PT101
Rapid detection, epidemiology and control of tomato spotted wilt virus (TSWV) in seed and processing tomatoes

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Know-how for Horticulture™

PT101

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1. Summary

(a) Industry summary

This study confirms that Spotted Wilt disease of potatoes is of sporadic importance and that TSWV is carried-over in seed tubers but only at a very low frequency. A number of management strategies for this disease have been identified. Certain techniques developed for insect monitoring and recommendations for their control are compatible with other principles of Integrated Pest Management systems that are currently being developed in other research projects.

Several techniques were evaluated for the rapid detection of TSWV. This has assisted in the early detection of Spotted Wilt in potato crops. One of these techniques has the potential to identify particular strains of TSWV. This information is important to establish area freedom from exotic and severe strains of the virus. It will help distinguish TSWV from certain related viruses that have been described in other countries.

Three years of monitoring seed crops in the Crookwell district revealed a low disease incidence (below 0.1%). Only one of the known insect TSWV vectors, *Thrips tabaci*, was found associated with these crops. A wider survey throughout NSW identified three crops with 50–100% infection levels: one site near Crookwell; another near Coleambally and a further one in western Sydney. This emphasises the fact that TSWV has the potential to infect a large proportion of a crop. With each of these instances and in a recent study in tomato crops with high virus infection levels, another thrips vector, *Frankliniella schultzei* was detected. This suggests that more years of monitoring could be necessary for a more accurate picture of virus-vector interactions in any particular production district. Infection of plants soon after their emergence probably explains why there was a perception in the potato industry that TSWV was carried-over at a high frequency in seed tubers.

A stereoscopic dissecting microscope was provided in this project and will remain located in the Crookwell district to assist with pest and disease identification.

Management strategies identified that growers may adopt to reduce the incidence and severity of TSWV are:

* Apply granular insecticides at sowing
* Remove all weeds from the crop vicinity before sowing
* Monitor crops early for thrips and the disease
* Rogue infected plants
* Monitor flowers for black thrips
* Apply strategic insecticide sprays (3 sprays x 5 days apart)

(b) Technical summary

Three relatively rapid detection techniques for TSWV were evaluated: a bioassay using excised *Petunia* leaves; an ELISA test based on an antiserum produced from a American isolate; and a reverse transcriptase Polymerase Chain Reaction (RT-PCR) using primers that amplified the nucleocapsid protein gene. All three techniques successfully detected a range of Australian TSWV isolates from solanaceous crops and the RT-PCR method has the potential to distinguish different virus strains in future studies. A positive reaction to an antiserum derived from an American strain of TSWV confirms that it a the cause of Spotted Wilt disease and not a sereologically distinct virus as has been described from some overseas research (German *et al.*, 1992). This result was supported by electron microscopy which showed that the arrangement of virus particles inside infected tissue conformed with that described for TSWV (Ie, 1971).

Results from ELISA tests demonstrated that seed tubers were not a significant source of TSWV in potato crops since tuber carry-over frequency of TSWV was low (0-7%). Further ELISA results suggested that TSWV did not translocate very well within infected plants. Often only a single stem became infected whilst the remainder of the plant stayed healthy. In some cases infected stems died such that the virus had aborted from these plants altogether. There was no correlation between the number of infected stems and the number of infected tubers in individual plants. This is further evidence that TSWV does not move readily throughout potato plants.

Monitoring of three seed crops in the Crookwell district of NSW over three seasons identified *Thrips tabaci* as the only known vector. Sticky traps confirmed their presence soon after plants had emerged. Disease symptoms also appeared at this time. Such early infections may explain why there was a perception in the industry that seed was a primary source of TSWV. There was a distinct pattern, between properties and between seasons, in the appearance and dynamics of thrips populations: numbers peaked in late January and were no longer detected by early March. Further studies are required to determine the virus-vector relationship of the other potential vector, *Frankliniella schultzei*, which was found associated with higher infection levels in a wider survey of potato crops.

Management strategies for Spotted Wilt should employ the use of granular insecticides to reduce the impact of thrips moving into crops soon after emergence. Removal of weeds that neighbour crops will reduce the risk of infection since they are potential hosts of TSWV and thrips. Crop monitoring for thrips and disease symptoms indicate appropriate timing of strategic insecticide sprays and the roguing of infected plants.

2. Recommendations

(a) Extension/adoption by industry of research findings

* An improved understanding of Spotted Wilt disease symptoms should assist seed growers with early detection and improved monitoring practices.

* Monitoring crops for insects and disease should become a standard management practice. Monitoring for TSWV should be intensified soon after plants have emerged and at flowering.

* The yellow sticky traps developed for this project have clear advantages over those commercially available as they are easier to handle and appear to be more robust in harsh climatic conditions, particularly strong winds. Furthermore, traps are reusable which may confer a significant cost advantage.

(b) Directions for future research and/or activities supported by the HRDC

* The RT-PCR technique developed in this project should be used in further studies to assess the genetic relatedness of TSWV isolates that occur on all Australian horticultural crops.

* The development and adoption of integrated crop management strategies (of which pest and disease management are but a part) needs to be facilitated by trained horticulturists who can regularly visit crops in each production area. Laboratories that can give rapid and accurate identification of pests and diseases need to be supported. Active training programs for growers and consultants need to be included into the objectives and budgets of future projects.

* Management strategies identified in this report need to be evaluated in further studies across all production areas. Vigilance will be required to prevent the introduction of the western flower thrips (*Frankliniella occidentalis*) into production areas.

* The technology is emerging to produce transgenic potato cultivars that are resistant to TSWV. Current research projects that are developing potato cultivars with resistance to other viruses (Potato Leaf Roll Virus and Potato Virus Y), should include TSWV resistance in their objectives. There is little benefit to IPM adoption, particularly to a reduction in pesticide usage, by having resistance to aphid-borne viruses but growers still using potent insecticides for thrips control.

* Further studies are required to evaluate the potential of new insecticides that can be applied at planting. Identifying the duration of their efficacy is of particular importance.

* Evaluation is required for chemical sprays that reduce the risk of insecticide cross-resistance. Petroleum spray oils are one example of such chemicals.

(c) Financial/commercial benefits of adoption of research findings

* Seed potatoes were identified in this study not to be a significant source of TSWV and therefore all seed producers should be able to contain this disease through progressive field generations. Similarly, processing and ware potato growers should be aware that TSWV can be introduced into their crops very quickly with thrips vectors and monitoring practices are necessary for early detection and the prevention of serious losses.

* Better management strategies for TSWV will benefit all sectors of the potato industry by improving product quality.

* Reduced incidence of early infections of TSWV in crisping crops will result in a more even crop stand and therefore fewer large tubers on neighbouring plants that compensate their growth. This will benefit both crisping growers and processors.

* Close monitoring of insect pests and disease symptoms leads to strategic insecticide spraying. This may lead to an overall reduction in the number of spray applications and therefore a direct economic benefit. Less chemical use also reduces the risk of resistance problems developing which ultimately reduces the pesticide costs. Less machinery movement through crops may also reduce the potential for the mechanical spread of two other potato viruses, PVX and PVS.

3. Technical report

(a) Introduction

Spotted Wilt or Bronze Wilt was first described in Australia on tomatoes in 1915 (Brittlebank, 1919). Pittman (1927) showed that this disease was caused by a "virus" and could be transmitted by an insect vector (*Thrips tabaci*). This was later confirmed by Samuel *et al.* (1930) in South Australia but with a different thrips species (*Frankliniella schultzei*). Tomato Spotted Wilt Virus (TSWV) is now known to occur worldwide on more than 500 plant species in some 50 different plant families and includes many important ornamental, fruit and vegetable crops (German *et al.*, 1992). This list has grown with each new published survey. High infection rates commonly reaching 50–100% (Cho *et al.*, 1987) and the dramatic expansion in geographical distribution of on of the major vectors, the Western Flower Thrips (*Frankliniella occidentalis*), makes this one of the most harmful plant viruses. This species of thrips has been intercepted by Australian quarantine authorities on imported produce (G. Brown, pers. comm.), and has been found in W.A., N.S.W. and Qld. since national surveys were conducted in 1993. Table 1 shows the confirmed vectors of TSWV-like viruses and their occurrence in Australia.

TABLE 1 Confirmed Thrips vectors of TSWV-like viruses and their distribution in Australia (Houston *et al.*, 1991; German *et al.*, 1992; Mound & Houston, 1987; Malipatil *et al.*, 1993; Palmer *et al.*, 1989.)

Vector		Distribution in Australia (December 1994)
Scientific Name	**Common Name(s)**	
Frankliniella occidentalis (Pergande)	Western flower thrips	W.A., N.S.W., Qld.
F. fusca (Hinds)	Tobacco thrips	not recorded
F. schultzei (Trybon)	Common blossom thrips, Cotton bud thrips, Tomato thrips	widespread in warmer climates
Scirtothrips dorsalis Hood	Assam or Chilli thrips	Qld.
Thrips tabaci Lindeman	Onion thrips	widespread
T. setosus Moulton	No common name	not recorded
T. palmi Karny	Melon thrips	N.T., Qld.

PT 101 TSWV on potatoes

A disease of NSW potato crops caused by TSWV was recorded in 1935 (Magee, 1936). Affected plants are characterised by brown spots or rings on leaves (Figures 1 & 2). When infected soon after emergence, plants appear bunched and leaves are slightly yellow and rolled back (Figure 3). Plants become noticeably stunted (Figure 4). Older leaves may have zoned brown spots that are indistinguishable from the fungal disease, Target Spot (Early Blight). The shoot apex may be blighted and younger plants may be killed.

TSWV affects potato crops in all NSW production areas, albeit sporadically. It has been suggested that dry seasons favour the disease, and this is thought to coincide with an increased movement of thrips (Conroy *et al.*, 1949). TSWV is of particular concern in seed production areas of Tableland districts where four plant generations have the potential to spread the virus in two ways: from infected to healthy plants within a crop and through infected tubers, thus multiplying the level of the virus. Earlier studies (Conroy *et al.*, 1949) estimated that yields of infected plants were reduced by 75 percent. A severe epidemic was recorded in 1946–47 in the Central Tablelands and resulted in the rejection for certification of 523 out of 541 acres of seed potatoes.

No conclusive studies have determined the extent of transmission of TSWV through tubers. Norris and Bald (1943) demonstrated that 31 percent of tubers (cv *Factor*) showing obvious cracking and deformation (symptoms thought to be due to TSWV) gave rise to plants with typical symptoms. These affected plants survived long enough in the field to act as reservoirs for the further spread of the disease. Conroy *et al.* (1949) compared field symptoms in plots sown from a heavily infected crop versus plants from apparently healthy tubers. They found some 16% of plants became infected from the 'suspect' tubers, while just over 7% of plants were affected when 'healthy' seed were sown. These results suggest that the rate of tuber carry-over was quite low, even allowing for secondary spread of the virus. However, neither of these studies could separate primary from secondary infections and did not take into account the stage of development that mother plants had become infected and therefore to what extent the virus had translocated to tubers.

TSWV has several unique qualities when compared with other plant viruses. It has a negative-sense single stranded RNA genome that is tightly associated with proteins forming pseudo-circular nucleocapsids that are bound by membrane envelopes with surface projections. Particles are 80–110 nm in diameter and are more closely related to the arthropod-borne Bunyaviridae family of viruses than to other plant viruses. This phylogeny was first suggested in 1984 by Milne and Francki and was officially confirmed at the 1990 meeting of the International Committee on Taxonomy of viruses. TSWV is the prototype of the genus Tospovirus within the Bunyaviridae. More recent studies have shown that there are several related but distinct viruses to TSWV (Law *et al.*, 1992). Impatiens Necrotic Ringspot Virus (INSV, formally TSWV-I), Watermelon Silvery Mottle Virus, Peanut Bud Necrosis Virus and Peanut Yellow Spot Virus are now recognised as serologically distinct viruses from TSWV (German *et al.*, 1992).

PT 101 TSWV on potatoes

The major aims of this study were:

(i) to confirm the identity of virus isolates causing Spotted (Bronze) Wilt Disease of NSW potato crops;

(ii) evaluate different methods for virus detection;

(iii) establish field monitoring procedures for this disease and potential insect vectors; and

(iv) determine the extent of virus carry-over in potato tubers.

Figure 1. Typical TSWV symptoms on potato : dark patches on leaves with characteristic ringspots.

Figure 2. Milder symptoms of TSWV are expressed only as black spots and rings on potato (cv. Atlantic).

Figure 3. Young leaves with TSWV infection appear bunched, thickened, slightly yellow and rolled back. Dark marking develop on their upper surface.

Figure 4. TSWV infected potato (centre) is noticeably stunted compared with neighbouring healthy plants.

(B) MATERIALS & METHODS

(i) Detection of TSWV

Bioassays

Several methods were used to detect TSWV in plants. Four cultivars of *Petunia hybrida* Vilm. were tested in bioassay experiments. They were cultivars: *High Society, Rose of Heaven, Colour Parade* and *Celebrity White*. TSWV isolates were held as systemic infections on certain indicator plants (*N. tabacum* and *N. glutinosa* [Figure 5]). Mechanical inoculations were performed using crude sap ground in a neutral phosphate buffer with 0.01 M sodium sulphite (Francki and Hatta, 1981). Excised *Petunia* leaves (cv. *High Society*) were inoculated as described above and placed on 9 cm petri plates containing 10 ml of tap water agar. Three dilution ratios of tissue to buffer were used: 1:10, 1:25 and 1:50. Petioles of inoculated leaves were pressed into the agar to maintain turgor. Plates were covered with lids and incubated in a stepped temperature incubator with fluorescent lighting. Local lesions were scored over 5 days (Figure 6).

Enzyme Linked Immuno-Sorbant Assays (ELISA)

ELISA was performed on potato plants collected from the major production areas of NSW as well as tomato crops from the Riverina and the Sydney Metropolitan Area. Plants were selected for their spotted wilt symptoms. which were also used as positive controls for ELISA. A double antibody sandwich method (Clark and Adams, 1977) was used with commercial antisera obtained from Agdia Inc., Elkhart, IN 46514, USA (Figure 7). Two serologically distinct antisera, TSWV-L and INSV (TSWV-I) were used.

Electron Microscopy (EM)

Transmission electron microscopy was used to confirm the virus morphology and arrangement in potato cells. Standard tissue preparation methods were used (Ie, 1971). Thin sections were viewed with a Philips EM 300.

A study was undertaken by Dr Ray Pares (Plant Virologist, BCRI) to investigate TSWV detection with Immuno-Electron Microscopy (IEM) using previously described methods (Pares and Whitecross, 1983).

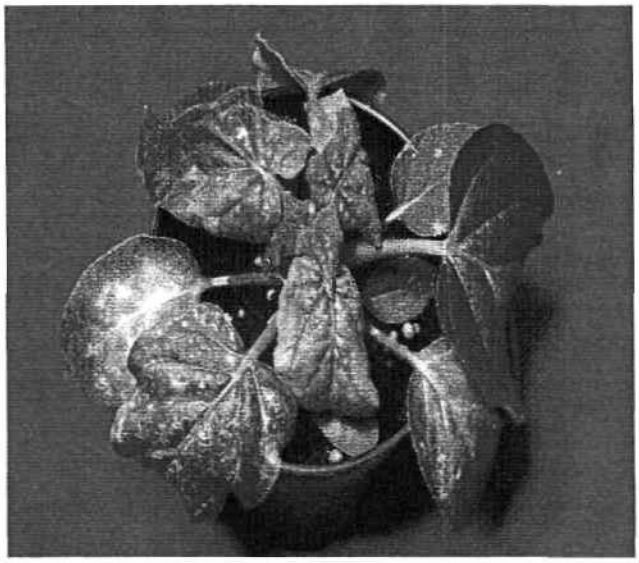


Figure 5. Sap from TSWV infected plants was inoculated into indicator plants (*Nicotiana glutinosa*) causing severe disease symptoms in 5 days.

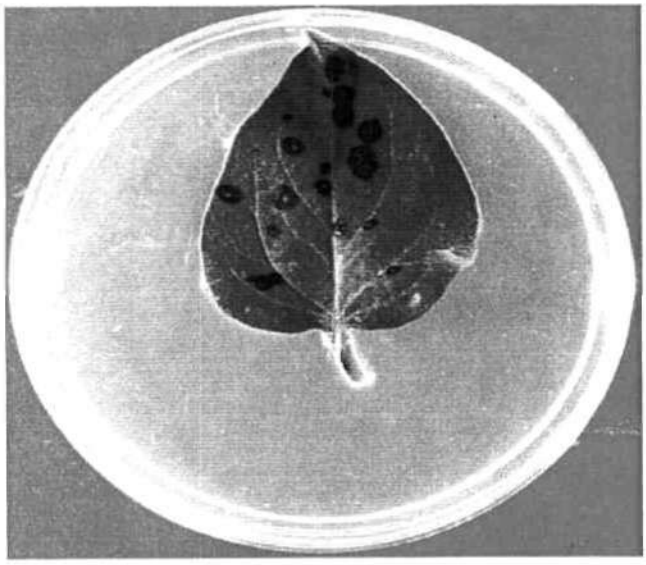


Figure 6. Petunia bioassay : This leaf was inoculated with infected sap and characteristic local lesions developed in 48-72 hours.

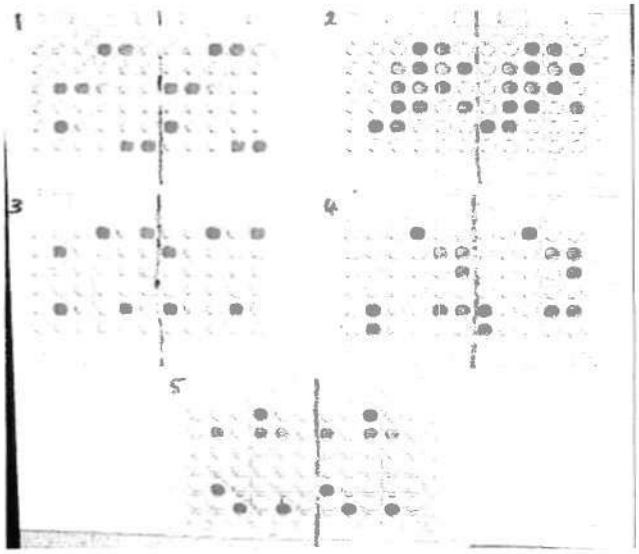


Figure 7. ELISA was used successfully to detect TSWV in infected plants. The yellow dye indicates presence of the virus in sap samples.

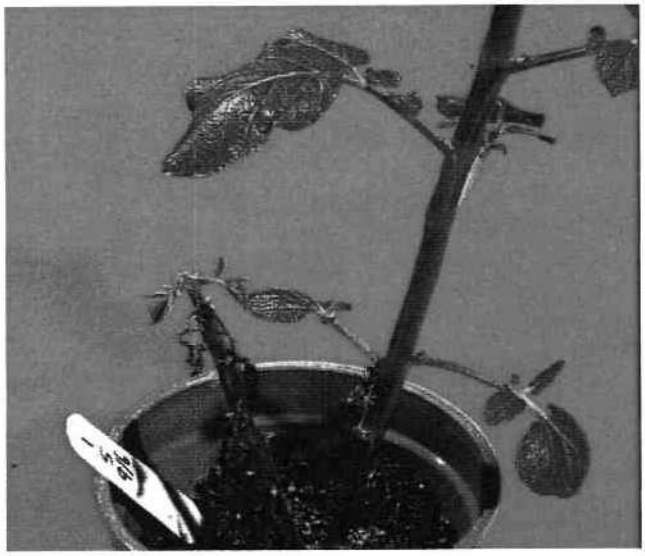


Figure 8. Blighted and blackened stems indicated this potted tuber was carrying TSWV. This was confirmed by ELISA.

Analysis of Double Stranded RNA by Electrophoresis

Double stranded RNA (dsRNA) was extracted from TSWV-infected tomato, potato or indicator plants (*Nicotiana glutinosa*) using a modification of the CF 11 cellulose chromatography procedure of Morris and Dodds (1979). Five grams of tissue were ground in liquid nitrogen and mixed in a 50 ml centrifuge tube with 9 ml of phenol:chloroform:isoamyl alcohol (25:24:1 v/v/v, Sambrook *et al.* 1987), 15 ml extraction medium (1.67 x STE, 1.25% SDS, 1.0% PVP) (STE = 50 Mm Tris-HCl, 100 Mm NaCl, 1 Mm EDTA, Ph 7.2), and 200 ul B-mercaptoethanol. The tube was held at room temperature for 30 min, after which it was centrifuged at 12,000 rpm (Sorvall SS34 rotor) for 20 min. The aqueous phase was recovered, adjusted to 16% (v/v) ethanol and diluted to 40 ml with STE/16% ethanol (v/v). The sample was applied to a 2 ml column of CF 11 cellulose (Whatman) equilibrated in STE/16% ethanol, and the column washed with 80 ml of the same buffer. DsRNA was eluted from the washed column with 10 ml of STE buffer, and precipitated by adding 3 volumes of absolute ethanol. After overnight storage at -20°C, dsrna was pelleted by centrifugation as above. The dsRNA pellet was resuspended in 100 ul of TE buffer (Sambrook *et al.* 1987).

DsRNA samples were electrophoresed on 1.2% agarose gels cast and run in TBE buffer (Sambrook *et al.* 1987). Gels were run at 100 volts for 2 hours and were stained with 1 μg/ml ethidium bromide for 30 min. Gels were photographed using transmitted UV light and an orange filter onto Polaroid Type 55 instant film.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Primers for amplifying the TSWV coat protein gene were designed using the DNASIS program (Hitachi) and were based on the sequence of the S RNA of TSWV from several isolates of TSWV lodged in GenBank (de Haan *et al.* 1989, 1990, Maiss *et al.* 1991, Pang *et al.* 1993). The primers were synthesised using a Gene Assembler Plus (Pharmacia-LKB) and were: TSWVCP1 (5' atggttaagctcactaagg), and TSWVCP3 (5' ttaacacactaagcaagcac). The forward primer TSWVCP1 was homologous to bases 3 to 21 of GenBank # TSPNUCAPC (Pang *et al.* 1993). The reverse primer TSWVCP3 was complementary to bases 841 to 860 of GenBank # TSPNUCAPC (Pang *et al.* 1993). These positions correspond to the start of the nucleocapsid gene (ATG) through to the termination codon (TAA).

The cDNA synthesis and amplification of the TSWV coat protein gene was performed using the Perkin Elmer Cetus Geneamp RNA PCR kit. A 2ul aliquot of dsRNA or 2ul of total nucleic acids extracted from TSWV infected plants (100 mg tissue equivalent) was boiled for 5 min and snap chilled on an ice slurry. Reverse transcription mix (16 ul: 50 mM KCl, 10 mM Tris pH 8.3, 5 mM $MgCl_2$, 1 mM each dNTP, 0.5 U/ul RNAse inhibitor, 1.25 U/ul reverse transcriptase, 1.5 uM TSWVCP1) was added, and the tubes held at room temperature for 5 min. The cDNA reaction was performed at 42°C for 20 min, after which the reverse transcriptase was inactivated by heating to 99°C for 5 min. The forward primer TSWVCP3 in 80 ul of PCR master mix (prepared as specified by the manufacturer) was added to the reverse transcription reaction. cDNA was amplified using

the following temperature program in a Hybaid thermal cycler: 94°C 1 min (1 cycle), 94°C 1 min, 50°C 1 min, 72°C 1 min (35 cycles), 72°C 5 min. The success of the amplification was monitored by analysing an aliquot on 1.2 % agarose gels cast and run in TBE buffer, Ph 8.3 (Sambrook *et al.* 1987). Gels were run at 100 volts for 90 min and stained with ethidium bromide.

Restriction analysis of RT-PCR products

PCR products were separated from the overlain paraffin oil by extraction with chloroform. Products were selectively precipitated from the supernatant by the addition of 0.6 vol of 20 % (w/v) PEG 6000 in 2.5 M NaCl. Tubes were held at 37°C for 10 min and then at 4°C for 30 min. PCR products were collected by centrifugation (15 min microfuge), washed with 80 % ethanol, air dried and resuspended in 50 ul TE buffer. Amplified capsid protein genes were digested with *Hinf* 1 according to the manufacturer's instructions. Digests were analysed by electrophoresis on 4% Nusieve 3:1 agarose gels (FMC) cast and run in TBE buffer (Sambrook *et al.* 1987). Gels were stained with ethidium bromide and photographed using transmitted UV light with an orange filter and Polaroid film.

(ii) Epidemiological Studies of TSWV

Transmission of TSWV through tubers

An initial experiment was conducted in a glasshouse. Fifty healthy tubers (cultivars *Sebago* and *Atlantic*) were sown in 15cm pots and mechanically inoculated with sap from an TSWV infected plant. Plants were observed for symptoms and sampled leaves were tested by ELISA for TSWV. Only plants that had been successfully infected were carefully repotted into 30cm pots and grown on to maturity. Tubers were harvested, stored and replanted after dormancy had broken. Shoots were noted for symptoms (Figure 8), sampled and tested by ELISA.

A survey of crops in the Crookwell district identified young plants infected with TSWV. Plants (cultivars *Sebago*, *Atlantic* and *Coliban*) were dug up, carefully repotted to 30cm pots and grown on to maturity in a glasshouse. Tubers were harvested, stored and repotted after dormancy had broken. Tissue samples were collected and tested by ELISA.

A field plot of sixty plants (cv. *Sebago*) were grown to maturity at BCRI in a further experiment. Plants were not sprayed with insecticides to increase the risk of thrips and TSWV infections. Tubers were collected from individual plants. They were sprouted and tested for TSWV by ELISA.

Distribution of TSWV within infected plants

Eight plants that began to exhibit TSWV symptoms at the early flowering stage (cv. *Atlantic*) were marked with stakes and allowed to grow-on until vines began to die-off. Plants, with tubers attached, were collected and processed in the laboratory. Leaflets from the upper three leaves and the lower three leaves were sampled separately from each stem and tested by ELISA. Tubers were stored and replanted after dormancy had broken. Tissue samples were tested by ELISA.

Monitoring for TSWV and vectors in NSW potato crops and their environs

Surveys for TSWV were conducted in major NSW production regions: Central Tablelands (Orange & Millthorpe), Southern Tablelands (Crookwell), Riverina (Griffith-Coleambally) and the Sydney Basin (Western Sydney & Gosford). A survey for TSWV was also conducted on ornamental plants growing in the homestead garden of a foundation seed potato grower in Crookwell. Twenty different plants were collected and tested for TSWV by ELISA.

Three seed potato crops in the Crookwell district were monitored for insects vectors for TSWV during the 1991-2, 92-3 and 93-4 seasons. Traps consisted of a 15 cm petri plate held vertically above the crop canopy on a central pole. Four traps facing the cardinal points were attached to each pole. Each property had four poles giving a total of 48 traps in the district. Traps were changed weekly and insects counted with the aid of a dissecting microscope. Two shades of yellow and the direction traps were facing were assessed in the

1991–2 season. A number of staff members assisted with this phase of the project. Technical assistants: Dr Mohamid Nabi (1992), Jenny Thompson (1993), were employed to score insect numbers on traps. Mary Ann Terras and Dr Alan Clift (Entomologists, Rydalmere) collated and statistically analysed insect data. Dr Murray Fletcher (Entomology/Taxonomy, Rydalmere) and Dr Mali Malipatil (Victorian Department of Food & Agriculture, Knoxfield) confirmed the identity of insects.

A microscope (Leica Zoom 2000 with built–in light source) was purchased and located in the Crookwell district to facilitate grower adoption of insect monitoring.

(C) RESULTS

(i) Detection of TSWV

Bioassays

Bioassays for TSWV on four *Petunia* cultivars gave no significant differences in local lesion scores or the dilution limit of detection. An incubation temperature for excised *Petunia* leaves (cv. *High Society*) of 22.5°C resulted in highest number of local lesions scored (Figure 9). Controlling environmental conditions during the incubation period increased the reliability of the bioassay.

High summer glasshouse temperatures often resulted in indicator plants (*N. glutinosa* and *N. tabacum*) 'growing out' of virus infections. In many cases freshly inoculated plants either only exhibited local lesions or failed to be infected altogether.

Enzyme Linked Immuno-Sorbant Assays (ELISA)

ELISA tests detected TSWV (TSWV-L) in all plants showing typical symptoms. The TSWV-I antiserum failed to recognise the virus, resulting in negative ELISA results. This confirms that the spotted wilt disease of potatoes and tomatoes in NSW is caused by TSWV and not INSV or another sereologically distinct virus with similar morphology.

Electron Microscopy (EM)

Transmission electron microscopy confirmed that virus morphology and their cellular arrangement conform to that of TSWV (Ie, 1971) (Figure 10). No highly ordered paracrystalline arrays of virus bundles were observed as have been described for INSV (Law & Moyer, 1990).

TSWV could be detected by the IEM technique (Figure 11), but was not reliable since all preparations also contained plant cell fragments of a similar size and appearance to TSWV particles. Detection was made easier when particles were grouped (Figure 11).

Figure 9 Local lesion scores in petunia bioassay with varying sap dilutions and incubation temperatures

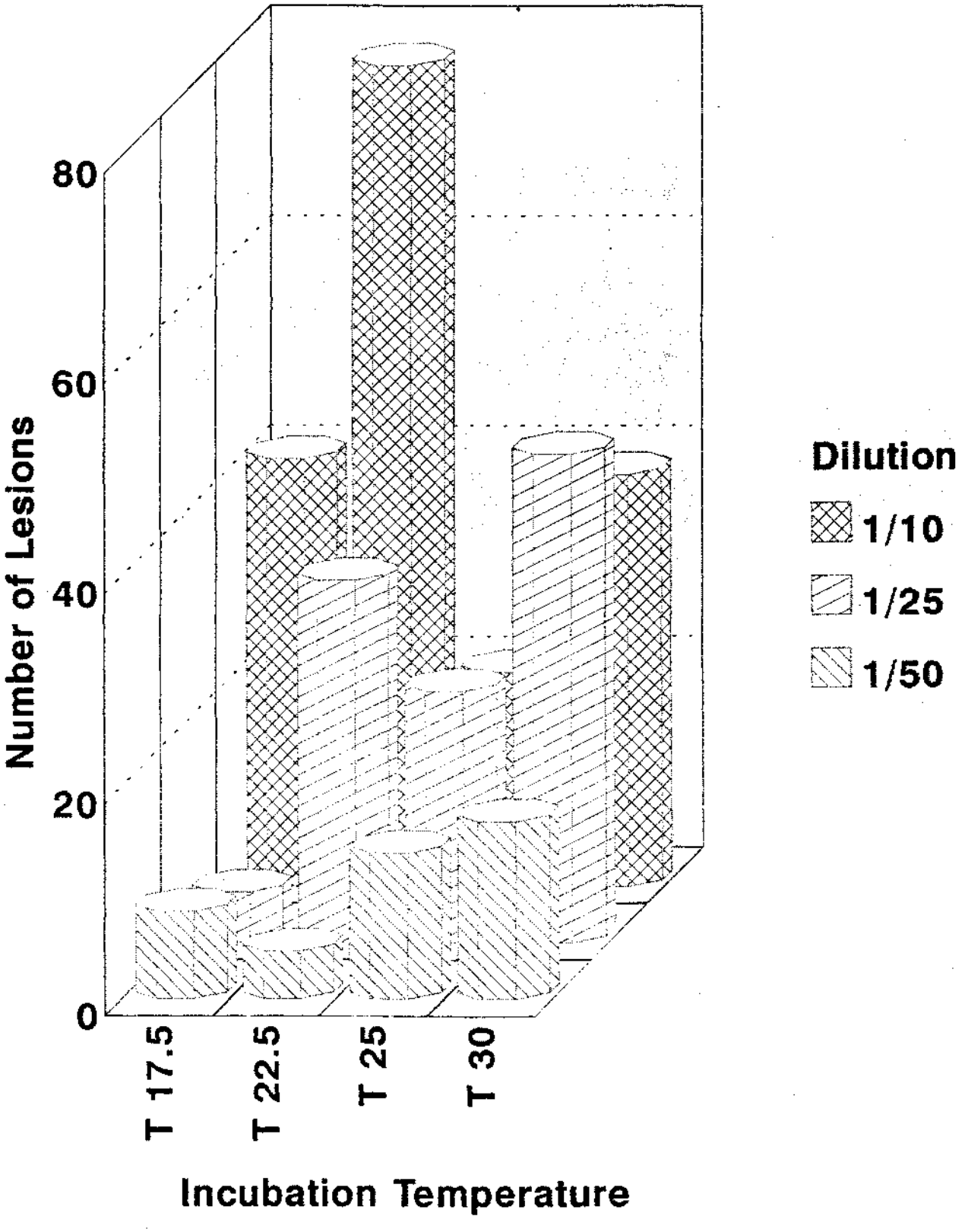


Figure 10 Transmission Electron Micrograph of TSWV in potato cell.

Figure 11 Immuno-electron Micrograph of TSWV particles.

Analysis of TSWV-specific dsRNA

The dsRNA extracted from TSWV-infected plants was analysed by electrophoresis on 1.2% agarose gels. No dsRNAs were detectable on any gels, despite using dsRNA extracted from the equivalent of 2 g of tissue, and despite analysing dsRNAs extracted from tissues known to be strongly positive for TSWV by ELISA. A minor contamination with high molecular weight DNA was observed, showing that the nucleic acids were not lost during the dsRNA purification process. No potential dsRNA bands were seen in electrophoretic separations of total nucleic acids, although the DNA and ribosomal RNA bands were clear and sharp, showing that there was no degradation of the material. It was assumed that dsRNA was indeed present in both the total nucleic acids and the purified samples, but at levels below the sensitivity of the ethidium bromide staining.

Amplification of the TSWV capsid protein gene

The dsRNA and total nucleic acid samples were used as templates for production of cDNA. Primers TSWVCP1 and TSWVCP3 were both tested for their ability to produce specific cDNA from dsRNA templates. The cDNAs were then tested for efficiency of the subsequent amplification by PCR. It was found that primer TSWVCP1 produced the better yield of specific product from TSWV dsRNA. After reverse transcription and amplification (RT-PCR), samples prepared from TSWV-infected plants produced a DNA fragment of approximately 860 base pairs (bp), indistinguishable from the known size of the capsid protein gene (858 bp, Pang *et al.* 1993). Amplifications using TSWVCP3 as the cDNA primer produced an additional band of about 900 bp generated from priming down stream from the capsid protein gene.

Amplifications were successfully performed on dsRNA prepared from infected tomato, potato and *Nicotiana glutinosa*, even though no dsRNA could be detected in any of the samples. DsRNA from the 12 TSWV isolates tested each produced a DNA fragment of the same size after RT-PCR, regardless of the area of origin. No amplification products were detected using samples prepared from uninfected plants or from reaction mixes to which no RNA was added. No amplification was observed using total nucleic acids as a template for RT-PCR.

Restriction Analysis of the Amplified Capsid Protein Gene

RT-PCR products from TSVW-infected plants were purified and digested with the restriction enzyme *Hinf* 1. This enzyme generated fragments of approximately 485, 170 and 95, 75 and 40 base pairs, in agreement with the sizes predicted by the database sequences of 486, 162, 93, 77 and 42 base pairs. No polymorphism was detected in the 12 isolates tested, but this is not surprising given the limited number of samples and restriction enzymes tested.

(ii) Epidemiological Studies of TSWV

Transmission of TSWV through tubers

Carry-over of TSWV was low. Experimental results are summarised in Table 2.

TABLE 2 Carry-over of TSWV through tubers

Time of Infection	% Tuber Carry-over
Early (emergence-six leaf stage)	2.3
Mid-Season (early flowering)	6.9
Late (late-post flowering)	0

Distribution of TSWV within infected plants

A study on the distribution of TSWV in infected plants suggests that the virus is partitioned in certain plant tissues. Results are summarised in Table 3.

TABLE 3 Proportion (%) of stems and tubers of individual plants (numbered 1-8) infected with TSWV

Plant	1	2	3	4	5	6	7	8
Stems	25	78	17	25	14	83	50	33
Tubers	0	0	0	0	10	23	0	33

Monitoring for TSWV and its vectors in NSW potato crops and environs

Three surveyed crops had TSWV infection levels of over 50%. One site was at Rosyln, near Crookwell (cv. *Atlantic*); the second site at Coleambally in the Riverina (cv. *Sebago*); and the third site in western Sydney (cv. *Sebago*). The common blossom thrips, *Frankliniella schultzei*, was found associated with these crops. The survey of ornamental plants in the homestead garden at Crookwell failed to detect a reservoir for the virus.

Seed crops monitored at Crookwell had very low infection rates of TSWV (<0.1%) in all three years of monitoring. Figures 12 and 13 show traps being changed and plants being scored for TSWV symptoms. TSWV infections were detected soon after plants had emerged and showed a typical stunting and necrotic pattern on the upper surface of leaves.

There was no significant difference in thrips counts between the two shades of yellow used on sticky traps (visible on Figure 14) or the direction traps faced. The identity of brown thrips scored on sticky traps (Figure 15) were confirmed by clearing and mounting on microscope slides (Figure 17). Both *Thrips tabaci* (a TSWV vector) and *Thrips imaginis* (not a TSWV vector) were identified. Tubular Black Thrips (*Haplothrips victoriensis*), another non-vector of TSWV, were easily distinguished on sticky traps (Figure 16).

Thrips were detected early in each growing season on all properties (peaking in late January), but fell to insignificant numbers by the eighth week of monitoring (Figures 18 & 19) (early March). Statistical analysis of data showed that thrips counts were not significantly different between properties.

Figure 12. Sticky traps were designed and used in potato crops in Crookwell and Blayney. Traps were changed weekly in the 1991-92 season by Dennis Payne.

Figure 13 (above). Potato crops being monitored by scouts. Mary Ann Terras (left) assisted with insect counting.
Filomena de Almeida (centre) changed traps weekly in 1993-94.
Sandra Lanz (right) is a crop consultant working in the Robertson district.

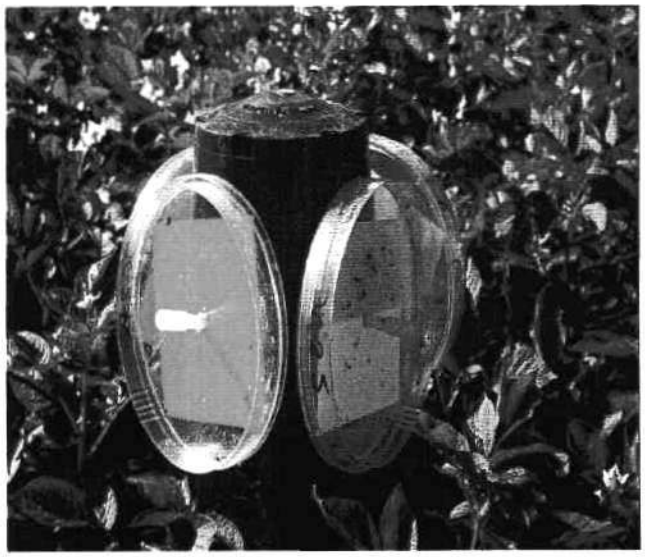


Figure 14. Sticky traps were inexpensive and based on 15 cm plastic petri dishes which could be reused after counting simply by washing and adding more sticky gum.

Figure 15. Brown thrips when viewed on a sticky trap with a dissecting microscope. it is not possible to distinguish between *Thrips* species but their presence was accompanied by TSWV symptoms on plants.

Figure 16. Tubular Black Thrips (*Haplothrips victoriensis*) is not a vector of TSWV and could be easily distinguished by a dissecting microscope on sticky traps.

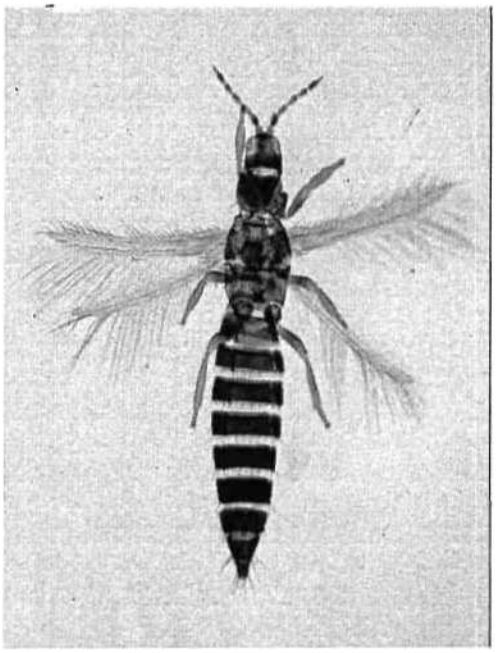


Figure 17. *Thrips tabaci* was the only TSWV vector found in monitored potato crops over 3 years.

Figure 18 Brown thrips on sticky traps in the 1991–2 season at Crookwell

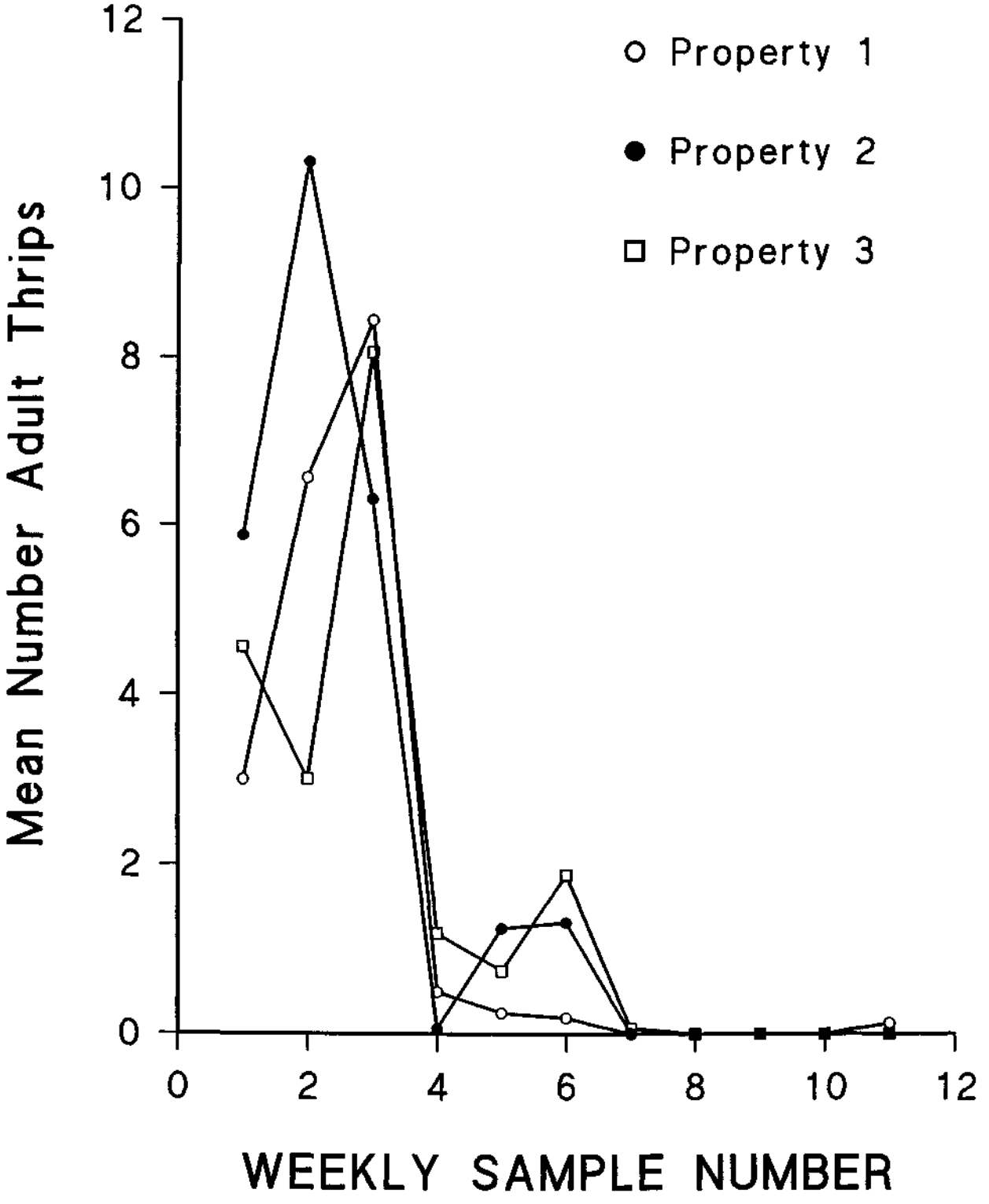
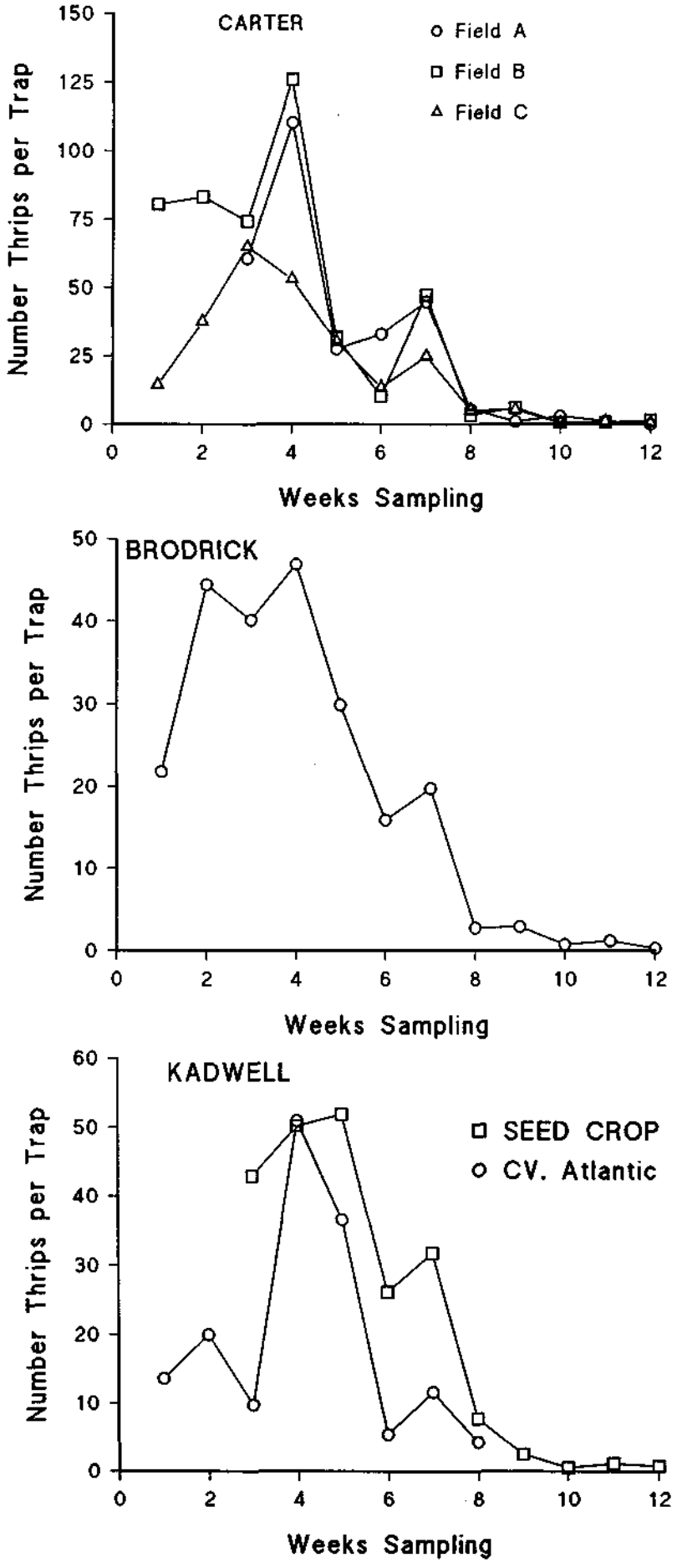


Figure 19 Brown thrips on sticky traps in the 1993–94 season at Crookwell



(D) DISCUSSION

All techniques successfully detected TSWV in plants showing typical virus symptoms. Bioassay (using excised *Petunia* leaves), ELISA and RT-PCR are relatively rapid and therefore any one may be chosen for the resource level of the diagnostic laboratory and the number of samples to be tested. RT-PCR could be used to distinguish TSWV strains and correlate their virulence, host ranges and geographical distributions. This information may be useful in the future to establish area freedom from exotic and severe strains of the virus.

The low infection levels in seed crops and the low level of tuber carry-over of TSWV suggest the seed tubers are not a significant source of TSWV. Evidence that TSWV was not widely distributed within infected plants also strengthens this argument. These relatively low levels of infection in seed crops probably also reflects containment of the disease due its early detection, roguing and regular spray programs such that no significant secondary spread occurred.

The fact that *Frankliniella schultzei* was only found associated with high levels of infection in processing and ware crops implies that it is a far more important vector of TSWV than *Thrips tabaci* which was only found associated with the monitored seed crops.

Infection of crops soon after emergence probably explains why there was a perception in the potato industry that TSWV is carried-over at a high frequency in seed tubers. Results in this study do not support this view. The use of granular insecticides at planting is seen as one management strategy that will reduce early TSWV infections. Other commonsense measures such as the removal of weeds from the crop vicinity and the regular monitoring for thrips and disease symptoms should also be encouraged. Infected plants should be rouged and an insecticide spray program should be initiated to minimise the secondary spread of TSWV.

Recent developments overseas have shown that plants may be genetically engineered with resistance to TSWV (Gielen *et al.*, 1991). Tobacco plants were successfully transformed with the viral nucleocapsid protein gene and were shown to be resistant to TSWV. Until such technology is successfully applied to the potato cultivars grown here, monitoring of crops for thrips and the disease, together with an effective and responsive spray program are seen as the best control strategies.

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