind, prompt removal of virus sources, avoidance of side-by-side plantings, and deploying intervening non-host barrier crops as control measures within an integrated disease management strategy for TSWV in field vegetable crops.

The roles of different thrips species as vectors of TSWV were assessed through field monitoring of thrips populations and TSWV incidence in vegetable crops over three growing seasons in Victoria, New South Wales and Queensland. In lettuce crops, low levels of WFT were often found without TSWV incidence, but 6 to 8 weeks after the arrival of onion thrips TSWV reached damaging levels often leading to crop abandonment. In capsicums *T. palmi*, tomato thrips, onion thrips and WFT were all present in crops infected with TSWV and *Capsicum chlorosis virus*, but a crop with WFT and tomato thrips was not infected with TSWV. In tomatoes, tomato thrips were present in crops with TSWV incidence up to 40%.

Forecasting and prediction model for WFT

A simple day-degree model based on temperature was developed to predict outbreaks of the vector WFT. Developing a model to predict TSWV is difficult since the vectordisease relationship is affected by many variables. These include abundance of the vector, planting date, abundance and types of host plants, presence and distribution of plants affected by TSWV, movement of the vector, and efficiency of transmission of the virus by the vector. Insufficient data on these variables were available to develop into a predictive model for TSWV soothe topic is discussed generally.

Integrated disease management strategies for TSWV in vegetables

Effective integrated management strategies were devised for TSWV in vegetables growing in seedling nurseries, protected cropping or field systems. Selecting the ideal mix of measures for each production situation required detailed knowledge of the epidemiology of TSWV and the mode of action of each individual control measure so that diverse responses could be devised that were tailored to meet the unique features of each of the different scenarios under consideration. The strategies developed were robust and cause minimal extra expense, labour demands and disruption to normal practices.

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Refereed Scientific Papers

Jones, R A C. (2003). Using epidemiological information to develop effective integrated virus disease management strategies. *Virus Research* **100**:5-30.

Coutts, B.A., Thomas-Carroll, M. and Jones, R.A.C. (2004). Patterns of spread of *Tomato spotted wilt virus* in field crops of lettuce and pepper: spatial dynamics and validation of control measures. *Annals of Applied Biology* (submitted for publication)

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Cook D, and Jones R. (2000). Proactive Research and Extension Avoids Damaging Outbreak of WFT and TSWV in WA Vegetable Crops. In *WA Grower Vol. 31. No. 2* pages 2-3. W.A. Vegetable Growers' Association (inc.).

Workshops/Growers meetings

Vegetable growers meeting at Wanneroo, Perth. National Vegetable Pathologist Working Group, May 2002

Vegetable growers meeting on TSWV and WFT at Wanneroo, Perth. December 2002

FINANCIAL ANALYSIS OF THE PROJECT

HAL Funding Budget vs Actuals

Life of Project 1999/2000 onward

Funding received	Budget	Actual	Variance
2000/01	\$88 000.00	\$88 000.00	\$0.00
2001/02	\$88 000.00	\$88 000.00	\$0.00
2002/03	\$88 000.00	\$88 000.00	\$0.00
Totals	\$264 000.00	\$264 000.00	\$0.00
Expenditure Actuals	Operating	Capital	Total
Expenditure Actuals 2000/01	Operating \$71 155.60	Capital \$0.00	Total \$71 155.60
Expenditure Actuals 2000/01 2001/02	Operating \$71 155.60 \$78 095.71	Capital \$0.00 \$0.00	Total \$71 155.60 \$78 095.71
Expenditure Actuals 2000/01 2001/02 2002/03	Operating \$71 155.60 \$78 095.71 \$114 748.69	Capital \$0.00 \$0.00 \$0.00	Total \$71 155.60 \$78 095.71 \$114 748.69

SECTION 1.0

IMPACT OF GRANULAR AND DRENCH APPLIED SYSTEMIC INSECTICIDES ON TSWV SPREAD

Summary

Two preliminary field experiments were done over two consecutive years to help assess the use of the granular insecticide acephate, the wettable granular insecticide thiamethoxam and drenching soil with the flowable insecticide imidacloprid, to control thrips vectors and TSWV spread in capsicums. There were low overall final levels of TSWV spread in both field experiments (15% and 4% TSWV). However, both experiments indicated an early effect of suppression of TSWV spread from insecticide treating the tomato 'infector' source plants, which subsequently resulted in delayed spread of TSWV to capsicum plants.

Subsequently, three field experiments were done to determine whether drenching plants with the systemically active neonicotinoid insecticides thiamethoxam and imidacloprid is effective in suppressing spread of TSWV by thrips vectors in lettuce. Separate treatments to TSWV 'infector' tomato (source) and healthy lettuce (recipient) plants provided information on the relative importance of targeting control at virus acquisition by nymphs verses virus transmission to healthy plants by adults. Drench application was either to seedlings just before transplanting or to soil around plants. The vectors found were WFT, tomato and onion thrips, but tomato and onion thrips predominated. Overall ratios of external to internal TSWV spread in plots without insecticide varied from 1:2.3 to 1:2.8 between field experiments. Applying thiamethoxam as a soil drench to young source plants and recipient seedling transplants together suppressed TSWV incidence by 86%, while such application to young source or recipient seedlings alone diminished it by 67-70%. When thiamethoxam was applied either as a soil drench to old source plants and at the same time as a seedling drench to recipient plants or as a seedling drench to recipient plants alone, incidence was suppressed by 65-73% and 54-73% respectively. Its application as a soil drench to old source plants alone diminished incidence by only 13% or not significantly. When imidacloprid was applied either as a soil drench to old source plants and at the same time as a seedling drench or as a seedling drench alone, it suppressed TSWV incidence by 90-92% and 80% respectively. Although adult vector thrips and nympal thrips numbers were low, diminished numbers of adult vector thrips and/or nymphal thrips were sometimes recorded due to insecticide application. Drenching healthy seedlings with neonicotinyl insecticides just before transplanting can be an effective chemical control measure to include in integrated disease management strategies that suppress TSWV epidemics in short-lived crops like lettuce

1.1 Preliminary experiments on the impact of granular or drench applied insecticides on TSWV spread in capsicums

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Summary

Two preliminary field experiments were done over two consecutive years to help assess the use of the granular insecticide acephate, the wettable granular insecticide thiamethoxam and drenching soil with the flowable insecticide imidacloprid, to control thrips vectors and TSWV spread in capsicums. There were low overall final levels of TSWV spread in both experiments (15% and 4% TSWV). However, both experiments indicated an early effect of suppression of TSWV spread from insecticide treating the tomato 'infector' source plants, which subsequently resulted in delayed spread of TSWV to capsicum plants.

Introduction

Systemic insecticides applied at transplanting may prove more effective than foliar applications in decreasing spread from TSWV infected plants. This is because the treated plants are then insecticide-protected from the beginning and the insecticide can tackle the first and early second nymphal stages of vector thrips, these stages being the only ones that can acquire the TSWV. Two preliminary field experiments were done over two consecutive years to assess the use of the granular insecticide acephate, the wettable granular insecticide thiamethoxam and drenching soil with the flowable insecticide imidacloprid, to control thrips vectors and TSWV spread in capsicums.

Methods

Expt. 1

Except for one control treatment, which did not have any introduced tomato infector plants (= TSWV source plants), each plot had five TSWV-infected tomato infector plants transplanted into its centre surrounded by 20 healthy capsicum cv. Rialto plants (= healthy recipient plants). Oat buffers were sown around the perimeter of each plot to help minimise any viruliferous thrips movement between treatments. Acephate (Orthene® at 1kg/ha) granular insecticide was applied to the soil at transplanting and imidacloprid (Confidor® at 1330gai/ha) soil drench was applied by syringe to the base of each plant at the same time. Within different plots, the insecticides were applied to both tomato infector plants and capsicum recipient plants, capsicum recipient plants only or tomato infector plants only. Separate treatment of the TSWV-infected source plants (tomatoes in the centres of each plot) and the capsicum recipient plants in the rest of the plot helped identify where insecticides were having the greatest effect (i.e. on the infected virus source plants or the healthy plants becoming infected). In the experiment, there were eight treatments, each with six replicate plots arranged in a randomised block design. The eight treatments were: 1) acephate to capsicums only, 2) acephate to tomatoes and capsicums, 3) acephate to tomatoes only, 4) imidacloprid to capsicums only, 5)

imidacloprid to tomatoes and capsicums, 6) imidacloprid to tomatoes only, 7) no insecticide to capsicum or tomatoes, and 8) no insecticide and no tomatoes.

Starting 2 months after transplanting of the recipient plants, weekly sampling of tomato and capsicum flowers was done to determine if the insecticides were having an effect on thrips numbers. From each plot on each occasion, 10 capsicum and 3 tomato flowers were taken. Fortnightly sampling of individual capsicum recipient plants was done in which a young leaf was taken from each plant on each occasion. The sampling started 6 weeks after the recipient plants were transplanted. The samples were tested by ELISA for TSWV and percentage TSWV plant infection calculated for each plot. In addition, counts were made visually of the capsicum plants with TSWV symptoms and leaf samples from each plant were tested for TSWV by ELISA to confirm its presence.

Expt. 2

Except for one control treatment, which did not have any introduced tomato plants (= TSWV source plants), each plot had 3 TSWV infected tomato infector plants and 14 marigold plants transplanted into its centre surrounded by 50 healthy capsicum cv. Rialto plants (= healthy recipient plants). Oat buffers were sown around the perimeter of each plot to help minimise viruliferous thrips movement between treatments. Thiamethoxam (Actara® at 1820gai/ha) wettable granules was applied by syringe to the soil at the base of each plant being treated at transplanting. Within different plots, the insecticides were applied to both tomato infector plants and capsicum recipient plants, capsicum recipient plants only or tomato infector plants only. Separate treatment of the TSWV-infected source plants (tomatoes in the centres of each plot) and the capsicum recipient plants in the rest of the plot helped identify where insecticides were having the greatest effect (i.e. on the infected virus source plants or the healthy plants becoming infected). Due to poor growth of capsicums at one side of the experimental area, the plots within 2 replicates were removed, so the data presented are for 6 replicates. In the experiment there were five treatments, each with eight replicate plots arranged in a randomised block design. The treatments were 1) thiamethoxam to capsicums only, 2) thiamethoxam to tomatoes and capsicums, 3) thiamethoxam to tomatoes only, 4) no insecticide to capsicum or tomatoes, and 5) no insecticide and no tomatoes. To determine the numbers of thrips vectors present during the experiment 10 capsicum flowers were sampled from each plot each week starting 2 months after transplanting.

The capsicum plants were individually sampled and tested fortnightly for presence of TSWV, starting 3 weeks after transplanting. In plots were TSWV was present, individual plants showing symptoms were tagged and the numbers of infected plants recorded within each plot.

Results and Discussion

Expt. 1

Three TSWV vectors (WFT, tomato and onion thrips) were present in flower samples from the tomatoes and capsicums in the experiment. Initially, overall numbers of WFT and onion thrips were at higher levels than those of tomato thrips, but subsequently WFT

and onion thrips numbers declined while those of tomato thrips increased with higher temperatures as summer approached. Total nymph numbers declined somewhat over time. However, neither insecticide altered measurable thrips levels during the sampling period. Possibly earlier sampling closer to the start of the experiment would have revealed effects of insecticide on thrips numbers, but as no flowers were present until 2 months after transplanting such sampling was not possible.

There was a low overall level of TSWV spread to capsicum plants in the experiment. By 19 weeks after transplanting, spread of TSWV reached a maximum of 14% in plots with confidor applied to both 'infector' and recipient plants and plots were confidor was applied to 'infectors' alone. All other insecticide treated plots had 9-12% TSWV spread these levels being similar to those in plots without insecticide or 'infector' plants (11%). Despite this, a statistically significant decrease (P < 0.05) in TSWV incidence was still obtained 13 wks after transplanting between plots with acephate granules applied to both infector tomato and capsicum recipient plants (no TSWV) and control plots with infector plants and no insecticide (3.6% TSWV). Also, infection was slower to develop in this acephate treatment than in all the others with infector plants. The least TSWV infection incidence (3.3% TSWV 19 wks after transplanting) was in the control plot that had no tomato infector plants showing that the experimental approach of using TSWV source plants in the centre of each plot was valid. None of the other insecticide treatments significantly (P < 0.05) decreased TSWV spread compared to that in the control plots with infectors but without insecticides. Thus, the only effective insecticide in controlling TSWV spread was acephate granular insecticide applied to both the infector and recipient plants. However, the decrease in TSWV spread due to this chemical was temporary as, although TSWV incidence was initially low in these plots up until 3 months after planting, it subsequently shot up to similar levels to those in the control plots without insecticide but with infector plants (11% TSWV, 19 wks after transplanting). Presumably, its effect wore off as plants grew bigger and the interval between its application and sampling increased.

We do not consider that a general recommendation for use of acephate granules is appropriate for TSWV control in capsicums based on these preliminary results.

Expt. 2

The TSWV isolate used to infect the tomato transplants was found to be very severe and resulted in early death of the infector plants. By 4 weeks after capsicum transplanting, most plots had 1 or 2 tomato infector plants left and by 15 weeks all tomato infector plants had died. Three thrips TSWV vectors, tomato, onion and WFT were present in the flower samples

By 15 weeks after transplanting, spread of TSWV to capsicum was very small only reaching 4.4% in the plots with tomato infector plants and no thiamethoxam applied (Fig. 1.1). When insecticide was applied to the capsicums only and tomato infector plants were present, there was a 3.6% TSWV level. Plots with insecticide applied to the tomato infector plants only or to both the tomato infector and capsicum plants had 2% TSWV

spread. Plots with no tomato infector plants and no insecticide had 1.3% TSWV, this representing the extent of spread between, as opposed to within, the plots.

The levels of infection at 15 weeks after transplanting were not significantly different between treatments (at P < 0.05) due to the overall low level of infection in the experiment presumably resulting from the tomato infector plants dying out early. However, Fig. 1.1 does indicate an early effect of suppression of spread from treating the tomato infector plants before they died out, which subsequently resulted in delayed spread of TSWV.

No recommendations over the use of thiamethoxam wettable granules can be made based on these preliminary results with capsicum.



Fig. 1.1. Expt. 2 - Effect of thiamethoxam soil drench on spread of TSWV in capsicum. Treatments: A) thiamethoxam to tomato infector plants only; B) thiamethoxam to tomato infector and capsicum plants; C) thiamethoxam to capsicum plants only; D) no insecticide to tomato infector or capsicum plants; E) no tomato infector plants present or insecticide used.

1.2 Suppressing spread of *Tomato spotted wilt virus* by drenching infected source or healthy recipient plants with neonicotinyl insecticides

(draft of paper submitted for publication to the Annals of Applied Biology)

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Running title: Controlling TSWV with insecticide drenches

Summary

Field experiments were done to determine whether drenching plants with the systemically active neonicotinoid insecticides thiamethoxam and imidacloprid is effective in suppressing spread of Tomato spotted wilt virus (TSWV) by thrips vectors. Separate treatments to TSWV 'infector' tomato (source) and healthy lettuce (recipient) plants provided information on the relative importance of targeting control at virus acquisition by nymphs verses virus transmission to healthy plants by adults. Drench application was either to seedlings just before transplanting or to soil around plants. The thrips vectors found were Frankliniella occidentalis, F. schultzei and Thrips tabaci, but F. schultzei and T. tabaci predominated. Overall ratios of external to internal TSWV spread in plots without insecticide varied from 1:2.3 to 1:2.8 between field experiments. Applying thiamethoxam as a soil drench to young source plants and recipient seedling transplants together suppressed TSWV incidence by 86%, while such application to young source or recipient seedlings alone diminished it by 67-70%. When thiamethoxam was applied either as a soil drench to old source plants and at the same time as a seedling drench to recipient plants or as a seedling drench to recipient plants alone, incidence was suppressed by 65-73% and 54-73% respectively. Its application as a soil drench to old source plants alone diminished incidence by only 13% or not significantly. When imidacloprid was applied either as a soil drench to old source plants and at the same time as a seedling drench or as a seedling drench alone, it suppressed TSWV incidence by 90-92% and 80% respectively. Although adult vector thrips and nympal thrips numbers were low, diminished numbers of adult vector thrips and/or nymphal thrips were sometimes recorded due to insecticide application. Drenching healthy seedlings with neonicotinyl insecticides just before transplanting can be an effective chemical control measure to include in integrated disease management strategies that suppress TSWV epidemics in short-lived crops like lettuce

Key words: TSWV, vegetable, lettuce, systemic insecticide, neonicotinoid, drench, source, recipient, epidemic, incidence, spread, suppression, control, integrated disease management.

Introduction

Tomato spotted wilt virus (TSWV; family *Bunyaviridae*, genus *Tospovirus*) has a host range that includes over 900 dicotyledonous and monocotylonous plant species worldwide (Peters, 1998). It is transmitted by several different thrips species, but *Frankiniella occidentalis* (western flower

thrips) is the most efficient vector (German *et al.*, 1992; Ullman, 1996; Mound, 2002). Following introduction of *F. occidentalis*, major upsurges in TSWV epidemics occurred in vegetable and ornamental crops in several parts of the world (Peters *et al.*, 1996). For example, , after it appeared in the early 1990's in south-west Australia (Malipatil *et al.*, 1993), damage increased considerably with lettuce (*Lactuca sativa*), pepper (*Capsicum annuum*) and tomato (*Lycopersicum esculentum*) amongst the worst affected crops (Latham & Jones, 1996, 1997). TSWV spreads to crops from nearby infected crops, volunteer crop plants and weeds (e.g. Latham & Jones, 1997; Gitaitis *et al.*, 1998; Wilson, 1998; Groves *et al.*, 2001, 2002). With field grown crops, integrated disease management (IDM) strategies against TSWV emphasise phytosanitary and agronomic measures that minimise the source of virus infection, chemical control measures against thrips vectors, and, when available, deployment of TSWV-resistant cultivars (Cho *et al.*, 1989; Brown *et al.*, 1996; Latham & Jones, 1996, 1998; Culbreath *et al.*, 1999; Riley & Pappu, 2000; Jones, 2003; Thomas-Carroll & Jones, 2003; Coutts *et al.*, 2004a).

In developing suitable chemical control measures to include in an IDM package for TSWV, it is important to target the first and early second larval stages of the thrips vector. This is because virus acquisition occurs only in these stages of the life-cycle, later nymphal stages and adults being unable to acquire TSWV (Moritz et al., 2004). It is also important to compare the effectiveness of treating TSWV-infected (source) versus healthy (recipient) plants in limiting Chemical control of TSWV has traditionally involved treating recipient plants with spread. foliar applied insecticides, but these are often ineffective in decreasing its incidence because of lack of adequate penetration into the plant parts where the nymphs hide and the soil where the pupal stage develops, and the ready development of resistance to certain chemical classes in thrips vectors, especially F. occidentalis (Heyler & Brobyn, 1992; Immaraju, 1992). Systemic insecticides applied to seedlings just before transplanting, or to the soil just afterwards, are more likely to reach early parts of the thrips life cycle than foliar applications as the chemical is taken up by the roots and distributed throughout the plant. If such a chemical is long lasting and the thrips vectors are not resistant to it, it has the potential to prevent both virus acquisition by nymphal thrips from infected source plants and its transmission to healthy recipient plants by adult thrips. When the systemic insecticides disulfoton, fenamiphos, carbofuran, terbufos and aldicarb were applied separately in-furrow to tomato plants, they decreased TSWV incidences (Treverrow & Mutton, 1990). In-furrow application of phorate to peanut plants controlled the vector thrips F. fusca and F. occidentalis and gave moderate suppression of TSWV (Culbreath et al., 1999), but similar applications of aldicarb or acephate controlled F. fusca without decreasing TSWV incidence (Todd et al., 1996). When the neonicotinyl insecticide, imidacloprid was sprayed onto tobacco seedlings just before transplanting, it sometimes diminished not only numbers of F. fusca but also TSWV incidence (Pappu et al., 2000; Csinos et al., 2001).

This paper reports results of field experiments that examined the effects of applying two systemic insecticides, thiamethoxam and imidacloprid, as seedling or soil drenches in suppressing TSWV epidemics. Imidacloprid and thiamethoxam belong to the choloronicotinyl and thianicotinyl classes of neonicotinyl insecticides respectively. Imidacloprid was the first such insecticide to be released while thiamethoxam is a second-generation member of the group (Maienfisch *et al.*, 2001; Jeschke *et al.*, 2002). Neonicotinyl insecticides are taken up readily by plant roots when applied directly to them, the soil around them or as seed dressings. They have high activity against sucking and chewing insects and act by interfering with the nicotinic acetylcholine receptor sites in the insect nervous system. They also have long lasting residual activity and a favorable safety profile (Maienfisch *et al.*, 2001; Jeschke *et al.*, 2002). Separate

applications to TSWV 'infector' tomato (source), and healthy lettuce (recipient) plants provided information on the relative importance of targeting control at virus acquisition from TSWV-infected plants by nymphal thrips verses virus transmission to healthy plants by adult thrips.

Materials and Methods

Virus isolates, inoculations and antiserum

The isolates of TSWV used were LeWA-3 and LeWA-4, both from infected lettuce cv. Raider in south-west Australia. Inoculations to maintain TSWV cultures and provide 'infector' plants were done by grinding infected leaves in 0.05M phosphate buffer, pH 7.2, with 0.01M sodium sulfite (German *et al.*, 1992). The sap was then mixed with 'celite' being before rubbing it onto leaves of tomato cv. Grosse Lisse. These cultures were used as positive controls in enzyme-linked immunosorbent assay (ELISA) for which polyclonal antiserum specific to TSWV was obtained from Bio-Rad, France.

Plants

Virus culture plants and 'infector' plants of tomato cv. Grosse Lisse were grown in a steam sterilised potting mix containing soil, sand and peat in air-conditioned, insect-proofed glasshouses kept at 15-20°C. Marigold (*Tagetes patula*) plants in pots and lettuce cv. Raider seedlings growing in trays were purchased from commercial seedling nurseries. Although 100 leaf samples collected at random from each batch of lettuces and marigolds were tested for TSWV presence by ELISA before transplanting, none was ever detected. To produce 'infector' plants for transplanting into the field experiments, tomato plants were inoculated at the 5-8 leaf stage with infective tomato sap containing one of the TSWV isolates. Tip leaf samples from each potential 'infector' plant were tested by ELISA before transplanting to confirm the presence of TSWV.

Enzyme-linked immunosorbent assay

Leaf samples were extracted (1g 20ml⁻¹) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5ml litre⁻¹ of Tween 20 and 20 g litre⁻¹ of polyvinyl pyrrolidone using a leaf press (Pollahne, Germany). The extracts were collected in

abeled, plastic sample tubes and tested by double antibody sandwich ELISA using paired wells in immunoplates as described by Clark & Adams (1977) using 0.6 mg ml⁻¹ of *p*-nitrophenyl phosphate in 10ml litre⁻¹ of diethanolamine, pH 9.8, as substrate. Absorbance values (A_{405nm}) were measured in a Titertek Multiskan immunoplate reader (Flow Laboratories, Finland). The 'two-times rule' for absorbance values from test sample sap versus healthy leaf sap was used to determine whether individual samples came from healthy or infected plants.

Details of field experiments

For each experiment, details of year, location, plot size, number of replicates, TSWV isolate introduced, transplanting dates for 'infector' plants, marigolds and lettuces, insecticides (active ingredient and trade names), application rates (g.a.i./ha) and volume of product applied (g/l and g/plant) are in Table 1. The locations used were Department of Agriculture field plots at South Perth and the nearby Research Station at Medina, both of which have sandy soils. Irrigation was daily by overhead sprinklers and each experiment was rigorously hand-weeded. Lettuces were fertilised according to standard commercial practice. 'Infector' plants of tomato acted as the primary TSWV source and flowering marigold plants helped to increase thrips numbers.

In Expt 1, except with a control treatment that lacked any 'infector' plants, six 'infector' plant foci were introduced per plot, each focus consisting of two 'infector' tomato and two marigold

plants. Each plot was surrounded by a 1.5 m wide oat buffer sown when the 'infector' and marigold plants were introduced. Inside each plot, 48 lettuce seedlings were planted 40 cm apart into raised beds 27 days after the 'infector' and marigold plants. At time of transplanting, insecticide was applied with a 50ml syringe as a drench to the soil surface at the base of each 'infector' plant. It was later applied in the same way to lettuce seedlings immediately after they were transplanted and reapplied to the 'infector' plants at the same time. Experimental treatments were a) thiamethoxam applied to 'infector' and lettuce plants; b) thiamethoxam applied to 'infector' plants only; c) thiamethoxam applied to lettuce plants only; d) no insecticide applied to 'infector' plants.

For Expts 2 and 3, except for a control treatment which lacked any 'infector' plants, six 'infector' plant foci were introduced into each plot, each focus consisting of one 'infector' tomato and two marigold plants. There were two plantings of lettuce in each experiment but the marigolds were all removed before the second planting and not replaced. As in Expt 1, each plot was surrounded by a 1.5m wide oat buffer sown when the 'infector' plants were introduced. Within each plot, 48 healthy lettuces were planted 40 cm apart into raised beds. The first planting was 28 days (Expt 2) and 23 days (Expt 3) after the 'infector' and marigold plants. One day before transplanting (DBT), both 'infector' and marigold plants were drenched by applying 50 ml of insecticide with a watering can to the soil within each pot. When the first lettuces were transplanted, insecticide was reapplied in the same way to the soil at the base of each 'infector' and marigold plant. At 7 DBT with the second planting, insecticide was reapplied similarly to each 'infector' plant. With lettuces, at 1 DBT each of the seedling trays was immersed into a 12 cm deep 30 x 40cm tub, containing 5 litres of the appropriate insecticide for 30 sec (i.e. until the soil was thoroughly saturated). The travs were then allowed to drain on mesh benches. Experimental treatments in the first planting were a) thiamethoxam applied to 'infector', marigold and lettuce plants; b) thiamethoxam applied to 'infector' and marigold plants; c) thiamethoxam applied to lettuce plants only; d) imidacloprid applied to 'infector', marigold and lettuce plants (both Expts); e) imidacloprid applied to lettuce plants only (Expt 2 only); f) no insecticide applied to 'infector', marigold or lettuce plants; and g) no insecticide applied and no 'infector' or marigold plants introduced (both Expts). Experimental treatments in the second planting were the same except that marigolds were absent.

Assessment of TSWV spread

In Expts 1-3, beginning 7 days after transplanting (DAT), lettuces were assessed weekly for presence of necrotic symptoms typical of TSWV infection (Cho *et al.*, 1989; Latham & Jones, 1997; Coutts *et al.*, 2004a). On each occasion when characteristic TSWV symptoms were first seen in a plant, this was noted and its position recorded on a map. Whenever there was doubt that the symptoms were caused by TSWV, leaf samples were taken and tested for TSWV by ELISA.

Assessment of vector thrips numbers and species

To identify vector thrips species and count their numbers, in Expt 1 following the transplanting of lettuces, two marigold flowers were collected weekly from different plants within each plot. In Expts 2 and 3, in planting 1, one marigold flower was collected from each plot weekly following transplanting of 'infector' and marigold plants, while in planting 2, 1-2 tomato flowers were collected from each plot weekly following transplanting of lettuce (marigold plants had been removed). Flowers of the same type were combined within all plots of the same treatment

before being transported to the laboratory. For this, marigold flowers were placed into polythene bags, while tomato flowers were placed into labelled vials containing 60% ethanol. Adult vector thrips were identified to species immediately (marigold) or using preserved specimens (tomato) using a dissecting microscope with reference to Mound & Gillespie (1997).

Statistical analyses

Genstat for Windows, release 5.4.2 was used for all the statistical analyses. To compare treatment effects on TSWV incidence, lettuce data for both area under the disease progress curve (AUDPC) and angular transformed final percentage of symptomatic plants were subjected to analysis of variance.

Results

TSWV spread

Expt 1

TSWV spread was substantial and by final assessment, the incidence of symptomatic lettuces reached 63% in plots with 'infector' plants without insecticide (Fig. 1a). With both final incidence and AUDPC, this value was significantly greater than those in all other plots (Table 2). The most effective treatment was thiamethoxam application to both 'infector' and lettuce plants, the final incidence value with it being significantly smaller than those in all other treatments with insecticide. There were no significant differences between incidence values for the insecticide treatments targeting 'infector' or lettuce plants alone. When the incidence values for plots without insecticide application to 'infector' or lettuce plants were compared with those for plots where insecticide was applied to both, TSWV spread was suppressed by 86%. Application to 'infector' plants alone suppressed spread by 67% while application to lettuces alone diminished it by 70%. The TSWV incidence in lettuce in plots without 'infector' plants or insecticide (19%) indicates the extent of its movement in between plots. The incidence difference (44%) between these plots and those without insecticide application but with 'infector' plants indicates the extent of spread to lettuce from within plot sources in the absence of insecticide. The overall ratio of external to internal spread was therefore 1:2.3. A truer picture of the effectiveness of treating 'infector' plants with thiamethoxam can be obtained by subtracting the final incidence value for plots without insecticide or 'infector' plants from the value for plots in which it was applied to 'infector' plants alone, giving a 3% incidence value. This is because the lettuce plants in such plots were untreated and therefore exposed to spread from untreated 'infector' plants in nearby plots.

Expt 2

With planting 1, at final assessment, there was only a 2% incidence of symptomatic lettuces in plots with 'infector' plants without insecticide (Table 2). However, despite this low overall incidence, there were still some significant differences between treatments, which showed more clearly with final incidence than AUDPC. When thiamethoxam or imidacloprid were applied to 'infector', marigold and lettuce plants, or thiamethoxam was applied to 'infector' and marigold plants only, there were no symptomatic plants, and this zero incidence value was significantly smaller than the incidence values for plots with 'infector' plants without insecticide. However, application of either chemical to lettuce plants alone did not suppress TSWV incidence significantly.

With planting 2, the incidence of symptomatic lettuces reached 30% in plots with 'infector' plants without insecticide (Fig. 1b). In general, AUDPC did not differentiate between incidence values to the same degree as final incidence, but the trends were similar. With both, incidence was significantly greater in plots with 'infector' plants without insecticide than in any others. The most effective treatment was imidacloprid application to both 'infector' and lettuce plants. With final incidence data, its incidence value was significantly smaller than those for all three insecticide treatments with thiamethoxam. However, this value was not significantly different from the one for imidacloprid application to lettuces alone. TSWV incidence in plots with thiamethoxam applied to 'infector' plants alone was significantly greater than the incidence values for all other insecticide treatments. When the incidence value for plots with 'infector' plants without insecticide was compared with the values for imidacloprid applied to both 'infector' plants and lettuces or to lettuces alone, suppression of TSWV spread was 90% and 80% respectively. When the same comparision was made for thiamethoxam, suppression was 73% for application to both 'infector' and lettuce plants or to lettuces alone, but only 13% for application to 'infector' plants alone. In plots without insecticide, the difference between the TSWV incidences with and without 'infector' plants (23%) indicates the extent of spread to lettuce from within plot sources. The overall ratio of external to internal spread was therefore 1:2.8. Substrating the final incidence value for plots without insecticide or 'infector' plants from the value for plots where thiamethoxam was applied to 'infector' plants alone gives an incidence value of 19%.

Expt 3

With planting 1, at final assessment, there was only a 6% incidence of symptomatic lettuce plants in plots with 'infector' plants without insecticide (Table 2). However, despite this low overall incidence, with both final incidence and AUDPC, this value was significantly greater than those in all other plots, except where imidacloprid was applied. Application of imidacloprid to 'infector', marigold and lettuce plants was significantly less effective in diminishing TSWV incidence than applying thiamethoxam to 'infector' and marigold plants, but not to lettuce alone.

With planting 2, at final assessment, the incidence of symptomatic lettuce plants was 48% in plots with 'infector' plants without insecticide (Fig. 1c). In general, AUDPC values did not differentiate between incidence values to the same extent as final incidence. With both, incidence was significantly greater in plots with 'infector' plants without insecticide than in any others, apart from where thiamethoxam was applied to 'infector' plants alone (Table 2). The most effective treatment was imidacloprid application to both 'infector' and lettuce plants. With final incidence, this value was significantly smaller than those for all other types of plot. With both types of data, where thiamethoxam was applied to 'infector' plants alone, incidence was significantly greater than where this insecticide was applied to both 'infector' and lettuce plants but not where it was applied to lettuce plants alone. When the value for the plots with 'infector' plants without insecticide was compared with that for imidacloprid, TSWV spread was suppressed by 92%. When the same comparision was made for thiamethoxam, application to both 'infector' and lettuce plants suppressed spread by 65% while applying it to lettuces alone did so by 54%. In plots without insecticide, the difference between TSWV incidence with and without 'infector' plants (34%) indicates the extent of spread to lettuce from within plot sources. The overall ratio of external to internal spread was therefore 1:2.4. Subtracting the final incidence value for plots without insecticide or 'infector' plants from the value for plots where thiamethoxam was applied to 'infector' plants alone gives an incidence value of 23%.

Thrips vectors

In Expt 1, adults of *F. schultzei* and *T. tabaci* were both found in marigold flowers, but *F. occidentalis* was absent. *T. tabaci* predominated throughout with highest numbers (15/flower) being found at 9 DAT. Peak mean numbers of adult vector (16/flower) and nymphal (10/flower) thrips were reached at 9 DAT, numbers decreasing to <1 adult vector and <1 nymphal thrips/flower after 29 DAT, before increasing again to 5-8 adult vector and 2-5 nymphal thrips/flower at 35-41 DAT. As insecticides were not applied to the marigolds, as expected, there were no trends evident between thrips numbers in plots with or without insecticide.

In Expt 2, adults of F. schultzei, F. occidentalis and T. tabaci were all found in marigold and tomato flowers collected during plantings 1 and 2 respectively. During planting 1, adult T. tabaci predominated throughout with highest numbers (4 to 16/flower) across treatments being found at 20 DBT. Adult F. schultzei were present on 10/12 sampling dates, but only at <1/flower. Adult F. occidentalis were only found at 1 DAT with <1/flower. Nymphal thrips were found on 6/12 sampling dates (<1/flower). At 20 DBT, there were more adult vector thrips on untreated (14-16/flower) than on insecticide-treated (4-10/flower) plants, but there were no differences in numbers of nymphs (<1/flower). Imidacloprid treated plants had the smallest adult vector thrips number (4/flower). At 7 DBT, there were 3-4 adult thrips/flower on untreated marigolds compared to 1-2/flower on treated plants, but no nymphal thrips were found. On the 10 subsequent sampling dates, numbers were <1-4 adult vector thrips/flower, and <1 nymphal thrips/flower regardless of whether insecticide was used. During planting 2, few tomato flowers developed to gather thrips information from. Adult thrips were present on 3/7 assessment dates, with F. schultzei, T. tabaci and F. occidentalis found on one occasion each, always at <1/flower regardless of insecticide presence. Nymphal thrips were found on 6/7 assessment dates at <1/flower in all treatments.

In Expt 3, adults of F. schultzei, F. occidentalis and T. tabaci were all found in marigold and tomato flowers collected during plantings 1 and 2 respectively. During planting 1, adult T. tabaci and F. schultzei were present throughout but did not exceed 1-2/flower. Adult F. schultzei predominated at 24 DAT with 1-2/flower. Adult F. occidentalis were found on 6/9 sampling dates but always at <1/flower. No effect of insecticide was apparent on adult vector thrip numbers or on the number of occasions when adults were found. However, at 4 DBT nymphal thrips were absent from flowers treated with thiamethoxam but present (0.2-0.7/flower) when imidacloprid was used or no insecticide was applied. From 4 DBT to 59 DAT, nymphal thrips were at low levels (0.1-<2/flower) in all plots. During planting 2, adult F. schultzei predominated up to 55 DAT but never exceeded 0.5/flower. Adult T. tabaci and F. occidentalis were present on 3/10 sampling dates each but at only 0.1/flower. Adult vector thrips were present on 6-7/10 of the assessment dates in plots without insecticide compared to 2/10 and 0/10 assessment dates where thiamethoxam or imidacloprid were applied to 'infector' plants, respectively. Nymphal thrips were present on 6/10 of the assessment dates in plots without insecticide compared to 1/10 assessment dates each where thiamethoxam or imidacloprid were applied to 'infector' plants.

Discussion

When TSWV spreads in plantings of a short-lived crop like lettuce, most of the infections are primary and result from feeding activity of vector thrips migrating from nearby external infection sources. However, limited within-crop spread also occurs (Coutts *et al.*, 2004a). We tested the

hypothesis that drenching source plants with systemic insecticides to kill the early larval stages of vector thrips, and thereby prevent TSWV acquisition, would be a suitable approach in controlling its epidemics. Treating source instead of recipient plants with systemic insecticide to suppress TSWV incidence is apparently novel. Although migrating viruliferous vector thrips can transmit the virus in feeds of as little as 5 min (Peters et al., 1996), we also tested the hypothesis that drenching recipient seedlings with systemic insecticide just before transplanting might suppress TSWV transmission. The insecticides used were imidacloprid and thiamethoxam. Both hypotheses proved correct with suppression of TSWV incidence by up to 66% from treating young source plants alone and up to 80% from treating recipient plants alone, while treating both young source and recipient plants together suppressed incidence by up to 92%. Moreover, the effectiveness of treating source plants alone was underestimated due to spread from untreated sources in nearby plots to the untreated recipient plants. Such exposure was considerable as within plots without insecticide, overall ratios of external to internal TSWV spread varied from 1:2.3 to 1:2.8 between field experiments. Separating the plots with wider non-host buffers than the 1.5m wide ones used would be necessary to minimise spread from untreated sources in other plots.

Although applying systemic insecticides as soil drenches to young source plants suppressed TSWV incidence well, such applications to large, old source plants were less effective. This is presumably because older plants need to take up much more chemical through the roots to achieve sufficient concentration of active ingredient than young, small plants. However, given the ability of viruliferous thrips to infect plants during brief feeds while migrating through a crop, what was surprising was the effectiveness of seedling drenches in curtailing TSWV incidence in recipient plants. Such effectiveness presumably reflects rapid take up by adult thrips, excellent efficacy and long-lasting residual activity of the neonicotinyl insecticides used (Maienfisch et al., 2001; Jeschke et al., 2002). Drenching seedlings growing in trays just before transplanting is much easier to do than drenching the soil around them just after transplanting or using foliar applications to the growing crop. Such insecticide treatments are therefore an attractive way of addressing the need for chemical control measures against TSWV in short-lived vegetable and other crops. The ideal approach to dealing with TSWV source plants is to remove them and this would normally be done with infected weeds or old crops. However, where a young crop of high value that is tolerant of TSWV damage becomes infected but needs to be kept until harvest, drenching the soil with insecticide is an option to help suppress spread of TSWV to neighbouring susceptible crops.

Although not evaluated as thoroughly as thiamethoxam in our field experiments, at the rates of application used imidacloprid proved more effective at suppressing TSWV spread. It gave suppression in TSWV incidence of up to of 80% and 90-92% when applied as a drench to recipient plants alone or to both recipient and old source plants respectively. The comparable figures for thiamethoxam were 57-73% (recipient plants alone) and 65-73% (both recipient and old source plants). We did not investigate the effectiveness of different application rates but used standard recommended rates for each chemical. Possibly thiamethoxam might have performed comparably at higher application rates. Lower application rates of imidacloprid also warrant investigation to see if adequate control is obtainable with them. Although numbers of adult vector and nymphal thrips tended to be small in the flower samples collected in our field experiments, diminished numbers of both were sometimes recorded following insecticide drench application, with imidacloprid causing greater decreases in their numbers than thiamethoxam. When tobacco seedlings were sprayed with imidacloprid before transplanting, the suppression of

TSWV incidence obtained was smaller than with lettuce in our studies and not always statistically significant (Pappu *et al.*, 2000; Csinos *et al.*, 2001). This may reflect the longer growth period of tobacco than lettuce such that the concentration of active ingredient within the plant has sufficient time to decline to an ineffective level for thrips vector control.

In our field experiments, we placed flowering marigold transplants near to the TSWV source plants to help boost thrips numbers. As the tomato source plants produced few flowers and the lettuces none, marigolds were also useful in recording thrips numbers in flowers. The marigolds were left untreated in Expt 1 but treated with insecticide in Expts 2 and 3. They tended to die relatively quickly so the survivors were removed before the second plantings in Expts 2 and 3, which, for these two experiments, were the ones in which sufficient spread of TSWV occurred in lettuce to obtain useful data on the effects of the insecticide applications. Insufficient TWSV spread in their first plantings was presumably because time was insufficient for enough thrips generations to develop upon the source plants. In Expt 1, TSWV spread to the untreated marigolds from the source plants: at the end of this experiment, ELISA tests on marigold petal samples from plots without insecticide but with source plants detected 37% infection. As Expt 1 was the experiment with the greatest TSWV spread and in which thiamethoxam had its greatest effect in diminishing TSWV incidence, there was no evidence that their presence diminished the effectiveness of the insecticides in suppressing the TSWV epidemic in lettuce. As with previous field studies on TSWV in lettuce (eg. Wilson, 1998; Coutts et al., 2004a), TSWV-infected lettuces were readily identified by their characteristic necrotic symptoms (Cho et al., 1989; Latham & Jones, 1997). Where symptoms were atypical, leaf samples were confirmed as being TSWV infected by ELISA. No other virus was present in the lettuce in these insecticide experiments but Lettuce necrotic yellow virus and lettuce big-vein disease were both found infecting lettuce in other studies at the site where Expts 1 and 2 were done. Infections with these two viruses were easily distinguished by their different symptomology in lettuce complemented by ELISA tests on leaf samples (Coutts et al., 2004b).

The vector species in all three of our experiments were F. schultzei and T. tabaci, along with F. occidentalis in two of them. Numbers of adult vector thrips found never exceeded 16/flower with the former, and 1/flower with F. occidentalis. The insecticides were therefore acting predominantly against mixed vector populations of F. schultzei and T. tabaci. Whether the chemicals used would have been as effective if F. occidentalis had been the predominant vector is unknown and tolerance to imidacloprid has been recorded in this species (Denholm *et al.*, 2002). Further field experimentation is required to determine their effectiveness in controlling TSWV epidemics under circumstances where F. occidentalis is abundant.

Demonstration of the principle that neonicotinyl insecticides are effective in suppressing TSWV spread when applied as drenches to source plants or to vegetable seedlings just before transplanting is of considerable interest for future chemical registration studies. If neonicotinyl insecticidal seedling drenches are deemed suitable by chemical registration authorities for use in TSWV control in short-lived vegetable or other crops, their incorporation as chemical control measures within IDM strategies (eg. Jones, 2003) would help to abeled TSWV control at minimal extra cost to the grower.

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Legends

Fig. 1. Disease progress curves for TSWV incidence in lettuce cv. Raider plants in

a) Expt 1; b) Expt 2, planting 2; and c) Expt 3, planting 2. Treatments applied: (•)

thiamethoxam to 'infector' and lettuce plants; (\blacksquare) thiamethoxam to 'infector' plants only; (\blacktriangle)

thiamethoxam to lettuce plants only; (•) no insecticide to 'infector' or lettuce plants; (X) no

'infector' plants present or insecticide used; (\circ) imidacloprid to 'infector' and lettuce plants; and (\Box) imidacloprid to lettuce plants only.

Table 1

General details of field experiments*

pplication	g product/	plant	0.05	0.05	0.17	0.05	0.17	
insecticide ap	g product/	litre	1.07	1.07	3.5	1.07	3.5	
Rate o		g ai/ha		1820	1820	1050	1820	1050
used		Trade	name	Actara	Actara	Confidor	Actara	Confidor
Insecticides		Active	ingredient	Thiamethoxam	Thiamethoxam	Imidacloprid	Thiamethoxam	Imidacloprid
		ansplants.	Planting 2	-	7 Mar		5 Mar	
lanting dates		Lettuce tr	Planting 1	20 Nov	19 Nov		19 Nov	
d		'Infector'	plants†	24 Oct	22 Oct		28 Oct	
TSWV	source	isolate	introduced	LeWA-3	LeWA-4		LeWA-4	
Replication				8	7		7	
Plot size	(m)			3 x 3	4.5 x 2.1		4.5 x 2.1	
Location				Medina	Medina		South Perth	
Year				2001/02	2002/03		2002/03	
Expt.				1	2		3	

*Lettuce cv. Raider used for all experiments. +Marigolds transplanted at same time as 'infector' plants in Expts 1-3, and treated with insecticide as for 'infector' plants but removed before planting 2 in Expts 2 and 3.

Table 2

Effects of different insecticide drench treatments on the incidence of TSWV in lettuce in Expts 1-3

Treatments*	Insecticide applied to		% symptomatic 1	ettuce plants**	AUDPC	
	'Infector' plants	Lettuce plants	Planting 1	Planting 2	Planting 1	Planting 2
Expt 1						
Thiamethoxam +	yes	yes	18.0 (9)		175	
Thiamethoxam +	yes	no	27.4 (21)		461	
Thiamethoxam +	no	yes	25.9 (19)		382	
No insecticide +	no	no	52.6 (63)		1338	
No insecticide -	-	no	25.9 (19)		357	
SED			3.83		133.3	
Significance (P)			< 0.001		< 0.001	
df			28		28	
Expt 2 [†]						
Thiamethoxam +	yes	yes	1.8 (0)	16.8 (8)	0	178
Thiamethoxam +	yes	no	2.6 (0.2)	26.7 (26)	5.5	567
Thiamethoxam +	no	yes	7.2 (1)	16.4 (8)	31.1	185
Imidacloprid +	yes	yes	1.8 (0)	10.5 (3)	0	115
Imidacloprid +	no	yes	6.8 (1.1)	14.1 (6)	30.0	196
No insecticide +	no	no	7.9 (2)	33.3 (30)	54.1	852
No insecticide -	-	no	2.6 (0.2)	15.2 (7)	5.0	227
SED			1.92	2.69	16.17	97.8
Significance (P)			0.002	< 0.001	0.009	< 0.001
df			42	36	42	36
Expt 3†						
Thiamethoxam +	yes	yes	2.5 (0.2)	24.4 (17)	6.8	372
Thiamethoxam +	yes	no	2.5 (0.2)	37.6 (37)	4.3	975
Thiamethoxam +	no	yes	5.5 (1)	28.3 (22)	20.7	612
Imidacloprid +	yes	yes	9.6 (3)	10.8 (4)	36.8	75
No insecticide +	no	no	14.4 (6)	43.8 (48)	58.6	1358
No insecticide -	-	no	4.6 (0.6)	21.7 (14)	9.3	323
SED			2.42	4.73	10.84	194.7
Significance (P)			< 0.001	< 0.001	< 0.001	< 0.001
df			30	30	30	30

2.1 +, with introduced 'infector' plants; -, without 'infector' plants **Final assessment was at 83 (Expt 1), 29 (Expt 2, planting 1), 53 (Expt 2, planting 2), 55 (Expt 3, planting 1) and 39 (Expt 3, planting 2) DAT. Values upon which analyses are based are angular transformed percentage infection. Values in parentheses are back transformed percentage incidences.

n.s.=not significant

*Marigold plants were insecticide treated in the same way as 'infector' plants in planting 1 but were removed from all treatments before planting 2.


SECTION 2.0

ROLES OF TSWV SOURCE PLANTS AND THRIPS VECTORS

Summary

Patterns of spread of TSWV were examined in lettuce and capsicum plantings in Western Australia into which thrips vectors spread the virus from external virus sources. After an initial trial which paved the way for the work, these plantings were: 1) eight separate field trials into which TSWV 'infector' plants of tomato were introduced alongside or near to plantings of lettuce or capsicum, and 2) three commercial lettuce plantings into which spread from nearby external infection sources was occurring naturally. The vector species were WFT, onion and tomato thrips, at least two of which were always present. Spatial data for plants with TSWV infection collected at different stages in the growing period were assessed by plotting gradients of infection, and using Spatial Analysis by Distance IndicEs (SADIE) and maps of spatial pattern.

Despite the persistent nature of TSWV transmission by thrips vectors, in both lettuce and pepper plantings there was a steep decline in TSWV incidence with distance from external infection sources that were alongside them. The extent of clustering of infected plants increased over time and was greatest closest to the source. In lettuce, the relationship between percentage infection and assessment date was more typical of monocyclic than polycyclic spread. Significant clustering of infected plants distant from TSWV sources confirmed that limited secondary spread was occurring within both crops in addition to the primary introductions that predominated. Spread to lettuce was greater downwind than upwind of the virus source, with magnitude and proximity of source determining the amount of spread. When 15 m wide fallow or non-host (cabbage) barriers separated TSWV sources from lettuce plantings, spread was slower and there was much less clustering with the latter. In commercial plantings, spread was favoured by TSWV movement within successive side-by-side plantings.

The spatial data from the diverse scenarios examined enabled recommendations to be made over 'safe' planting distances between external infection sources of different magnitudes and susceptible crops that were short-lived (eg. lettuce) or long-lived (eg. capsicum). They also helped validate the inclusion of isolation and 'safe' planting distances, planting upwind, prompt removal of virus sources, avoidance of side-by-side plantings, and deploying intervening non-host barrier crops as control measures within an integrated disease management strategy for TSWV in field vegetable crops.

The roles of different thrips species as vectors of TSWV were assessed through field monitoring of thrips populations and TSWV incidence in vegetable crops over three growing seasons in Victoria, New South Wales and Queensland. In lettuce crops, low levels of WFT were often found without TSWV incidence, but 6 to 8 weeks after the arrival of onion thrips TSWV reached damaging levels often leading to crop abandonment. In capsicums *T. palmi*, tomato thrips, onion thrips and WFT were all

present in crops infected with TSWV and *Capsicum chlorosis virus*, but a crop with WFT and tomato thrips was not infected with TSWV. In tomatoes, tomato thrips were present in crops with TSWV incidence up to 40%.

2.1 Preliminary observations on the roles of TSWV source plants and thrips vectors

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Summary

A preliminary field trial was done with lettuce to provide initial information on the amount of spread of TSWV over increasing distance from a virus infection reservoir.

Methods

The lettuce trial was located at Medina Research Station near Perth. The dimensions of the irrigated bay used were 12 x 61m. The source of TSWV was infected tomato plants and the trial included a band (12m wide x 3m length) of tomato infector plants across the middle of the bay used. This band also contained marigold plants planted in between the tomato plants to increase thrips numbers. Two blocks of lettuce were planted along raised beds one above and the other below this TSWV infector band. The dimensions of each lettuce block were 12 x 29m. The tomato TSWV infector plants were transplanted into the site at the beginning of September. However, the lettuce transplants were not planted until the beginning of December. Within each of the seven raised beds, the lettuce plant spacing between rows was 40cm. There were three rows within each bed and within each of them lettuce was planted every one metre. Weeds were rigorously controlled and surrounding land was kept fallow (without weeds), so no other TSWV source was present. The lettuce plants were inspected for TSWV symptoms every week for 6 weeks after transplanting and each individual infected plant was tagged and recorded after confirmation of infection by ELISA. Thrips were sampled on a weekly basis from the marigolds and tomato infector plants, to quantify the numbers present.

The spatial data collected for the distribution of TSWV-infected plants in the lettuce trial were analysed and mapped using the spatial analysis program SADIE and a mapping program. The surfer maps in Fig 2.1 show the following:- Each dot represents a quadrat containing several plants. The size of each of the red dots indicates the level of significance of clustering of infected plants and the size of each of the blue dots indicates the level of significance of 'gaps of infection'. Small non-significant but positive red dots represents isolated infections. The black line indicates zero significance, the areas inside the red lines are significantly clustered and the areas inside the blue lines are significantly non-clustered.

Results and Discussion

The thrips population was greatest in November and the beginning of December. The majority of the thrips identified on the marigolds and tomatoes were onion thrips. A small population of tomato thrips was present until the end of November, followed by a large increase in early December which only lasted 3 weeks before returning to previous low levels. This high thrips level in early December was followed by a burst of TSWV spread recorded 3 weeks later, comprising of 46% of the total spread recorded. No WFT were found at this site.

The overall incidence of TSWV was low, only reaching 5% at final assessment. We originally expected that the TSWV infection would be higher closer to the TSWV source and that there would be more infection downwind of the prevailing wind direction. However, the wind direction had changed through 90° by windbreaks running along either edge of the overall site area. This resulted in most TSWV spread along the crop edges downwind of the source as indicated by the red spots and re contours (Fig. 2.1). It was resolved to repeat the trial in a situation where the prevailing wind was along the plot length.





2.2 Patterns of spread of *Tomato spotted wilt virus* in field crops of lettuce and pepper: spatial dynamics and validation of control measures

(draft of paper submitted for publication to the Annals of Applied Biology)

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Running title: Patterns of TSWV spread in lettuce and pepper

Summary

Patterns of spread of *Tomato spotted wilt virus* (TSWV) were examined in lettuce and pepper plantings into which thrips vectors spread the virus from external virus sources. These plantings were: 1) seven separate field trials into which TSWV 'infector' plants of tomato were introduced alongside or near to plantings of lettuce or pepper, and 2) three commercial lettuce plantings into which spread from nearby external infection sources was occurring naturally. The vector thrips species were Frankliniella occidentalis, F. schultzei and Thrips tabaci, at least two of which were always present. Spatial data for plants with TSWV infection collected at different stages in the growing period were assessed by plotting gradients of infection, and using Spatial Analysis by Distance IndicEs (SADIE) and maps of spatial pattern. Despite the persistent nature of TSWV transmission by thrips vectors, in both lettuce and pepper plantings, there was a steep decline in TSWV incidence with distance from external infection sources that were alongside them. The extent of clustering increased over time and was greatest closest to the source. The relationship between percentage infection and assessment date suggested that spread was predominantly monocyclic with only limited polycyclic spread. Development of isolated clusters of infected plants distant from TSWV sources within both crops was consistent with only limited polycyclic spread. Spread to lettuce was greater downwind than upwind of virus source, with magnitude and proximity of source determining the amount of spread. When 15 m wide fallow or non-host (cabbage) barriers separated TSWV sources from lettuce plantings, spread was slower and there was much less clustering with the latter. In commercial plantings, spread was favoured by TSWV movement within successive side-by-side plantings. The spatial data from the diverse scenarios examined enabled recommendations to be made over 'safe' planting distances between external infection sources of different magnitudes and susceptible crops that were short-lived (eg. lettuce) or long-lived (eg. pepper). They also helped validate the inclusion of isolation and 'safe' planting distances, planting upwind, prompt removal of virus sources, avoidance of side-by-side plantings, and deploying intervening nonhost barrier crops as control measures within an integrated disease management strategy for TSWV in field vegetable crops.

Key words: TSWV, vegetables, spread, pattern, clustering, gradients, spatial analysis, control measures, non-host barriers, fallow barriers, 'safe' planting distances, integrated disease management.

Introduction

Tomato spotted wilt virus (TSWV; family *Bunyaviridae*, genus *Tospovirus*) was first found in 1915 in Australia (Brittlebank, 1919). Since then, its known host range has increased to over 900 dicotyledonous and monocotylonous plant species worldwide (Peters, 1998). Many horticultural crops and weeds become infected. TSWV is transmitted by several thrips species, of which the western flower thrips (WFT; *Frankiniella occidentalis*) is the most efficient vector (German *et al.*, 1992; Ullman, 1996; Mound, 2002). Major upsurges in TSWV epidemics occurred in several parts of the world following the introduction of WFT (Peters *et al.*, 1996). For example, in south-west Australia, TSWV has caused sporadic losses to vegetable production since the 1920's (Carne, 1928). However, after the appearance of WFT in the region in the early 1990's (Malipatil *et al.*, 1993), an increase in epidemics of TSWV occurred in a wide range of crops, with pepper (*Capsicum annuum*) and lettuce (*Lactuca sativa*) amongst the worst affected, entire crops sometimes being lost (Latham & Jones, 1996, 1997).

Infection reserviors from which TSWV spreads to susceptible crops include nearby plantings of TSWV-susceptible crops, volunteer crop plants and weeds (Cho et al., 1989; Latham & Jones, 1996, 1997; Gitaitis et al., 1998; Wilson, 1998; Groves et al., With field grown crops, integrated disease management (IDM) 2001, 2002). strategies devised against TSWV place a major emphasis on phytosanitary and agronomic measures that minimise the source of virus infection for spread to susceptible plantings (Cho et al., 1989; Brown et al., 1996; Latham & Jones, 1996; Jones, 2004). In developing IDM strategies against the virus, information on spatial patterns of TSWV spread is required to provide reliable recommendations on the effectiveness in decreasing spread of control measures such as isolation between susceptible crops, 'safe' planting distances, planting upwind, prompt removal of virus sources, avoiding side-by-side plantings and deploying intervening barriers of nonhost crops or fallow land. Published data on spatial patterns of TSWV spread is limited and contradictory as regards the occurrence of secondary virus spread within infected crops (Bald, 1937; Thresh, 1983; Camann et al., 1995; Latham & Jones, 1997; Gitaitis et al., 1998; Wilson, 1998). This is because the pattern of spread of TSWV is complicated by the need for vector thrips to multiply on TSWV-infected plants before they become viruliferous. Acquistion only occurs in the first and early second nymphal stages of the life-cycle and adult thrips cannot acquire the virus (Moritz et al., 2004). Thus infected plants must be hosts for both virus and vector before they become sources for TSWV spread and secondary spread only occurs within infected crops when this is the case (Camann et al., 1995; Jones, 2004).

This paper describes spatial patterns of TSWV spread in: a) field trials in which TSWV 'infector' plants were introduced alongside plantings of lettuce and pepper, b) field trials in which intervening non-host barrier crops or bare earth fallow separated TSWV 'infector' plants from lettuce plantings, and c) commercial lettuce crops with naturally occuring external TSWV sources. Spatial Analysis by Distance IndicEs (SADIE) (Perry *et al.*, 1996, 1999) was used to assess cumulative infection data.

Contour maps based on clustering indices from SADIE and gradients of infection over distance from virus sources assisted with interpretation of the data. The information obtained assists in validation of cultural control measures and their incorporation into IDM tactics against the virus.

Materials and Methods

Virus isolates, inoculations and antiserum

The three isolates of TSWV used were LeWA-3 and LeWA-4 both from lettuce cv. Raider, and CaWA-3 from pepper cv. Azure, all from south-west Australia. Inoculations to maintain TSWV cultures and provide 'infector' plants were done by grinding infected leaves in 0.05M phosphate buffer, pH 7.2, with 0.01M sodium sulfite (German *et al.*, 1992). The sap was then mixed with 'celite' before rubbing it onto leaves of tomato (*Lycopersicum esculentum*) cv. Grosse Lisse. These cultures were used as positive controls in enzyme-linked immunosorbent assay (ELISA) for which polyclonal antiserum specific to TSWV was obtained from Bio-Rad, France.

Plants

Virus culture plants and 'infector' plants of tomato were grown in a steam sterilised potting mix containing soil, sand and peat in air-conditioned, insect-proofed glasshouses kept at 15-20°C. To produce 'infector' plants for transplanting into the field trials, plants of tomato cv. Grosse Lisse were inoculated at the 5-8 leaf stage with infective tomato sap containing the required TSWV isolate. Tip leaf samples from each potential 'infector' plant were tested by ELISA before transplanting to confirm the presence of TSWV. Lower leaves were removed at time of transplanting to minimise any wilting due to transplant shock. Except on two occasions when they were purchased from a commercial seedling nursery, lettuce seedlings for transplanting in the field were produced in insect-proofed glasshouses by germinating seeds in sterilised potting mix. Pepper and cabbage (*Brassica oleracea* ssp. *capitata*) seedlings and marigold (*Tagetes patula*) plants for transplanting out were always purchased from nurseries. When seedlings or plants were obtained from nurseries, 100 leaf samples collected at random from each batch were tested for TSWV presence by ELISA before transplanting, but none was ever detected.

Enzyme-linked immunosorbent assay

Leaf samples were extracted (1g 20ml⁻¹) in phosphate buffered saline (10 mM potassium phosphate, 150 mM sodium chloride), pH 7.4, containing 5ml litre⁻¹ of Tween 20 and 20 g litre⁻¹ of polyvinyl pyrrolidone using a leaf press (Pollahne, Germany). The extracts were collected in labelled, plastic sample tubes and tested by double antibody sandwich ELISA using paired wells in immunoplates as described by Clark & Adams (1977) using 0.6 mg ml⁻¹ of *p*-nitrophenyl phosphate in 10ml litre⁻¹ of diethanolamine, pH 9.8, as substrate. Absorbance values (A_{405nm}) were measured in a Titertek Multiskan immunoplate reader (Flow Laboratories, Finland). Absorbance values for positive test sample sap were always more than ten times those for healthy sample sap.

Details of field trials

For each field trial, details of year, location, areas assessed, crop type and cultivar, TSWV isolate introduced, presence or absence of a 15 m wide barrier, planting dates for 'infector', susceptible crop and non-host barrier crop, and number and frequency of assessments are in Table 1. The two locations used were Department of Agriculture field plots at South Perth and the nearby Research Station at Medina, both of which have sandy soils. All trials consisted of rectangular plots arranged west-east, the prevailing wind coming from the west. Irrigation was daily by overhead sprinklers and each trial was rigorously hand-weeded. Lettuce and pepper plants were fertilised according to standard commercial practice and no insecticide was applied. 'Infector' plants acted as the primary TSWV source and flowering marigold plants helped to increase thrips numbers.

Trials 1 and 2 each consisted of one rectangular plot of pepper plants. At their east ends there was a 3 m wide band into which 60 'infector' plants and 85 marigold plants (trial 1), or 180 'infector' plants and 160 marigold plants (trial 2) were transplanted; the 'infector' plants were spaced 30 cm apart and the marigolds evenly spaced between them. Within each plot, pepper plants were planted in rows running across the plot 1m apart with a 50 cm plant spacing within rows.

Trial 3 consisted of two rectangular plots of lettuce arranged end-to-end. In between them was a 4 m wide band into which 168 'infector' plants and 140 marigold plants were transplanted; the 'infector' plants were spaced 40 cm apart and the marigold plants evenly spaced between them. On the first planting date both plots were 24 m long, but on the second date the west plot was 30 m and the east one 57 m long. Each plot consisted of seven raised beds running lengthwise with three rows of lettuce 40 cm apart planted along each bed and 1 m plant spacing within rows.

Trials 4, 5 and 6 each consisted of one rectangular plot of lettuce. Trials 5 and 6 were in the same field and were 80 m apart, but trial 4 was at another site. At the west end of each plot was a 5 m wide band into which 76 TSWV 'infector' and 76 marigold plants were transplanted; the 'infector' plants were spaced 80 cm apart and the marigolds evenly spaced between them. In trial 4, lettuces were planted in 24 single rows spaced 40 cm apart running lengthwise with 1 m plant spacing within rows. In trials 5 and 6, they were planted in seven raised beds running lengthwise with three rows 40 cm apart planted along each bed and 1 m plant spacing within rows. The area planted to lettuce (both plantings) was 25 m long in trial 4, but 30 m long in trials 5 and 6. A 15 m wide 'barrier' zone separated the 'source band' from the lettuce. In trials 4 and 5, a non-host crop of cabbage was planted in this zone, but in trial 6 it was left fallow; in trials 4 and 5 the cabbage seedlings were planted in rows.

Trial 7 consisted of a rectangular block with a 1 x 2 m plot in its centre planted with 42 TSWV-'infector' plants spaced 30 cm apart. Twelve further plots were planted with paired rows containing seven lettuces each; plant spacing within and between rows was 40 cm. Six of the lettuce plots were arranged around the TSWV 'source plot' such that they all started 1 m away from it and radiated outwards, three each to the east and west, with a 40 cm space between them at their closest point to the source plot. Another six identical plots started 15 m away from the 'source plot'

and radiated outwards, three each to the east and west with a 3 m space between each of them at their closest point to the source.

Details of commercial lettuce crops

For each commercial lettuce crop assessed, details of year, farm location, area assessed, cultivar, planting date and age at assessment are in Table 1. The crops were all within the Perth Metropolitan area, sown in sandy soils and irrigated by overhead sprinklers. On farm 1, the virus source was an old tomato crop ($28 \times 94 \text{ m}$) with 100% TSWV infection, located 4 m east of the rectangular lettuce block. Just before the assessment was done an area of crop 12 m wide at the end closest to the infection source had been removed with herbicide by the farmer due to 100% TSWV infection. On farm 2, the virus source was a mature lettuce crop ($10 \times 20 \text{ m}$) with 90% TSWV infection, located 5 m to the west of the earliest lettuce planting which adjoined a later sown one; the later lettuce planting had a fallow area ($8 \times 20 \text{ m}$) 5 m to the west of it. On farm 3, the TSWV source was a mature celery crop with 10% TSWV infection ($5 \times 19 \text{ m}$) which was 15 m to the south of the oldest lettuce planting. There were three rectangular plantings of lettuce side-by-side, which were 3, 5 and 6 wks old with the 5 wk old planting in the middle.

Assessment of TSWV spread

In trials 1 and 2, a tip leaf sample was taken from each individual pepper plant at 2–4 wk intervals after transplanting and tested separately for TSWV presence by ELISA. When a sample first tested positive, the position of each infected plant was recorded on a map showing the position of the individual plants. Trials 3-7 were inspected weekly for presence of lettuces with necrotic symptoms typical of TSWV infection (Cho *et al.*, 1989; Latham & Jones, 1997). On each occasion when characteristic symptoms were first seen in a plant, this was noted and in trials 3-6 its position was then recorded on a map, as in trials 1 and 2. Whenever there was any doubt that the symptoms were caused by TSWV, leaf samples were taken and tested for TSWV by ELISA. At farms 1-3, recording the exact location of each symptomatic lettuce plant on a map was done on one occasion each. As in trials 3-7, these assessments were based on recording the presence of symptomatic plants, backed up by taking leaf samples from any plants with atypical symptoms, and testing each sample individually by ELISA. Disease progress curves for the percentage of plants with TSWV infection were plotted for trials 3-7.

Analysis of spatial pattern

For trials 1-3 and 5-7, and for farm 1, infection data for individual plants were used to plot gradients of infection over increasing distances from the primary virus source. Fitted linear or exponential lines were used as appropriate. With data from field trials 1-6 and farm 3, the counts for presence or absence of TSWV infection in each set of nine adjacent plants were combined together to provide a sample unit ('quadrat') figure. For farms 1 and 2, counts for 25 adjacent plants were combined to provide this figure. Spatial pattern of affected plants based on early and final cumulative 'quadrat' counts was quantified using Spatial Analysis by Distance IndicEs (SADIE) as described by Thackray *et al.*(2002). For a random arrangement of the observed counts amongst the given sample units, the expected value for the index of

aggregation (I_a), an index of the degree of clustering for the whole sample area, is one, while $I_a > 1$ indicates aggregation of counts into clusters (Perry *et al.*, 1996). For farm 1, SADIE was also redone as described by Thackray *et al.* (2002) for the areas containing the largest and smallest concentrations of symptomatic plants located at each end of the lettuce block.

The clustering indices, v, for cumulative infections were contoured using the computer program 'Surfer' (Anon., 1997) to provide maps of spatial pattern. The contouring levels used indicate where estimated indices are half as great again as expected by chance (v = 1.5 for infection patches and v=-1.5 for infection gaps). The resulting maps indicate the spatial location and extent of patches and gaps of infection. Spots represent individual quadrat sample units denoting infection patches with v>0 (red) and infection gaps with v<0 (blue). Small spots represent clustering indices of 0 to +/-0.99 (clustering below expectation), intermediate spots +/- 1 to +/-1.49 (clustering exceeds expectation) and large spots >1.5 or <-1.5 (half as much again as expectation). Red lines enclosing patch clusters are contours of v=1.5 and blue lines enclosing gap clusters are of v=-1.5. Black lines are zero-value contours, representing boundaries between patch and gap regions where the count is close to the sample mean.

Assessment of vector thrips numbers and species

To identify the species of adult vector thrips present and count their numbers in field trials 1-6 (thrips data not collected from trial 7), flower samples (1/plant) were collected at random every week within the TSWV 'infector' plant band. Five marigold flowers were collected on each occasion and, in addition, 12, five and five tomato flowers were collected in trials 1, 5 and 6 respectively; they were not collected in trials 2, 3 or 4 because the tomato plants died prematurely or failed to produce flowers. In trials 1 and 2, ten pepper flowers were also collected from each pepper row weekly. Before being transported to the laboratory for thrips identification, marigold flowers were placed into polythene bags, while tomato and pepper flowers were placed into labelled vials containing 60% ethanol. On farms 1-3, ten flowers were selected at random on the day when the TSWV assessment was done. They were of pepper at farm 1, lantana (Lantana camara) and bougainvillea (Bougainvillea glabra) from a nearby windbreak at farm 2, and basil (Ocimum basilicum) and sowthistle (Sonchus oleraceous) at farm 3. The flowers were placed into polythene bags for transport to the laboratory. The vector thrips species (adults) found were identified immediately (marigold, lantana, bougainvillea, basil, sowthistle) or using preserved specimens (pepper and tomato) using a dissecting microscope with reference to Mound & Gillespie (1997).

Results

Spatial patterns of spread in field trials

<u>Pepper</u>

In the two trials with pepper, survival of tomato 'infector' plants was relatively poor resulting in a small source and slow TSWV spread, final incidences of infected plants only reaching 3% at day 170 in trial 1 and 4% at day 182 in trial 2. *Ia* values at final

assessment revealed that clustering of infected plants was highly significant over the entire plot area in both trials (Table 2). Gradients of infection showed that the greatest concentration of infected plants was closest to the virus source (Fig. 1a,b). Within both trials, the red contours and spots on the contour maps at days 170 (trial 1) and 182 (trial 2) revealed significant clustering of infected pepper plants close to the virus source band and smaller central cluster (Fig. 2). The blue contours and spots indicated that areas distant from the introduced source were mostly occupied by gap clusters in both trials. When maps of earlier assessments were constructed, presence of the central clusters was found to have arisen from secondary spread from TSWV-infected pepper plants. Including the central clusters, the boundary between patch and gap areas (black line) extended just beyond the middle of each block (18 m).

<u>Lettuce</u>

In trial 3, there was no TSWV spread within the first lettuce planting. At final assessment in the second planting (day 55), 18% and 16% of lettuce plants were symptomatic, in the west and east plots, respectively. When the data for both plots were combined and used to plot a disease progress curve, the rate of increase in incidence accelerated slowly over time (Fig. 3a). Ia values at final assessment revealed that clustering of symptomatic plants was highly significant over the entire area of each plot (Table 2). Gradients of infection upwind (west) and downwind (east) of the virus source showed that the greatest concentration of symptomatic plants (>70%) was in a 3 m wide zone immediately downwind and a 1m wide zone immediately upwind of it (Fig. 1c). Decline in incidence further downwind was slow while upwind the gradient did not reveal any such decline. At 28 days, clustering of symptomatic plants was concentrated on either side of the source band, as indicated on the contour map by the red and black contours and distribution of red spots with only a few isolated infections elsewhere, which were often on plot edges (Fig. 4a). At 55 days, the east plot had greatest clustering closest to the source but there was also a large patch cluster on the northern edge as indicated by the red contours and spots (Fig. 4b). The main boundary line between patch and gap areas (black contour) extended to one third of the distance away from the source band (29 m). Comparison of the initial spread from the primary source (Fig. 4a) with subsequent spread in the same areas (Fig. 4b) suggested that secondary spread of TSWV had occurred from within-crop sources of infected lettuce plants, especially within the large cluster on the northern edge. The 55 day assessment in the west plot revealed more diffuse spread, the clustering occurring close to the source as indicated by the red contour at 28 days (Fig. 4a) not increasing further, although more red spots subsequently developed inside the black contour line (Fig. 4b). The main boundary line between patch and gap areas (black contour) did not extend quite as far away from the source as that in the east plot. Also, a small infection on the western edge of this plot expanded between the 28 and 55 day assessments indicating within-crop spread.

Intervening non-host crop or fallow

Trials 4 and 5

In trial 4, there was no spread of TSWV within the first planting. However, at final assessment (day 62) in the second planting 13% of the plants were symptomatic and there was a straight line relationship between percentage infection and assessment

date (Fig. 3b). The overall *Ia* value indicated no significant clustering over the entire plot (Table 2). Also, although a small amount of clustering was indicated by the red contour in the zone closest to the cabbage plot on the contour map, symptomatic plants were scattered across the plot (Fig. 5a). At final assessment in the first lettuce planting in trial 5, only 2/650 lettuce plants were symptomatic. At this stage in the second planting (day 55), however, 10% of lettuce plants were symptomatic and there was a straight line relationship between percentage infection and assessment date (Fig. 3c). Also, by this time, but not earlier, there was significant clustering over the entire plot area as indicated by the *Ia* value (Table 2). At day 35 there was no gradient of infection (Fig. 1d) and no concentration of symptomatic plants in the area closest to the cabbage plot as indicated by the red contour on the contour map, and as in trial 4, symptomatic plants were scattered across the entire plot (Fig. 5c).

Trials 6 and 7

At final assessment in the first planting in trial 6, only 12/650 lettuces were symptomatic. In the second planting, at final assessment (day 55) 18% of lettuces were symptomatic and the relationship between percentage infection and assessment date showed a very slow rate of acceleration over time rather than a straight line relationship (Fig. 3c). There was significant clustering over the entire plot at both of days 35 and 55 as indicated by the Ia values (Table 2). In contrast, as mentioned above, *Ia* values had revealed no clustering whatsoever in trial 4 while clustering only became significant at final assessment in nearby trial 5. In contrast to the situation in trial 5, at day 35 in trial 6, there was a gradient of infection that revealed a concentration of symptomatic plants next to the barrier with a gradual decline thereafter (Fig. 1d). Also, there was significant patch clustering adjacent to the intervening fallow as indicated by the red contour on the contour map and significant gap clustering in the half of the plot distant from it (Fig. 5d). By day 55, the distinction between patch and gap areas was less clear due to extensive virus spread. At final assessment (day 49) in trial 7, 15% of lettuces were symptomatic. Most spread was concentrated in the plots adjacent to the TSWV source with little being found in those 15 m away (Fig. 3d).

Commercial crops

<u>Farm 1</u>

Overall at farm 1, 43% of plants in the lettuce crop were symptomatic. Incidence of symptomatic plants was greatest close to the infection source, where it reached 100%, gradually declining to 22% over distance (Fig. 6a). The overall *Ia* value for the entire data set confirmed that clustering of symptomatic plants was highly significant (Table 2). When SADIE was redone focussing separately in the areas at opposite ends of the block, the resulting *Ia* values revealed significant clustering in the area closest to the source but not at the opposite end. The complete contour map revealed that the main block (=45 m from the main virus source) with greatest patch clustering closest to the virus source and greatest gap clustering furthest away from it (Fig. 6b). From the complete contour map, the spatial patterns of spread seemed homogenous for patch

and gap areas at either end of the block. However, separate contour maps for each end revealed that this was not the case (Figs 6c,d).

Farms 2 and 3

In farm 2, overall incidences of symptomatic plants were 20% and 3% in the 3 wk and 2 wk old lettuce plantings respectively, 2 wks being insufficient time for much TSWV spread to be observed in the latter. Also, 3 wks was insufficient time for any secondary spread to be observed so all infections were primary. *Ia* values for overall mean data revealed that clustering of symptomatic plants was highly significant (Table 2). As expected, the contour map revealed that clustering was greatest closest to the TSWV source and least furthest away from it, the boundary between patch and gap areas (black contour) coinciding with the boundary between the two planting dates (map not shown).

At farm 3, the three adjoining lettuce plantings had TSWV incidences of 14% (6 wk old), 5% (5 wk old) and 2% (3 wk old). *Ia* values for overall mean data showed that clustering was highly significant (Table 2). On the contour map, the main boundary between patch and gap areas (black contour) was within the 5 wk old planting (map not shown). In general, greatest patch clustering was in the oldest planting which was closest to the source and greatest gap clustering in the youngest planting furthest from it, while the 5 wk old planting was intermediate. As at farm 2, the distribution of infection found reflected not only proximity to source but also age of successive plantings.

Vector thrips species and numbers

Adult *F. schultzei*, *F. occidentalis* and *T. tabaci* were all found in trials 1 and 2. In trial 1, *T. tabaci* peaked in early December in pepper flowers but afterwards *F. schultzei* dominated (Fig. 7a). There was an early peak of *T. tabaci* on marigold flowers in November reaching 11/flower. Subsequently, however, *F. schultzei* was commonest in them peaking in December at 11/flower and being found throughout, while *T. tabaci* was not found after December. In tomato flowers, numbers of *F. schultzei* and *T. tabaci* remained low, not exceeding 1.1 and 0.9/flower respectively. *F. occidentalis* were only found on the pepper flowers but their numbers never exceeded 1/flower. In trial 2, numbers of *T. tabaci* were greatest peaking in January in marigold (11/flower) and pepper (4/flower) (Fig. 7b). As in trial 1, *F. occidentalis* were found only on the pepper flowers, their numbers never exceeding 1/flower.

Adults of both *F. schultzei* and *T. tabaci* were present in trials 3-6, but *F. occidentalis* was found only in trial 4. In trial 3, numbers of *T. tabaci* were greatest on marigold flowers, peaking in early November (8/flower). They predominated until late December, but after this numbers of *F. schultzei* and *T. tabaci* were similar, forming a second peak together in late December (Fig. 7c). In trial 4, overall thrips numbers remained low. They were greatest during November and December in marigold when *T. tabaci* was the predominant species reaching 2/flower. Over the duration of the second planting, all three species were present in marigold flowers but at very low numbers, never exceeding 0.3/flower. In trial 5, thrips numbers were

greater than in trial 4 (Fig. 7d). In marigold flowers, *T. tabaci* was predominant from October to February, with peaks in October, November and February (1-9/flower), while low numbers of *F. schultzei* were present at <2/flower throughout both plantings. In tomato flowers, thrips were only found on one occasion in March and *F. schultzei* (<1/flower) was the only species present. Thrips numbers were greater in trial 6 (Fig. 7e) in which *T. tabaci* was the predominant species in marigold flowers from October to December (2-13/flower). There were <5 *F. schultzei*/flower from November to January in marigolds. During the second planting, numbers of *T. tabaci* and *F. schultzei* were similar but low. In tomato flowers, thrips were only found on one occasion in April with *F. schultzei* (<1/flower) the only species present.

At time of TSWV assessment at farm 1, a TSWV-infected pepper crop had adult *F. occidentalis* (3/flower). At farm 2, there were <1 adult thrips/flower in samples collected from bougainvillea and lantana, with *F. occidentalis* and *F. schultzei* both present. At farm 3, there were <1 adult *F. occidentalis*/flower in basil and sowthistle.

Discussion

In lettuce and pepper plantings into which TSWV had spread from virus sources alongside them and 2-3 different vector thrips species were present, clusters of infected plants were mostly concentrated closest to the virus source but with some isolated ones further away. Also, gradients of infection showed a rapid drop off in occurrence of infected plants with increasing distance from the source, confirming the sharp gradients over distance from virus sources previously recorded with TSWV in lettuce, pepper and several other crops (Latham & Jones, 1997; Gitaitis *et al.*, 1998; Wilson, 1998). A marked effect of proximity to infection source upon the extent of virus spread over distance is typical when the type of spread is predominantly monocyclic (eg. Thresh, 1976, 1983; Jones, 1993).

Our studies using SADIE and maps of spatial pattern to analyse the distribution of TSWV-infected plants, contribute towards resolving the long-standing controversy over whether any thrips-vectored secondary spread of TSWV takes place from within-crop infection sources. Previous studies on its spread in tomato (Bald, 1937) and lettuce (Wilson, 1998) in Australia suggested that all infections were primary, while ones with tobacco (Nicotiana tabacum) in Greece suggested secondary spread (Thresh, 1983), and others with pepper, tomato and groundnut (Arachis hypogaea) in the USA suggested that most infections were primary although limited secondary spread also occurred (Camann et al., 1995; Gitaitis et al., 1998). In our trials, isolated clusters of symptomatic plants gradually developed distant from the introduced infector plants not only in a long-lived crop (pepper) but also in a shortlived crop (lettuce). The most plausible explanation for these isolated clusters is that limited localised secondary spread of TSWV occurred due to activity of thrips that developed on isolated primarily infected plants, acquired the virus from them in the early larval stage, matured and then flew to adjacent plants. In the lettuce, this was apparently so despite the absence of flowers (which favour thrips activity). Possible alternative explanations for the development of isolated infection clusters include: (1) they might have arisen by spread of TSWV from other external sources rather than from the introduced infector plants transplanted in bands across the trial plantings; (2) each isolated cluster might have been due to a single viruliferous thrips arriving and then infecting a group of neighbouring plants in which expression of TSWV

symptoms was staggered; (3) that viruliferous thrips gradually leaving the band of infector plants might always move in the same direction within a planting and alight at the same distant point causing a cluster of infections there. However, none of these alternative explanations are likely to be correct. With (1), at both sites used for the trials the land is mostly kept fallow and TSWV has never been found in susceptible plantings except when infector plants were introduced deliberately. With (2), lettuces always developed necrotic symptoms quickly following infection with TSWV and staggered symptom development was never observed. With (3), thrips dispersing from the infection source could not always have followed the same course within a planting because wind directions changed often and the introduced infector plants from which they came were not isolated point sources but were arranged in a band across each planting. Moreover, when disease progress was followed in a trial in which a lettuce planting bordered onto the TSWV source, although the rapidly accelerating sigmoidal shapes typical of the polycyclic spread that occur in epidemics of many insect-borne viruses (Thresh, 1974, 1983; Nutter, 1997) did not develop, there was a slow rate of acceleration (Fig. 3a) indicating that limited secondary spread was occurring. Lack of any secondary spread would have produced a linear relationship between incidence and assessment date as seen in the two trials with intervening non-host buffers separating the TSWV sources from the lettuce plantings (Figs 3b,c).

We did not attempt to determine the roles played by individual thrips vector species in shaping the patterns of TSWV spread found at individual sites so which species were responsible for the limited secondary spread found in lettuce and pepper was not determined. However, *F. occidentalis, F. shultzei* and *T. tabaci* all complete their life cycles on lettuce, pepper and tomato (Yudin *et al.*, 1988; Tommasini & Maini, 1995). *T. tabaci* and *F. shultzei* were present in flowers of marigold and/or pepper in all our trials, and were also sometimes found at low levels in tomato flowers; lettuce leaves were not examined for thrips. Although their numbers fluctuated, in general, *T tabaci* populations were often greater earlier when the climate was cooler, while *T. shultzei* tended to predominate later. When it was present, populations of the third vector species, *F. occidentalis*, were mostly very low.

Camman et al. (1995) suggested how TSWV spread that is predominantly primary but with limited secondary infections, might occur in groundnut, and Jones (2004) provided an explanation of why spatial patterns of TSWV spread often suggest monocyclic patterns or only limited secondary spread of the virus in other monoculture crops. Critical considerations include: 1) the delay of at least 3 wks imposed by the need for a thrips vector to complete its life-cycle on a TSWV-infected plant before the early larval stage can acquire the virus and then grow to maturity, and for the adult produced to fly to other plants so that transmission can occur; 2) the ability of viruliferous thrips to infect many plants in probes as brief as 5 mins as they migrate through a crop; and 3) repeated cycles of secondary spread only occur if a thrips vector is present that can multiply upon infected crop plants, volunteer crop plants or weeds within the crop and if the crop is of sufficient duration for several additional generations of thrips to develop. The growing period of lettuce crops is too short for 3) to apply, but this is not so with pepper yet there was no rapid secondary (polycyclic) spread of TSWV in our two pepper trials despite the presence of three different thrips vector species. Possibly, the climatic conditions over the hot summer period did not favour vector thrips multiplication on pepper sufficiently for such TSWV spread to occur.

Gitaitis et al. (1998) recorded gradients of TSWV infection in tomato when planted next to a TSWV source but not when the source was 200 m away, while Groves et al. (2001) did not detect gradients of TSWV infection in weed hosts 35 m from a localised TSWV source. In a trial in which a 15 m-wide fallow barrier separated 'infector' plants from a lettuce planting, SADIE, the spatial pattern of spread (Fig. 5d) and gradient of infection (Fig. 1d) resembled those obtained in TSWV spread situations in lettuce without barriers. However, in an otherwise identical trial only 80 m away from it, when the 15 m-wide barrier was planted instead with a non-host crop (cabbage), spread occurred more slowly with no gradient of infection visible at 35 days (Fig. 1d) and the pattern of TSWV spread was different. Here, SADIE found much less clustering over the entire plot, and maps of spatial pattern revealed a greater proportion of scattered infected plants than clustering including at the end closest to the source (Fig. 5c). In an identical trial at the second site, the differences were even greater with SADIE revealing no significant clustering over the entire plot. These results suggest that deploying a non-host cabbage barrier crop is likely to be more effective as a control measure that delays TSWV spread than using a fallow barrier. Presumably, this is because, as thrips migrate away from the original source, some of the viruliferous vector thrips land on the cabbage plants, remain and colonise them rather than flying further; F. occidentalis, F. shultzei and T. tabaci are all able to complete their life cycles on cabbage (Shelton, 1995; Tommasini & Maini, 1995). Also, as it is a non-host for TSWV, when their progeny fly on to susceptible hosts, they will be non-viruliferous and therefore not cause any new infections.

As expected from previous research (eg. Latham & Jones, 1997; Gitaitis et al., 1998; Wilson, 1998), our studies revealed marked effects of magnitude of virus source on the extent of TSWV spread. Thus, the heavily infected tomato crop at farm 1 was a much more potent source for thrips vectors to spread TSWV to nearby lettuce than the localised virus sources consisting of bands of tomato 'infector' plants used in our trials. As previously reported for lettuce by Wilson (1998), orientation of the virus source in relation to prevailing wind is important with viruliferous thrips being blown downwind. In our studies, when bands of 'infector' plants separated upwind and downwind blocks of lettuce, greater spread downwind than upwind was revealed by both maps of spatial pattern and gradients (Figs 1c and 4). These results help emphasise the potential benefits if virus source removal and planting upwind are used as control measures (Jones, 2004). The value of isolation as a further control option, was demonstrated in a trial in which small plots of lettuce were planted 1 m and 15 m from a small TSWV source, most spread being concentrated in the plots next to it, with little occurring to those only 15 m away (Fig. 3d). Isolation is effective because when fallow areas intervene in between localised virus sources and susceptible plants, dispersal of vectors by wind and flight increases with increasing distance from the source (Thresh, 1974, 1983). Avoiding successive side-by-side plantings is another important control measure (eg. Latham & Jones, 1996; Jones, 2004). The influence of different planting times of adjacent crops, and resulting unequal periods of exposure to thrips vectors, on TSWV spread were shown at farms 2 and 3, where there were successive, side-by-side plantings of lettuce. As expected, there was much less infection in younger than older plantings exposed to the same virus source, and, when lettuces were exposed for 3 wks or less, there was insufficient time for any secondary spread to develop.

Although their effectiveness also depended on factors like magnitude of virus source, prevailing wind direction and presence of non-host barrier crops, the need to establish 'safe' planting distances to help diminish spread of TSWV in vegetable crops was demonstrated in these studies. Our results permit tentative estimates to be made over such distances between virus sources of different potencies and susceptible crops with different growing periods. Thus, with a small nearby, upwind TSWV source as little as 20 m of separation contributed considerably towards diminishing TSWV incidence in lettuce plantings, so a suitable 'safe' planting distance recommendation that errs on the side of safety would be 25 m for this short-lived crop. Moreover, despite its longer growing period, our trials with pepper did not suggest that a greater distance was needed with it at the time of year they were done. In contrast, with a massive TSWV source, as at farm 1, the boundary between patch and gap clustered areas within the lettuce planting (Fig. 6) was up to 45 m away from the virus source suggesting that, should prompt removal of such a large source not be possible, a 'safe' planting distance of 75-100 m would be more appropriate in these extreme circumstances.

Jones (2004) proposed an integrated disease management strategy for TSWV in vegetable field crops, that included a wide range of phytosanitary and agronomic control measures as well as deploying TSWV-resistant cultivars, if available, and applying appropriate insecticides to suppress thrips vectors. Our studies involving spatial analysis of diverse TSWV infection scenarios with lettuce and pepper help validate inclusion of isolation from potential sources, using 'safe' planting distances, prompt removal of virus sources, avoiding successive side-by-side plantings, planting upwind, and deploying intervening non-host barrier crops among the control measures recommended.

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Figure legends

Fig. 1. Gradients of TSWV infection away from the introduced TSWV source: a) trial 1 (pepper) with fitted exponential line $2.15+67.9(0.43^{x})$; b) trial 2 (pepper) with fitted exponential line $1.48+127.3(0.23^{x})$; and c) trial 3 (lettuce), planting 2 upwind (left) and downwind (right) from the source with fitted exponential lines $14.42+110.1(1.91^{x})$ and $10.11+104.4(0.76^{x})$ respectively; and d) trials 5 (• and solid line) and 6 (\blacktriangle and broken line), planting 2 with fitted linear 4.12-0.0365x and exponential $2.68+23.7(0.852^{x})$ lines respectively (lettuce separated from source by 15 m wide cabbage barrier in trial 5 and fallow barrier in trial 6).

Fig. 2. Maps of clustering indices for cumulative numbers of plants with symptoms of TSWV in pepper: a) trial 1 at assessment days 123 and 170, and b) trial 2 at assessment days 118 and 182. Axes show 'quadrat' numbers not distances in metres. Spots represent units denoting infection patches with v>0 (red) and infection gaps with v<0 (blue). Small spots represent clustering indices of 0 to +/-0.99 (clustering below expectation), intermediate sized spots +/-1 to +/-1.49 (clustering exceeds expectation) and large spots >1.5 or <1.5 (half as much again as expectation). Red lines enclosing patch clusters are contours of v=1.5 and blue lines enclosing gap clusters are of v=-1.5. Black lines are zero-value contours, representing boundaries between patch and gap regions where the count is close to the overall sample mean.

Fig. 3. Relationship between the percentage of lettuce plants with TSWV symptoms and assessment date in planting 2: a) trial 3; b) trial 4; c) trials 5 (\blacksquare) and 6 (\bullet); and d) trial 7 at two distances from the virus source, $\bullet=1m$ and $\bullet=15m$.

Fig. 4. Maps of clustering indices for cumulative numbers of plants with symptoms of TSWV in lettuce in trial 3, planting 2 at assessment days a) 28 and b) 55. Symbols, contours and axes are as for Fig. 2.

Fig. 5. Maps of clustering indices for cumulative numbers of plants with symptoms of TSWV in lettuce in planting 2: a) trial 4 at assessment day 62; trial 5 at assessment days b) 35 and c) 55; d) trial 6 at assessment day 35. Width of intervening barrier

between TSWV source and plot is 15 m. Symbols, contours and axes are as for Fig. 2.

Fig. 6. TSWV incidence in lettuce over distance from the virus source at farm 1: a) gradient of infection with fitted exponential line $15.92+178.1(0.95^{x})$. Broken line represents distance between TSWV source and lettuce planting, including a 4 m wide vehicle track and the first 12 m of the lettuces killed with herbicide. Maps of clustering indices for cumulative numbers of lettuces with symptoms of TSWV for b) entire block; c) an area defined by $\{1-5 x \text{ axis}; 21-27 y \text{ axis}\}$; d) an area defined by $\{1-5 x \text{ axis}; 1-7 y \text{ axis}\}$. In b), c) and d) symbols, contours and axes are as for Fig. 2.

Fig. 7. Incidence of adult vector thrips species in pepper flowers in a) trial 1 and b) trial 2; and in marigold flowers in c) trial 3, d) trial 5 and e) trial 6. \blacktriangle =mean number of *T. tabaci*/flower, \blacksquare =mean number of *F. schultzei*/flower, \blacklozenge =mean number of *F. occidentalis*/flower. Broken lines in c), d) and e) represents periods between plantings 1 and 2.

No. and	assessments			17, 2 wkly	26, 2-4wkly	7, wkly	5-7, wkly	5-6, wkly	5-6, wkly	5, wkly		1,4 wk old	1, 3 wk old	1, 2 wk old	1, 3 wk old	1, 5 wk old	1, 6 wk old
Planting dates*	Non-host transplants					17 Oct	16 Oct, 12 Feb				ı						
	ısplants	2	-			29 Nov	6 Mar	21 Feb	21 Feb								
	Crop trar	1		28 Sept	3 Oct	3 Oct	12 Nov	18 Nov	18 Nov	16 Jan		27 Feb	12 Nov	19 Nov	23 Dec	1 Jan	16 Jan
	TSWV 'infector' plants		9 Aug	16 Aug	13 Aug	17 Oct	16 Oct	16 Oct	16 Dec								
15 m wide	trials	No	No	No	Yes (cabbage)	Yes (cabbage)	Yes (fallow)	Yes (fallow)	cial crops								
TSWV source	a) Field	Yes (CaWA-3)	Yes (LeWA-3)	Yes (LeWA-3)	Yes (LeWA-4)	Yes (LeWA-4)	Yes (LeWA-4)	Yes (LeWA-4)	b) Commer	No	No		No				
Crop type and cultivar				Pepper cv. Rialto	Pepper cv. Rialto	Lettuce cv. Raider	Lettuce cv. Raider	Lettuce cv. Raider	Lettuce cv. Raider	Lettuce cv. Raider		Lettuce cv. Raider	Lettuce cv. Raider		Lettuce cv. Raider		
Area assessed (m)				10 x 30	10 x 30	12 x 48 (a) 12 x 87 (b)*	10 x 25	12 x 30	12 x 30	10 x 50 #		10 x 59	10 x 20	8 x 20	5 x 19	5 x 19	4 x 19
Location				South Perth	South Perth	Medina	South Perth	Medina	Medina	South Perth		Gnangara	Landsdale		Wanneroo		
Year				2000/01	2001/02	2001/02	2002/03	2002/03	2002/03	2002/03		2001	2001		2002		
Trial/ farm number				1	2	c,	4	5	9	7		1	2		3		

Table 1. General details of lettuce or pepper plantings assessed

*(a) and (b) refer to first and second dates of planting of crop transplants. Non-host transplants were cabbage cv. Sugarbowl.

total trial area

Table 2.

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18.		1.30	1.57	2.11	2.06	1.71	1.71	3.06	1.82	0.94	1.18	0.94	1.48	2.01	1.35		7.41	2.31	1.07	2.57	2.26	TSWV symp
Cumulative no. of plants with symptoms	ls	11	16	10	22	11	15	196	116	37	61	23	65	46	118	crops	1595	674	207	273	122	e numbers of plants with
I otal no. of plants assessed	a) Field tria	580	580	009	009	1197	630	1197	630	460	460	651	651	651	651	b) Commercial	3717	875	875	2200	1800	tion for cumulative
Assessment day		123	170	118	182	28	28	22	22	41	62	35	22	35	55							Index of Aggregat
I rial plot assessed						east	west	east	west													Overall Mean
l rıal		1	1	2	2	3	3	3	3	4	4	5	5	9	9		1	1-insert a#	1-insert b#	2	3	* $Ia = SADIE$

licates randomly arranged affected plants and Ia

>1 indicates clustering of affected plants. # indicates areas within the entire block defined by a) 1-5 x axis, 21-27 y axis, b) 1-5 x axis, 1-7 y axis.



Distance from TSWV source (m)









Fig. 4b

Fig. 4a

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20/11/02

18/12/02

Sampling date

26/2/03

2

0

16/4/03

27/3/03

2.2 Roles of thrips vectors

Alan Clift (University of Sydney), with Sandra MacDougall (Agriculture New South Wales), Len Tesoreiro (Agriculture New South Wales), John Hargreaves (Queensland Department of Primary Industry)

Summary

The roles of different thrips species as vectors of TSWV were assessed through field monitoring of thrips populations and TSWV incidence in vegetable crops over 3 growing seasons in Victoria, New South Wales and Queensland. In lettuce crops low levels of WFT were often found without TSWV incidence, however, 6 to 8 weeks after the arrival of *T. tabaci* into the crop TSWV reached damaging levels often leading to crop abandonment. In capsicums, *T. palmi*, *F. schultzei*, *T. tabaci* and WFT were all present in crops infected with TSWV and *Capsicum chlorosis virus*, however a crop with WFT and *F. schultzei* was not infected with TSWV. In tomatoes, *F. schultzei* were present in crops with TSWV incidence up to 40%.

2000/01

Vegetable crops

There was relatively little vector thrips activity and TSWV incidence in the Sydney Basin during the 2000/01 season. This was largely due to the very wet periods during November and December reducing outdoor thrips populations. TSWV was found on three lettuce farms, at Kellyville, Leppington and Camden during February/March. On each farm, both WFT and onion thrips, *T. tabaci*, were present. Based on previous evidence from monitored farms 1997 to 2000, there are frequently WFT populations on farms, but without TSWV. *T. tabaci*, which is a more mobile species, can carry the TSWV onto a farm, infecting either crop plants or weeds. Within a few generations a significant part of the WFT population can acquire the virus as first instar larvae, then as adults spread the disease within the farm. Known TSWV hosts, including capeweed, sowthistle and cobblers peg are common in the Sydney Basin and *T. tabaci* is also common.

In the Murray Irrigation Area (MIA) there was little TSWV in seed production or fresh lettuce crops, although both *T. tabaci* and tomato thrips, *F. schultzei* were present. There was continuing concern in the Griffith and Hillston areas about TSWV damaging seed crops of lettuce so monitoring traps were put in place by late October. Previous work in the Griffith area indicated volunteer lettuce plants as a potential reservoir for TSWV.

Glasshouse capsicum and chilli plants in the Dareton/Mildura areas have had a high incidence of TSWV during 2000/01. The vector was believed to be WFT, so a monitoring program was set up from late October to determine which vectors were present. Field crops were also be monitored to determine disease and vector incidence.

Sticky traps from chilli and capsicum crops in the Bowen area, north Queensland were counted. Both *T. palmi* and *F. schultzei* were present, as was *Capsicum chlorosis virus*, so both species could be vectors. All *F. schultzei* examined were the yellow form and their resemblance to WFT was considerable.

Other crops

Seed potato crops in the Crookwell area were sampled in January and February 2001. Up to 20% TSWV were found in some cultivars. *T. tabaci* was the only vector species found on traps and by direct sampling.

TSWV was found in kangaroo paw and flannel flowers at a cut-flower farm on the central coast. One cultivar of Kangaroo paw was especially susceptible to the disease. Detailed examination of traps in the crops indicated both WFT and *T. tabaci* present over July to August. Samples of plants, flowers and more traps were collected to determine which vector species was breeding on the flowers. This situation is important as it is the first significant incidence of TSWV in an Australian native plant. Gerberas in a cut flower farm about 5 km south of the previous property were infected with TSWV. Once again, both WFT and *T. tabaci* were present.

2001/02

Until December 2001, both WFT and *T. tabaci* were abundant in the Sydney basin, with at least one hydroponic lettuce farm so badly infected it closed down over the remainder of summer. On the basis of previously monitored crops, low levels of WFT persist, but when *T. tabaci* brings TSWV on the farm, the level of infection builds up very rapidly over 6 weeks.

The heatwave and bushfires knocked back thrips numbers over Chistmas/NewYear, 2001/02 but by March/April another lettuce farm was virtually closed down by the high levels of TSWV. When the disease was discovered, heavy spraying eliminated most thrips, but the usual two vectors were probably involved. Due to the hot weather and wind directions over December/January, there were abundant *F. schultzei* in the Sydney Basin over March and April, but they were not associated with TSWV. Mixed populations of WFT and *F. schultzei* were observed on the two vegetable market gardens properties monitored in the Schofields area during March to May 2002. The main crop plants infested were eggplant and capsicum/chilli: close inspection over that interval did not find TSWV.

Jerilderie and Darlington Point areas in the MIA experienced up to 60% infection levels with TSWV in solanaceous vegetables, with the much lower levels being in Victoria. Both *T. tabaci* and *F. schultzei* were present, but based on previous experiences in the area in 1995/96, we believe it was the *F. schultzei* that brought the TSWV into the area. No WFT were present during the 2001/02 season.

Traps from glasshouses at Mildura/Dareton/Buronga areas indicated abundant WFT and *T. tabaci* over January/April. Both glasshouse and field crops were infested. There was no *F. schultzei* found on any of the traps, including the field crops. Based on the Sydney scenario, this was a potentially lethal mixture if any of the *T. tabaci* have TSWV. The glasshouse crops were capsicum/chilli and the field crops were capsicum/chilli and eggplant.

Traps from Bundaberg area continued to show *T. palmi*, *T. tabaci*, some WFT and the yellow form of *F. schultzei*. Both TSWV and a second tospovirus, *Capsicum chlorosis virus* were found in the monitored crops.

A large wholesale nursery near Winmalee in the foothills of the Blue Mountains, producing both ornamental and vegetable seedlings was monitored since July 2002. This nursery was also the site for the NSW part of the ornamentals insecticide work. While WFT was present in varying numbers over the property, there was no indication of TSWV

2002/03

The 2002/03 season was a good year for thrips and TSWV in NSW. Although the spring, September to November was relatively dry, there were major *T. tabaci* migrations within the Sydney basin, carrying TSWV with them onto many hydroponic lettuce farms from Leppington/Bringelly in the south to Richmond in the north. Any of the farms with low level WFT populations experienced up to 80% losses over the January to May interval. It generally took 6-8 weeks for significant infection levels to develop after arrival of the *T. tabaci*.

In NSW the strain of WFT did not develop populations on tomato plants, but clearly F. *schultzei* are very attracted to these plants. During 2002/03 at least one tomato grower in the Leppington/Bringelly area experienced TSWV in tomato plants. There were major populations of F schultzei present in the Sydney basin from November 2002 to April 2003 and this was the most likely vector species.

There were major levels of TSWV in processing tomatoes in the MIA, especially Jerilderie and Yanco in NSW. Average TSWV incidence in these areas was 40%. However, in contrast to previous seasons, growers around Echuca experienced 25% infection levels, reducing to about 10% at Rochester and Boort in Victoria. WFT was found in the area during the 2002/03 season, but there is no evidence they were involved in disease transmission. The vector was *F. schultzei*, the same vector from the 1995/96 season.

If WFT become established in the Processing Tomato production area, incidence of TSWV is likely to increase and be far more difficult to manage. *F. schultzei* is susceptible to insecticides and so as a vector is easily controlled; WFT is clearly a different situation.

Excerpt from Western Flower Thrips newsletter no. 29, March 2003.

During the latter part of 2002 there were several instances of TSWV on hydroponic lettuce farms in western Sydney. The excessively hot and dry conditions December 2002-early February 2003 reduced thrips numbers and TSWV incidence generally. However with rains in early February, at least one hydroponic lettuce farm in southwestern Sydney experienced TSWV. The vectors involved were most likely the combination of *T tabaci* introducing the virus onto the farm, then the WFT population acquiring the disease as first instars and spreading the infection around the farm.

Further, during February, TSWV was found in glasshouse tomatoes, also in south western Sydney. This is very interesting as the NSW WFT population is rarely found on tomatoes, except as occasional individuals, so it is possible there was another vector involved. Tomato and cucumber chemical trials were done on a farm within 800m during the previous season and there were virtually no WFT on the tomato plants, but they were present on the cucumber and capsicum plants.

Excerpt from Western Flower Thrips Newsletter no. 30, June 2003

The 2002/03 season featured significant thrips and TSWV activity.

Within the Greater Sydney basin, lettuce growers were reporting increased TSWV from October onwards. At the start, the main vectors were *T. tabaci* onion thrips and WFT. Generally, as the surrounding areas dried, the *T. tabaci* moved into the remaining green areas. A small proportion of them were infected with TSWV and these introduced the virus onto farms. Previous experience indicated a six week interval between introduction of the disease and significant infection levels. In the Sydney area, it is common to have low levels of WFT, usually without TSWV. It is after the virus has been introduced that WFT will become infected as first instar larvae and then effectively spread the disease within the farm.

One of the WFT trial farms had severe TSWV during December-February, but only isolated infected plants by mid-April. However by mid May, infection levels were as high as 80% within some areas. The infections were very patchy, with the infection moving long the hydroponic pipes rather than between different pipes. There were very distinct gradients between neighbouring racks with different aged plants.

Processing tomato growers in northern Victoria/southern NSW also experiences significant levels of TSWV. The vector in this situation was *F schultzei* and WFT was also found in the production areas, but was not associated with TSWV.

TSWV infection levels were higher around Jerilderie, up to 40% infection in some crops, but usually 20-30%. Levels of 20-25% could be found near Echuca and even in the Corop and Rochester area levels of 10% were reported. This is a more serious situation

than the last severe epiphytotic in 1994/95, when the highest levels Victoria experienced was 3.5%.

The presence of WFT has the potential to make this situation far worse. Just having WFT does not automatically mean TSWV, but with both *T. tabaci* and *F schultzei* to bring the disease onto farms, the probability of severe infection levels is now greater, with levels as high as 80% in sprayed situations over wide areas possible.
SECTION 3.0

PREDICTION MODEL FOR WESTERN FLOWER THRIPS

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Summary

A simple day-degree model based on temperature was developed to predict outbreaks of the vector WFT. Developing a model to predict TSWV is difficult since the vectordisease relationship is affected by many variables. These include abundance of the vector, planting date, abundance and types of host plants, presence and distribution of plants affected by TSWV, movement of the vector, and efficiency of transmission of the virus by the vector. Insufficient data on these variables were available to develop a predictive model for TSWV, so the topic is discussed generally.

Introduction

Relationship between development and temperature

WFT (like other insects) cannot regulate their own temperature and their development depends on the temperatures to which they are exposed in the environment. Figure 3.1 demonstrates the relationship between temperature and development of WFT on two different hosts – cucumber and chrysanthemum. As temperature increases, development time (time taken to develop from an egg to adult) decreases. The amount of available pollen also affects developmental time, with WFT completing development at lower temperatures on chrysanthemum more quickly. For this reason, it is important to clear weeds, particularly flowering ones, from around the affected property.



Figure 3.1. Relationship between temperature and development time of WFT on two different hosts – cucumber and chrysanthemum.

The Day-degree Model

The day degree model is based on the minimum developmental temperature below which the insect does not develop, and an upper temperature above which development stops, or the insect dies. The total amount of heat required between the lower and upper thresholds for an organism to develop from one point to another in its life cycle is calculated in units called degree-days (°D). Day-degree models are frequently used for predicting the development of insect pests and pathogens in IPM programs (for examples of use, see the University of California's IPM on-line guide http://www.ipm.ucdavis.edu/default.html). The day-degree model is a useful tool to determine when monitoring and control needs to be carried out, but development times will vary each year depending on the local weather pattern.

Data required to generate a day degree model

The following data are required to generate a day-degree model:

- 1. Daily minimum (Tmin) and maximum temperatures (Tmax) for each calendar day. Temperature data (minimum, maximum) were obtained from the Meteorological Bureau for Wanneroo, Perth for 1993-2003. This area was chosen since WFT and TSWV are regular seasonal problems there in capsicum, tomato and lettuce. Tenyear average temperatures were calculated from the data.
- 2. Threshold temperature. This is the temperature above or below which no perceptible development occurs. Threshold temperatures are normally determined from laboratory studies under controlled conditions. From the available published literature, the minimum threshold temperature for WFT is 10.91°C (Katayama 1997). No maximum threshold temperatures were available. For the purpose of this model, it is assumed that WFT are not limited in their growth by high temperatures.
- 3. Number of accumulated day degrees. Number of day-degrees required for the organism to develop from egg to adult. One life cycle (egg to adult) of WFT is completed after the accumulation of 317 DD above the threshold temperature of 10.91°C (Katayama 1997).

WFT model for Wanneroo

The day-degree model of WFT for the Wanneroo area is shown in Figure 2. Points of the model to note:

- the higher the numbers of generations of WFT per month, the higher the final population of WFT.
- the model is based on outside (ambient) air temperature. For indoor crops, air temperature is usually higher, particularly through winter. Development of WFT is therefore likely to be much quicker in a greenhouse than in the field.
- WFT field populations begin to increase in September and continue in high numbers until April.

 Air-temperatures in the Wanneroo area are never sufficient to kill off WFT and WFT can survive temperatures below 0°C. Growers with winter crops should therefore still monitor for WFT.



Figure 3.2. Day-degree model for WFT in the Wanneroo area in Western Australia.

Limitations of the day-degree model

- The model assumes that a single generation of WFT is present at the start of each month. In the field there are likely to be overlapping generations of WFT (i.e. eggs, adults, larvae and pupae from different generations are present).
- The model is based on ambient air-temperature. In sheltered areas such as glasshouses, the model will underestimate WFT population growth. In crops that are canopied, e.g. tomatoes, the crop will also 'buffer' WFT against extremes in temperature.
- The day-degree model is a predictive tool only and it has not been tested in the field. To determine its validity for a particular region, the model needs to be compared with actual WFT trapping data. At least three year's data are required to test the validity of a model.

What about a day-degree model of WFT for my growing region?

- Simialr models can be developed for other regions of Australia, provided that daily air temperatures are available for that area. Models can also be developed for a particular farm or glasshouse.

Measuring TSWV Risk

Researchers at the University of Georgia, USA have developed a tomato spotted wilt virus index for peanuts (=groundnuts) <u>http://sacs.cpes.peachnet.edu/spotwilt/index.htm</u>). The index was derived from research on resistant varieties, planting densities, effect of herbicides and insecticides on amount of crop affected by TSWV. It is interactive so that growers can learn which practices will reduce their TSWV index rating.

Growers are asked to go through a list of 7 factors; factors are given an index rating from 0-50 (Figure 3.2). For each factor, 0 indicates that there is no risk and 50 a high risk. Answers are tallied to give an overall relative risk loss. The developers of the index stress that "Using preventative measures to reduce risk of TSWV losses is the only way to control the disease. After the crop is planted, there are no known control measures".

For Australia, it is suggested that a similar index be developed on a crop-by-crop basis for crops that are affected by TSWV. Below are a list of factors that need to be considered in the index:

Variety

By planting TSWV resistant varieties, growers can reduce the risk of TSWV. However, resistant varieties are only available for tomato and capsicum, and no variety is known to be totally immune to TSWV as resistance-breaking strains can appear.

Planting Date

The day-degree model for Wanneroo shows that WFT numbers will begin to increase in September. Susceptibility of crops to infection is also high at this time. For this reason, it is suggested that growers should commence planting during the middle of the season (where possible). If this is not possible, then the risk of TSWV can be reduced by planting resistant varieties, increasing planting density, planting in twin rows rather than single, etc.

Plant Density

Plants that are closely spaced are less likely to be affected by TSWV than if they are widely spaced. Research has shown that the "higher plant population may not reduce the number of infected plants, but it will increase the number of healthy plants that can fill in and compensate for infected plants".

Insecticide Usage

In theory, lowering overall thrips populations with insecticides should effectively reduce the spread of TSWV. However, overseas research has shown that foliar applied insecticides are often ineffective at suppressing TSWV, especially at suppressing primary TSWV infection (Baldwin *et al.* 2003). Primary infection is likely to come from outside the crop. For example, from weeds that are harbouring thrips and TSWV. As described earlier in this report to HAL, systemic insecticides applied as drenches are more effective at suppressing TSWV spread.

Row Pattern

By planting in twin rows rather than a single row, the incidence of TSWV can be reduced. This method does not utilise more plants, but is based on planting the same number of plants. Early canopy cover shades over infected TSWV source plants thereby reducing spread.

Weed control

By controlling weeds, growers can reduce the risk of TSWV. Weeds are reservior hosts of TSWV and weeds, particularly flowering weeds can harbour populations of WFT and other thrips.

Category	Variety	Risk Index Points
Susceptible I	FlorunnerSunOleic 97RFlavorunner 458	O 50
Susceptible II	• Perry	040
Intermediate I	 Andru 93 NC-V11 NC-12C 	O 35
Intermediate II	• AT-201	O 30
Intermediate III	• Georgia Hi-OL	0 25
Moderately Resistant I	 Georgia Green Southern Runner FL MDR98 Virugard Gregory VC2 Norden Andru II 	0 20
Moderately Resistant П	 C-99R Hull Carver Georgia 02C 	O 15
Moderately Resistant III	• DP-1	© 10
	• Other*	O 50

FACTOR 1: PEANUT VARIETY*

Figure 3.3. Part of the interactive S.W.E.A.T. (Spotted Wilt Eradication Action Team, University of Georgia, USA) index for peanuts. This is the first of 7 factors that are used to calculate an overall risk. By using different factors, growers can learn how to reduce their risk to TSWV.

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SECTION 4.0

INTEGRATED DISEASE MANAGEMENT STRATEGIES FOR TOMATO SPOTTED WILT VIRUS

(from paper published in the journal 'Virus Research', Vol. 100, pages 5-30)

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Summary

Epidemiological information has been used to develop effective integrated disease management strategies for Tomato spotted wilt virus (TSWV) in vegetables growing in seedling nurseries, protected cropping or field systems. The virus causes serious losses in yield and quality of in vegetables growing in seedling nurseries, protected cropping or field systems worldwide. These losses and the resulting financial damage can be limited by controlling epidemics with measures that minimise the virus infection source or suppress virus spread. However, individual measures used alone may only have small effects and they often tend to become ineffective, especially over the long term. When diverse control measures that act in different ways are combined and used together, their effects are complementary resulting in far more effective overall control. Such experiences have led to development of integrated management concepts for virus diseases that combine available host resistance, cultural, chemical and biological control measures. Selecting the ideal mix of measures for each production situation requires detailed knowledge of the epidemiology of the causal virus and the mode of action of each individual control measure so that diverse responses can be devised that are tailored to meet the unique features of each of the different scenarios under The strategies developed must be robust and cause minimal extra consideration. expense, labour demands and disruption to normal practices. This section explains how the integrated strategies for TSWV were devised for each situation, and describes each measure within a summary that covers how is achieved, its mode of action and the situations in which it can be used effectively.

TSWV occurs worldwide causing damaging diseases in many vegetable and ornamental crops, and in others as diverse as potato, tobacco and goundnut (=peanut). It is transmitted persistently by eight different thrips species belonging to the genera Frankliniella (F. fusca, F. intonsa, F. occidentalis, F. shultzei) and Thrips (T. palmi, T. setosus, T. tabaci) but not through seed (eg. German et al., 1992; Peters et al., 1996). The first reports of TSWV and its transmission by a thrips species, T. tabaci (onion thrips), were from Australia (Brittlebank, 1919; Pittman, 1927), TSWV might. therefore, have originated in native Australian vegetation, first invading cultivated crops following their introduction by European colonists and then spreading through international trade to the rest of the world. However, extensive testing of native Australian plants failed to provide evidence for this hypothesis (Latham and Jones, 1997) and none of several hundred thrips species native to Australia are known to transmit it (Mound, 1996). Therefore, it probably originated elsewhere, was introduced with cultivated crops and was absent before colonisation. Its most efficient vector, F. occidentalis (the western flower thrips), originated in North America but recently spread to many other regions of the world, where its introduction often triggered a major upsurge in TSWV epidemics and resulting losses in diverse crops (Peters et al., 1996). For example, in south-west Australia, after TSWV was first reported (Carne, 1928), it caused sporadic epidemics and losses until 1993 when introduction of F. occidentalis was followed by a substantial increase in damage, particularly to lettuce, pepper and tomato crops (Latham and Jones, 1997).

TSWV has a very wide host range and many species of weeds become infected with it in the field. Except in climates with dry summer conditions, when they die from lack of moisture if no irrigation is used, or cold climates, in which herbaceous weeds growing outside heated glasshouses fail to survive winter frosts, TSWV-infected weeds upon which thrips vectors can multiply act as a 'green bridge' within which the virus survives in the absence of susceptible crop plants. Weed hosts of both vectors and virus constitute not only major external infection reservoirs for spread of TSWV to susceptible crops but also important 'within-crop' infection sources. However, the weed host species that play critical roles as virus reservoirs tend to vary from one geographic and climatic zone to another (eg. Cho et al., 1996; Bitterlich and MacDonald, 1993; Bautista et al., 1996; Latham and Jones, 1997; Groves et al., 2002). Crop species that TSWV infects and upon which thrips vectors multiply also play pivotal roles as virus reservoirs. Thus, nearby infected ornamental and vegetable crops provide potent external sources of infection for thrips to acquire the virus from and spread it to susceptible crops, as do infected volunteer crop plants growing from corms, tubers or bulbs left behind in the soil at harvest and infected perennial ornamental plants growing in nearby gardens. When present within crops, infected volunteer plants are also important internal infection sources. In addition, if (i) batches of vegetable seedlings containing some that were infected previously within the nurseries they came from or (ii) stocks of corms, bulbs or tubers that are partially contaminated are planted inadvertently, TSWV-infected crop plants can act as potent 'within-crop' infection sources (eg. Zitter and Simons, 1980; Latham and Jones, 1997).

TSWV is acquired only by the first or early second larval stage of the thrips life cycle so its vectors must multiply upon infected plants if winged adults are to spread the virus to healthy plants. It may take up to 3 or more weeks for the life cycle to be completed and for winged thrips within which the virus is replicating to be generated, but successful inoculation requires probes of as little as 5 minutes. Because viruliferous adults maintain an infectious state for the whole of their lifespan and can infect healthy plants with brief probes, they are capable of infecting many plants when they migrate from plant to plant (eg. German et al., 1992; Peters et al., 1996; Moritz et al., 2003). When thrips flying from external infection reservoirs introduce the virus to a TSWVsusceptible crop but are unable to multiply on crop plants or weeds within it, only primary infections and a monocyclic pattern of virus spread develops. If, however, they can multiply upon infected crop plants, volunteer crop plants or weeds within the crop and the crop is of sufficient duration for additional generations of thrips to develop, further cycles of TSWV spread can occur resulting in a polycyclic pattern with higher final virus incidences. Where more than one thrips species is involved, the situation is more complicated as one vector species may multiply on the crop plant while another When this occurs, greater abundance of the thrips vector species that may not. multiplies will result in greater TSWV spread, whereas, if relative numbers of the species that multiplies are small, spread will be slower and the pattern near monocyclic. Similarly, if vector thrips can multiply only on internal weed or volunteer crop TSWVhost plants but not on the susceptible crop itself, the magnitude of secondary spread will depend on the abundance of the weeds or volunteer plants relative to the actual crop plant and on how long the crop lasts. Such considerations may help explain why spatial patterns of TSWV spread reported in the literature often suggest monocyclic patterns of spread or only limited secondary spread of TSWV (eg. Camman et al., 1995; Coutts et al., 2003). They may also help explain why gradients of infection for the virus within crops often reveal a high concentration of TSWV-infected plants close to nearby external infection sources (eg. Latham and Jones, 1997; Coutts et al., 2003), rather than the greater magnitude of spread over distance that might be expected given the persistent nature of TSWV transmission. To obtain a better understanding of patterns of TSWV spread in the field, more information is needed over factors such as the frequency with which vector thrips change hosts and migrate between plants, what triggers increased dispersal when plants visited are rejected, what alters relative susceptibility of hosts and the probability that brief probes will lead to infection (Peters et al., 1996).

Knowledge that TSWV has a very wide host range within crop and weed species, is spread by thrips in a persistent manner but is not seed-borne, is acquired only by the first or early second larval stage of the thrips life cycle, of its temporal and spatial spread patterns of spread, of how different cultivars of susceptible crops perform in the field and of the features typical of increased virus spread scenarios is critical epidemiological information required to form a picture of how its epidemics develop and what factors drive them. Thus potency of an external virus infection source for spread to a TSWVsusceptible field crop is dictated by its size and how long it lasts; whether irrigation is prolonging its life; the proportion of TSWV-infected plants within it; if it is a planted crop itself, or consists of volunteer crop plants or weeds; what thrips vector species infest it; whether the TSWV-infected plants are also hosts upon which thrips vectors can multiply readily; whether the time of year and weather conditions, especially temperature, favour thrips population build-up; when vector thrips first arrive, how abundant they become and their period of activity; its proximity to the exposed crop; whether an intervening windbreak or non-host crop for TSWV or thrips vectors or both is present; whether it is upwind or downwind of the exposed crop; whether the exposed crop is protected by a cover crop, mulch or other groundcover, thrips predators or insecticide treatment; whether the exposed crop is planted with a TSWV-resistant cultivar; its plant density, planting date and row pattern; etc. When field crops are exposed only to internal infection sources, key considerations include: the proportion of TSWV-infected plants present; whether they are crop plants, volunteers or weeds; whether thrips vectors can multiply up on them; if climatic conditions are suitable for thrips population build up; when vector thrips first arrive, how abundant they become and their period of activity; whether cover crops, mulches, thrips predators or TSWVresistant cultivars are present; whether insecticides are used that kill thrips or selective herbicides which kill weeds or volunteer crop plants; if plants with visible TSWV symptoms are rouged out; if plant density is sufficiently high and row spacing narrow enough to shade out infected plants or dilute the rate of virus spread; the tillage system used at sowing time; etc. Similar considerations apply to outside crops in which early TSWV spread has introduced internal primary infection sources from which secondary spread is occurring. For protected crops and vegetable seedling nurseries, additional factors to consider include: if temperature is maintained all year round, inadvertently, at optimum levels for F. occidentalis population increase; the frequency of small side-byside, sequential plantings of susceptible crop plants packed closely together; the extent of exposure of plants to infection at their most vulnerable early growth stage; whether thrips-proof nets are present; if thrips predators have been introduce; if plastics that absorb ultraviolet light were used in tunnel house construction; etc. Advance warning of likely virus epidemics can be obtained by deploying TSWV and thrips 'trap plants' (Ullman et al., 1998) or getting representative leaf samples to detect virus presence. Culbreath et al. (1999) developed a 'spotted wilt risk assessment index' to help determine the relative risk from TSWV damage in field crops of groundnut in south-east USA. The 150 point 'index' is based on site history, cultivar planted, planting date, plant density, row pattern, tillage practices and whether soil-applied insecticide is used, with different weightings applied for each factor. There is scope for this kind of 'index' approach in key vegetable crops and for developing predictive models to forecast TSWV epidemics.

TSWV damages plants by decreasing overall yield and impairing the quality of produce. The magnitude of the losses it causes to marketable yield varies with crop concerned, and with the timing and incidence of infection. For example, TSWV-infected lettuce plants are killed so, within an infected crop, only the remaining healthy lettuces can be sold and if infection incidence reaches 100% there are none to sell. With pepper and tomato, yield is diminished most when young plants become infected early. Fruit from infected plants have visible surface blemish symptoms such that they cannot be sold, so heavily-infected crops are frequently abandoned (eg. Latham and Jones, 1997). In general, in parts of the world where intensive vegetable production occurs outside all year round, TSWV-epidemics cause most damage in situations where

susceptible crops such as lettuce, pepper and tomato are sown in sequential, side-by-side plantings throughout the year, finished crops are not removed promptly, control of weeds and volunteer crop plants is poor, and vegetatively-propagated ornamental plants are present in nearby gardens. Under such conditions, from their most vulnerable early growth stage on, plants near to infected crops are exposed to a barrage of infectious thrips moving from one crop to the next, and the amount of background infection builds up rapidly over time such that production of lettuce, pepper or tomato is no longer economic unless remedial action is taken (eg. Latham and Jones, 1996, 1997). Similar considerations apply in parts of the world where intensive vegetable production occurs within protected cropping situations inside glasshouses or plastic tunnel houses, with epidemics being favoured by sequential, side-by-side plantings of susceptible crops all year round, finished crops not being removed promptly, inadequate control of weeds and volunteer crop plants, and presence of vegetatively-propagated ornamental plants. Because the optimum temperature conditions for growth under which the crops are kept also tend to be optimal for population build up of F. occidentalis and the area available is restricted, such that plants must be grown in close proximity, TSWV epidemics can spread very fast and the need to take remedial action is more urgent than with outside crops. In vegetable production nurseries the need for remedial action is even more pressing as the plants are exposed to infection at their most vulnerable growth stage, sequential side-by-side sowings are packed tightly together and shielded from the weather, and infection with TSWV is often not evident until after they are sold and growing as crops on the purchasers' land. Thus, if control measures are not put in place, a single contaminated nursery can be responsible for spreading TSWV to many crops and farms.

With susceptible crops, such as lettuce, pepper and tomato growing in vegetable seedling nursery and protected cropping situations, the 'economic threshold' for TSWV is frequently exceeded (Fig. 1), so prophylactic use of multiple control measures against TSWV is highly cost effective. This is because of the relatively small areas involved, the high value of the produce and the severe penalties that arise when TSWV is allowed to damage marketable yield. The same applies where intensive vegetable production occurs outside and susceptible crops are sown in small sequential, side-by-side plantings throughout the year. However, where rotation with non-host crops is practiced and weed control is good or susceptible crops can only be grown for part of the year, the 'economic threshold' may only be breached occasionally such that additional control measures against TSWV may rarely be needed.

Since the early Australian research of Hutton and Peak (1949, 1952) and Finlay (1952, 1953) on resistance to TSWV in tomato and its inheritance, much effort has gone into identifying sources of resistance to the virus in a range of crops in different countries (eg. Stevens *et al.* 1992, 1994; Moury *et al.*, 1997). Of the resistances found in pepper and tomato, the hypersensitivity genes *Tsw* and *Sw-5* from *Lycopersion peruvianum* and *Capsicum chinense* respectively have proved most useful and are now present in commercial cultivars. In general, these genes perform well in conferring resistance in the field but strains of TSWV that overcome them limit their effectiveness (eg. Latham and Jones, 1998; Roggero *et al.*, 2002; Thomas-Carroll and Jones, 2003).

Because resistance-breaking strains develop quite readily, resistant cultivars with single gene resistance cannot be relied on alone but must be deployed with other control measures within an overall TSWV management approach. Cultivars with partial resistance are useful here as they can diminish the epidemic rate substantially without resistance breakdown, eg. with partially TSWV-resistant groundnut cultivars (Culbreath *et al.*, 1999).

There is a substantial international literature describing the results of field experiments evaluating the effectiveness of diverse types of cultural and chemical control measures against epidemics of TSWV in susceptible crops (eg. McPherson et al., 1995a,b; Brown et al. 1996; Todd et al., 1996; Pappu et al., 2000; Riley and Pappu, 2001; Coutts and Jones, 2003). The most thorough and comprehensive research has been at the University of Georgia in south-east USA involving multiple control measures against its spread in groundnut, tobacco and tomato (eg. Culbreath et al., 1999; Riley and Pappu, 2000). In summary, the most effective cultural measures for field crops are manipulation of planting date to avoid exposure of young vulnerable plants at peak thrips population and flight times; promote crop canopy cover and higher plant populations by sowing at high seeding rates using optimum planting depth and narrow row spacing to permit growth of vigorous healthy plants that compensate for and shade over neighbouring infected source plants, provide earlier and greater canopy cover to diminish thrips landing rates and dilute the numbers of plants that become TSWV-infected; adding stubble mulch or direct sowing with minium tillage practices to ensure there is a groundcover of straw, stubble or crop debris on the soil surface to help decrease thrips vector landing rates; and covering the ground with silver reflective mulch to diminish thrips landing rates. Study of gradients and spatial patterns of virus spread over distance from external TSWV sources crops gives information on safe planting distances for new field crops or the benefits provided by intervening non-host barrier crops, eg. a distance of 50 metres from a potent TSWV source is normally sufficient, at least with a short-lived crop like lettuce, while a small TSWV source may only warrant a 15 metre gap (eg. Latham and Jones, 1997; Coutts et al., 2003). As regards chemical control measures, applying insecticides either directly to the soil, or, preferably, as seedling drenches just before transplanting, offers greatest promise in suppressing TSWV spread. The most effective chemicals tested so far are the neonicotinyl insecticides imidacloprid and thiomethoxam, which are effective against early larval thrips. Foliar applied insecticides have generally proved ineffective at suppressing TSWV spread by thrips but newer chemicals such as the microbial insecticide Spinosad to which F. occidentalis has so far not developed resistance, offer greater promise. To avoid selecting insecticide resistant F. occidentalis, consecutive foliar applications should be rotated with ones belonging to different insecticide groups (eg. Todd et al., 1996; Pappu et al., 2000; Riley and Pappu, 2001; Coutts and Jones, 2002, 2003). Within protected cropping situations, using plastics that absorb ultraviolet light in tunnel house construction and deploying thrips proof nets decrease thrips vector numbers (eg. Antignus, 2000).

Published integrated disease management strategies for TSWV in field crops (eg. Cho *et al.*, 1989; Latham and Jones, 1996, 1997; Culbreath *et al.*, 1999; Riley and Pappu, 2000; Thomas *et al.*, 2000; Coutts and Jones, 2002), and the critical knowledge described

above on its epidemiology, the factors driving its epidemics and the effectiveness of different types of control measures against it, were employed to develop a comprehensive integrated disease management approach against TSWV in lettuce, pepper and tomato in field crops, and new integrated strategies for protected crops of these species and vegetable seedling nurseries (Coutts and Jones, 2002). The individual component measures are: avoid spread from 1) finished and 2) nearby growing vegetable crops; minimise spread from 3) ornamental plants and 4) weeds or volunteer crop plants. 5) remove crop plants with viral symptoms (=roguing); 6) only introduce healthy transplants; 7) avoid spread within seedling trays; 8) certify the health status of seedling nurseries; 9) use mulches or minimum tillage at planting time; 10) promote early canopy cover and high plant densities; 11) manipulate planting date; 12) employ windbreaks, and barrier or cover crops; 13) deploy resistant cultivars; 14) apply insecticides; 15) introduce thrips predators; 16) diminish thrips build-up using ultraviolet light absorbing plastics; 17) install fine thrips-proof nets; 18) obtain advance warning; and 19), as an ultimate measure where all else fails, institute a 'susceptible crop and weed free period'. Table 5 lists each of these control measure, how it is achieved, its mode of action, and whether it is appropriate to use in nurseries, protected crops or field crops. The measures suitable for all three situations are 3), 4), 14) and 18); 15) can be used in nurseries and protected crops; 1), 2), 5), 6), 10), 13), and 19) are for protected and field crops; 7) and 8) are just for seedling nurseries; 16) and 17 are for protected crops; and 9), 11) and 12) are only appropriate with field crops.

In organic vegetable production, herbicides and insecticides are not admissible on environmental grounds. Unless weeds, volunteer crop and other TSWV source plants are suppressed in other ways, eg. mechanically or by hand removal, inability to control them with herbicides often leads to severe TSWV epidemics and losses. In such situations, rigorous application of as many as possible of the non-chemical control measures in the strategy is vital. In non-organic situations, chemicals should always be used judiciously on vegetables to avoid toxic residues, selecting insecticide resistant thrips and killing off beneficial insects. In protected cropping, insecticide use can be minimised or, in organic production, avoided by releasing thrips predators.

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Measure	How achieved	Mode of action	Nurseries	Protected	Field
			2	crops	crops
Avoid spread from finished crops	Promptly destroy finished crops with herbicide, burn, plough under or cut and remove.	Removes a potent external source of virus infection and thrins infestation for spread to crop.	No	Yes	Yes
Avoid spread from nearby crops	No overlapping crop sowings in close proximity or sequential plantings side by side. Employ safe planting distances from potentially infected crops and use intervening non-host crops or fallow. Plant unwind of potential sources.	Minimises a major external source of virus infection for spread to crop.	No	Yes	Yes
Avoid spread from ornamental plants	Remove all ornamental plants in vicinity, especially if vegetatively propagated.	Removes a key external source of virus infection and thrips infestation for spread to other plants.	Yes	Yes	Yes
Minimise spread from weeds or	Spray selective herbicides on target crop and any neighbouring crons to remove weeds and cron volunteers. Control weeds and	Removes a major internal and external source of virus infection and thrins infection for surread to	Yes	Yes	Yes
volunteer crop	crop volunteers on nearby unused land. Hand weed nurseries and organic crops.	other plants.			
Use roguing within crop	Remove all crop plants with visible virus symptoms. Most effective if removed before virus spread starts	Removes internal source of virus infection for spread to other plants.	No	Yes	Yes
Introduce healthy transplants	Purchase seedlings for transplanting from virus-tested nurseries. Alternatively, propagate directly from seed on site.	Avoids virus infection entering with transplants from contaminated nurseries. Virus is not seed-borne.	No	Yes	Yes
Avoid spread within seedling	Discard any trays in which seedlings show viral symptoms. If possible, get representative leaf samples from seedlings in trays	Removes a major virus infection source for spread to other seedling trays. Avoids virus introductions to	Yes	No	No
trays	virus-tested before consignment release. Promptly remove and destroy all trays of seedling transplants beyond saleable size.	commercial crops via inadvertent use of infected transplants.			
Certification of seedling nurseries	Institute regular inspection procedures for nurseries by impartial authority. Hygiene of operations and absence of visible symptoms certified. Leaf samples taken and tested by serology.	Certification provides end user with confidence over health status of seedling transplant consignments.	Yes	No	No
Use mulches or minimum tillage	Cover soil around plants with reflective plastic or spread straw mulches on soil surface at sowing time. Sow directly into crop debris or stubble without cultivation.	Reflective mulch diminishes thrips landing rates. Straw mulch, stubble and dry crop debris allows predators of thrips to build up.	No	No	Yes
Promote early canopy cover and high plant density	Plant seed or transplants at high seeding rates with close row spacing.	Early canopy cover shades out stunted, early-infected internal virus source plants. High plant density dilutes overall numbers of infected plants.	No	Yes	Yes
Manipulate planting date	Select planting dates to avoid exposure of young plants to peak thrips populations at their vulnerable, young growth stage. Transplant healthy plants instead of sowing seed.	Diminishes infection of plants at their vulnerable young growth stage. Plants becoming virus-infected later are less damaged and yield more.	No	No	Yes

			-	-			
Yes	Yes	Yes	No	No	No	Yes	Yes
No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
No	No	Yes	Yes	(Yes)	(Yes)	Yes	No
Windbreak re-directs incoming thrips way from crop area. Barrier and cover crops diminish thrips landings on crop.	Diminishes virus spread. [But overcome by resistance-breaking strains].	Kills thrips vectors. Particularly important to kill the young larval stages that acquire the virus from infected plants.	Diminishes numbers of thrips vectors.	Minimises thrips population increase rate within protected area.	Prevents thrips from entering protected area.	Provides early warning of increased thrips activity and TSWV spread. Control measures can then be applied as appropriate.	Breaks infection cycle over entire area by removing all herbaceous growing plant TSWV sources.
Erect shade cloth barrier that redirects prevailing winds away from vegetable growing area (fast growing trees another possibility). Surround crop with non-host barrier crop. Sow tall non-host cover crop with target crop.	Plant TSWV-resistant tomato and pepper cultivars.	Apply regular rotational sprays with different insecticides that are effective against thrips. Apply systemic insecticide as seedling drench just before transplanting or to soil at planting.	Introduce natural thrips predators regularly. Only apply insecticides that are ineffective against these predators.	Construct plastic walls of tunnel houses using ultra violet light absorbing plastic.	Protect all entrances and vents of plastic tunnel houses and glasshouses with thrips-proof netting.	Introduce potted plants of petunia or broad bean every week. Leave in place for 1 week. Numbers of necrotic spots on leaves indicate successful TSWV inoculations by thrips. Amount of thrips feeding damage provides measure of thrips activity.	Neighbouring properties in production district co-operate to provide weed and susceptible crop free period of at least 3 months over whole area, including inside any plastic tunnels or glasshouses. Leave district fallow. If soil erosion likely, plant non-host crops eg. green manure or brassica
Employ windbreaks, and barrier or cover crops	Deploy resistant cultivars	Apply insecticide	Introduce thrips predators	Diminish vector population growth	Install fine nets	Obtain advance warning	Institute 'susceptible crop and weed free period' [Ultimate measure when all else fails]

Sources of data used: Cho *et al.* (1989); McPherson *et al.* (1995a,b); Brown *et al.* (1996); Latham and Jones (1996, 1997, 1998); Todd *et al.* (1996); Ullman *et al.* (1998); Culbreath *et al.* (1998); Pappu *et al.* (2000); Riley and Pappu (2000, 2001); Thomas *et al.* (2000); Coutts and Jones (2002); Groves *et al.* (2002); Roggero *et al.* (2002); Thomas-Carroll and Jones (2003); Coutts *et al.* (2003); Roggero *et al.* (2003); Roggero *et al.* (2002); Roggero *et al.* (2002); Roggero *et al.* (2002); Thomas-Carroll and Jones (2003); Coutts *et al.* (2003).

Integrated disease management strategy for tomato spotted wilt virus in seedling nurseries

Integrated disease management strategy devised to minimise infection with TSWV in vegetables within seedlings nurseries

Control measure	How it works
Remove all weeds within and around the	Removes virus sources and thrips
nursery regardless of virus symptoms	infestation reservoirs for spread to
	seedlings
Remove ornamental plants in the vicinity of the	Removes virus sources and thrips
nursery, especially if vegetatively propagated,	infestation reservoirs for spread to
regardless of virus symptoms	seedlings
Discard any trays of seedlings showing viral	Helps eliminate virus reservoirs and
symptoms and promptly remove and destroy	minimises virus spread to other
trays of seedling beyond saleable size	seedling trays
Institute regular inspection procedures for thrips	Avoids TSWV infection being
and virus symptoms in seedlings, and virus test	transferred to other properties
seedlings prior to release	
Regular rotational use of different insecticides	Decreases thrips numbers and
that are effective against western flower thrips.	consequently virus spread
Apply systemic insecticide as soil drenches at	
planting	
Introduce natural thrips predators regularly and	Decreases thrips numbers and
only apply insecticides that are ineffective	avoids over-use of insecticides
against these predators	
Use ultraviolet light absorbing plastic on walls of	Minimises thrips population build up
tunnel houses	
Protect propagation areas and all entrances and	Prevents thrips from entering a
vents of tunnel houses and glasshouses with	protected areas
thrips-proof netting	
Introduce potted bait plants (e.g. susceptible	Provides early warning of increased
petunia or broad bean), change pots weekly	thrips activity (feeding damage) and
	TSWV spread (necrotic spots)







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Department of Agriculture Government of Western Australia

