Management of tomato spotted wilt virus in potatoes

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MANAGEMENT OF TOMATO SPOTTED WILT IN POTATOES

FINAL REPORT
(14th FEBRUARY 2005)

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DEPARTMENT of PRIMARY INDUSTRIES, WATER and ENVIRONMENT
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Media Summary

Tomato spotted wilt virus (TSWV) occurs sporadically in potato crops in Australia, sometimes causing severe losses. Very little is known about the disease in potato making it difficult to determine the underlying factors responsible for disease. Part of the problem stems from the sporadic nature of the disease in potato, which makes it hard to find a consistent data set for analysis, at the desired level of aggregation, both for disease incidence and intensity as the variables to be explained and for explanatory variables.

As part of a continuing effort to deepen our insights into the ecology and epidemiology of TSWV in potato crops, surveys were conducted from June 2001 to March 2004 in commercial potato fields in South Australia, Victoria and Tasmania. Mathematical models were developed to predict onion and tomato thrips (the only two known vector thrips species found in potato crops) population fluctuations. The models can be used as a hazard prediction for integrated TSWV disease management.

Among the most significant factors affecting the epidemiology of virus diseases is the inherent susceptibility of the cultivars being grown. The reaction of twenty-seven potato cultivars, with diverse genetic backgrounds, to TSWV were studied for two seasons in both glasshouse and field conditions, consistent with normal commercial growing practices, and to evaluate the effects of infections at different stages of plant growth. Variations in susceptibility to infection were exhibited by potato cultivars and were conditioned by the age of the plant at the time of infection as measured by symptom expression, shoot and tuber infections.

Viral movement restriction is a common natural resistance mechanism to infections in many infected plants. To further understand the observed resistance to both infection and virus systemic invasion in potato, the effects of temperature and its interaction with plant age at the time of inoculation on foliar and tuber infections of TSWV, and symptom expression in two commonly commercially grown potato cultivars Shepody (susceptible) and Russet Burbank (tolerant) was studied. Overall, there were variations in the virus movement patterns in early versus late inoculations at different temperatures used.

Factors that influence thrips dispersal determines if and when. Onion thrips preferences and performances on host plants were examined, in both choice and non-choice assays, to gain insights into the relative importance of different potential TSWV transmission patterns. Thrips raised on either TSWV-infected potato, tomato or *D. stramonium,* fed and reproduced preferentially on some test plants. Thrips did not reproduce on potato cultivars Bismark and Tasman and on the weed, *C. album,* confirming non-preference for these plants and suggesting the involvement of some non-contact cues involving antibiosis or antixenosis.

Insecticide trials across three seasons indicated the potential of both pre-plant and foliar treatments to reduce disease spread in Tasmania (where onion thrips is the only known TSWV vector active in potato). In contrast, a trial in South Australia where both onion and tomato thrips are present indicated little benefit of insecticide treatment.

In summary, the results from the above studies, collectively, contribute to uncover some ecological relationships and patterns of both TSWV and its vector thrips that can be integrated with plausible mechanisms to explain the epidemiology of the virus in potato crops and advance the rationale for future research in this direction.

Technical Summary

Tomato spotted wilt virus (TSWV) occurs sporadically in potato (S*olanum tuberosum*) crops in Australia, sometimes causing severe losses. Very little is known about the disease in potato as evidenced from the literature review (Chapter 1), making it difficult to determine the underlying ecological and epidemiological factors regulating inoculum and thus intensifying epidemics. Part of the problem stems from the sporadic nature of the disease in potato, which makes it hard to find a consistent data set for analysis, at the desired level of aggregation, both for disease incidence and intensity as the variables to be explained and for explanatory variables.

As part of a continuing effort to deepen our insights into the ecology and epidemiology of TSWV in potato crops, surveys were conducted from June 2001 to March 2004 in commercial potato fields in the southern States of Australia. Empirical descriptions of risk factors and statistical exploration of their interactions into functional complexes, which cause sporadic epidemics in potato crops was done based on the survey data. Mathematical models were developed, defining spatio-temporal oscillations, in both population structure and demography, of the *Thrips tabaci* and *Frankliniella schultzei* populations, the only two known vector thrips species found in potato crops, to regional weather variables (Chapter 2). The models can be used as a hazard prediction to orient integrated TSWV disease management.

The above study also indicated a more complex scenario than simply the effect of weather variables in thrips dispersal and population dynamics on one hand, and TSWV epidemics in potato crops on the other. Among the most significant factors affecting the epidemiology of virus diseases is the inherent susceptibility of the cultivars being grown. The reaction of twenty-seven potato cultivars, with diverse genetic backgrounds, to TSWV were studied for two seasons in both controlled (glasshouse) and naturally field conditions, consistent with normal commercial growing practices, and to evaluate the effects of infections at different stages of plant growth. Variations in susceptibility to infection were exhibited by potato cultivars and were conditioned by the age of the plant at the time of infection as measured by symptom expression, shoot and tuber infections (Chapter 3).

Viral movement restriction is a common natural resistance mechanism to infections in many infected plants. To further understand the observed resistance to both infection and virus systemic invasion in potato, the effects of temperature and its interaction with plant age at the time of inoculation on foliar and tuber infections of TSWV, and symptom expression in two commonly commercially grown potato cultivars Shepody (susceptible) and Russet Burbank (tolerant) was studied (Chapter 4). Overall, there were variations in the virus movement patterns in early versus late inoculations at different temperatures used.

Factors that influence thrips dispersal determines if and when external inoculum will become available for infection, and consequently the initiation, sustenance and spread of an epidemic. *Thrips tabaci* preferences and performances on host plants were examined, in both choice and non-choice assays, to gain insights into the relative importance of different potential TSWV transmission patterns (Chapter 5). The female *T. tabaci* population used, failed to transmit the TSWV isolate An_{WA}*-1* from the systemically infected potato, tomato, *Datula stramonium, Arctotheca calendula* (Cape weed) or *Solanum nigrum* (Blackberry nightshade) to seven potato cultivars; Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic and Victoria and three weeds; *A. calendula*, *Chenopodium album* and *S. nigrum*. Thrips raised on either TSWV-infected potato, tomato or *D. stramonium,* fed and reproduced preferentially on some test plants. Thrips did not reproduce on potato cultivars Bismark and Tasman and on the weed, *C. album,* confirming non-preference for these plants and suggesting the involvement of some non-contact cues involving antibiosis or antixenosis. The influence of experience of thrips on *S. nigrum* and *A. calendula* on the subsequent preferences and performances on potato cultivars were not significantly different ($P = 0.05$).

Insecticide trials conducted over three seasons were hampered by low disease incidences. However, useful trends were observed. In five trials in Tasmania, both pre-plant and foliar treatments appeared to offer some protection against TSWV infection. In contrast, in one trial in South Australia, little benefit of insecticide treatment was suggested. This difference may reflect different sensitivity to treatment between onion thrips (present in both Tasmania and South Australia) and tomato thrips (present in South Australia only) TSWV vectors. Late planting (with plants emerging after major thrips flights) also appeared effective in reducing disease levels.

In summary, the results from the above studies, collectively, contribute to uncover some ecological relationships and patterns of both TSWV and its vector thrips that can be integrated with plausible mechanisms to explain the epidemiology of the virus in potato crops and advance the rationale for future research in this direction.

Project aims

While valuable information has been obtained in prior research on TSWV in potato in Australia (Magee 1936, Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b; Wilson 2001), there are still many gaps in the available knowledge. Some of the issues of particular importance concern an understanding of the most important aspects of the potato agrosystem and how they combine to determine the dynamics of the vector thrips/TSWV ecology and disease epidemiology, and consequently giving insights to the sporadic nature of TSWV epidemics. This is essential for the successful development of sustainable control strategies suitable for different agro-ecological zones where potato is grown. Some areas that require study include the following: -

1. Identification and monitoring of factors associated with TSWV epidemics in potato through field surveys.

Many factors such as seed health, virus sources, thrips vector activity, virus levels and patterns of infection in crops over time, weather and potato plant resistance to TSWV infection and translocation, all under the influence of weather factors are thought to have a role in epidemics. There is, therefore, a need to ascertain the influence of each of these factors on the disease development and spread. The sporadic nature of TSWV epidemics raises questions.

- **What are the sources of TSWV?** In many vegetatively propagated crops, like potato, viruses are disseminated through planting material. In countries, such as Australia, where there are regulatory certification schemes, it is unclear whether the main spread of viruses which lead to sporadic epidemics in potato fields is between potato crops and/or from other sources. It is also likely that there are many plants from which viruses infecting potato spread. Many weed species remain untested and work is required to determine whether alternative hosts are of continuing epidemiological significance as sources of infection to potatoes across the diverse range of agro-ecosystems in which the crop is grown.
- **Which key vectors are associated with TSWV epidemics in potatoes?** What are the dynamics of the vector thrips in potato as affected by potato cultivar and weather parameters? Research is needed on the factors affecting the movement of vectors and survival within the potato field. This is vital in the understanding of disease build up. Little is known about the relative importance of short and long-range dispersal of vectors and the influence of environmental factors including wind and frontal systems on such movements in potato growing regions. There is no direct evidence that thrips behave the same way in potato fields as they do in other crops. The canopies of many host plants of vector thrips are different and studies on potential migration may be justified.
- **Is primary spread of TSWV more important than secondary spread in all potato cultivars?** The relative importance of primary spread of TSWV in potato and the subsequent secondary spread within the field has not been clearly established. It is essential to make detailed studies on the pattern and sequence of spread at a representative range of sites in different agro-ecologies and under different cropping systems over a period of several years. It will be important to monitor the numbers and infectivity of the vectors reaching potato fields.
- **How do the immigrating adult vector thrips from hosts other than potato behave on potato?** Are the adult vector thrips and subsequent progenies from off-season potato volunteers, non-solanaceous species and weeds within potato fields more efficient colonisers and vectors than the immigrant vector thrips due to trade-off in fitness?

2. Screening of potato cultivars for resistance to TSWV under glasshouse and field conditions.

The important question here is; How do different potato cultivars react to TSWV through mechanical inoculation (glasshouse) and field vector thrips transmission? There are long term benefits derived from selecting or breeding for virus resistance in released and future potato cultivars, as the costs of using insecticides to protect susceptible cultivars are likely to be much greater than the cost of the breeding programme in the long term. However, breeding for resistance against any pathogen relies on the knowledge of the aetiology, ecology and epidemiology of the pathogen in question. While potato breeding for aphid-transmitted viruses (Rodoni, 2003), yield and other qualities (Kirkham *et al.,* 2001; Isenegger *et al.,* 2001; Dawson *et al.,* 2002; Williams *et al.,* 2003) have been successfully carried in Australia, breeding for resistance to TSWV is yet to be done. Some potato cultivars such as Russet Burbank and Coliban (Wilson 2001) are believed to be virus-tolerant, the relative effects of these tolerant cultivars on disease distribution and progress over time and space, and their potential as one of the tools for management of TSWV needs to be evaluated. Further research is also needed on the most appropriate means of deploying TSWV-resistant cultivars. Spatial diversification of host resistance appears to be a major technique to achieve successful and durable management of crop pathogens by genetic means. The observation in potato (Norris 1951a, 1951b; Wilson 2001) that plants arising from second generation tubers exposed to TSWV have lower incidence of the disease is potentially enormous, since benefits of host gene-mediated resistance (gene-silencing mediated protection) can be combined with other desirable agronomic characteristics in the breeding programme. However, there is still a need to study the role of reversion and proteolysis in virus elimination in potato.

3. Determination of the effects of light, temperature and humidity on TSWV infection and symptom variability in different potato cultivars.

The appearance and severity of symptoms varies depending on the host, virus strain, age of the plant and environmental conditions (Francki & Hatta, 1981). Several unrelated plant pathogens produce very similar symptoms and strains of the same virus can produce very different symptoms in different hosts (Reddy 1990). There is reported evidence that TSWV produces different symptoms even in the same potato cultivar (Norris (1951a) and that these symptoms can easily be confused with those caused by the early blight fungus *Alternaria solani.* This can clearly cause confusion during crop inspections for certification purposes and may also lead to an underestimation of the disease, and consequently, inappropriate control responses. While temperature has been shown to have an effect on symptom expression in pepper (Moury *et al.,* 1998) and different hosts (Llamas-Llamas *et al.,* 1998), at present, there is no information about the impacts of temperature, light intensity and relative humidity on TSWV infection efficiency and subsequent disease development and symptom expression in different potato cultivars. And it is still unclear what role temperature plays in the formation of defective RNAs, which impede virus transmission by vector thrips (Nagata *et al.,* 2000a). Indirect evidence on the role of humidity in TSWV symptom expression has been obtained in studies on tomato by Córdoba *et al.* (1991). The role of light in TSWV symptom expression in potato has not been studied. Clearly, there is a need to document the whole range of TSWV symptoms encountered on potato in Australia and to develop improved methods of disease identification and monitoring. It is, also essential to document the independent and interactive effects of these environmental factors in order to understand the epidemiology of the disease and consequently, for the development of effective management strategies or screening for host resistance. Ideally, any plants showing atypical or suspect viral symptoms should be tested for TSWV to provide unequivocal results.

4. Quantitative evaluations of the relationships of TSWV infection in potato with the population dynamics of known vector thrips species and the determination of vector competency and virus transmission in potato and weeds.

Since the pioneering research on the transmission of TSWV by vector thrips was done in Australia (Pittman, 1927; Samuel *et al.*, 1930), additional information on several more aspects of vector transmission have been elucidated (Sakimura, 1963a, 1963b; German *et al.*, 1992; Wijkamp *et al*., 1995; Mumford *et al.,* 1996a; Goldbach & Peters, 1996; Ullman *et al.,* 1997; Chatzivassiliou *et al*., 1999, 2001, 2002; Nagata & Peters, 2001; Sakurai *et al.*, 2002; Nagata *et al.*, 2002; Inoue *et al.*, 2002; De Kogel; 2002). Many studies in Europe and North America (Jones 1959, Paliwal 1974, 1976; Wijkamp *et al.,* 1995; McPherson *et al.,* 1999; Chatzivassiliou *et al*., 1998a, 1999, 2001), South America (Nagata *et al.,* 2002) have indicated differences in TSWV transmission by thrips vector between species and sexes. From all these studies and reviews, it is evident that gaps in the available knowledge still remain, particularly those relating to host specificity and transmission competency and efficiency. Pertinent questions in the context of epidemics in potato will have to be;

- Whether vector thrips identified in field surveys differ in host-plant specificity and transmission efficiency. The relative importance of vectors and their transmission capacity from different host plants will have to be determined.
- Whether host preference and performance has a role in the transmission efficiency in the epidemics occurring.

5. The evaluation of the efficacy of insecticidal control of vector species associated with TSWV epidemics in potato and other strategies.

Complimentary to the use of resistant or tolerant potato cultivars, there is a need to evaluate the use of insecticides to control vector thrips as part of an integrated disease management system. While it is anticipated that insecticides can prevent secondary spread of the virus, it is currently unclear whether they have any effect on the transmission rate of TSWV given that viruliferous vector thrips from external sources can transmit the virus with minimum feeding. In such a scenario, consideration should also be given the evaluation of repellants. Many other pertinent questions regarding the use of insecticides to control TSWV in potato relate to: -

Which chemistries can effectively be used to control vector thrips without causing resistance,
The methods of application (foliar, soil-applied systemic, systemic seed treatment),
Timing of application (pre-emergence, planting time, post-emergence, viruliferous thrips population threshold),
Rates of application.

6. The collation of epidemiological data for the prediction of TSWV epidemics in potatoes.

Mathematical modeling and simulation have facilitated rapid advances in understanding many pathosystems (Kranz, 1974, 1990; McLean *et al.* 1986). Generally, a disease model is developed to estimate the probability of an undesirable event occurring at a given location and time (Kranz, 1974, 1990). Such models are useful due to their speed and ability to handle the more complex systems, especially in studies where the objective is disease forecasting. Disease forecasting can be developed through risk assessment and management (McLean *et al.* 1986). The development of a predictive model requires data from representative sites over sufficient multiple seasons (Thresh, 1974; Barnett 1986) and a careful evaluation and determination of individual risk factors playing a role in the epidemic (Duffus, 1971; Kranz, 1974, 1990; Thresh 1974; Plumb & Thresh, 1983; Gray & Banerjee, 1999).

Because of its sporadic nature, TSWV has proven to be a difficult plant disease to manage. Despite these difficulties, an ability to forecast the incidence of potato viruses with reasonable precision and ideally before or

soon after crops are planted, could provide potato growers, processors and farm advisers with reliable and timely management recommendations (Thresh 1986). A disease prediction and economic model based on conditional probability and linear regression has been devised for predicting TSWV incidence at harvest as a function of early disease incidence and cumulative vector thrips abundance in lettuce crops (Yudin *et al.*1990). However, this model has not been adopted and used widely elsewhere. Part of the reason could be due to the absence of local data to use in such a model. Accurate predictive models are obviously of greatest value to all concerned but they may not always be feasible, given the need for extensive quantitative epidemiological studies in different agroecologies, which are essential for understanding pathosystems and for their effective management. But since disease forecasting can be developed through risk assessment and management, an alternative method would be to use these risk assessment tools, which incorporate data from individual risk factors to formulate recommendations for management of TSWV. Such a tool has been developed in the USA for TSWV in peanuts (Culbreath *et al*., 2003). But the focus of such a risk assessment tool is only for management of TSWV in individual fields. In that context, the success of such a risk management tool is only feasible where all growers in the neighbourhood comply and use the tool. In the absence of total compliance in the use of the tool, reservoirs of both vector thrips and TSWV will remain in the environment, posing a potential danger for epidemics. To counteract such handicap, cognizant of the wide host range of both vector and virus and the sporadic nature of epidemics in potato, the design of an alternative risk management tool should be based on the formulation of recommendations for management that does not focus on individual fields but rather consider the dynamics of the disease in a whole locality; thus the concern should be with analysis for strategic rather than tactical management (Jeger & Chan 1995). Formulation of such a predictive model should allow the impact and interactions between management variables to be examined with a minimum number of parameters, all of which should have a clear biological interpretation. This kind of analysis allows consideration and inclusion of all factors likely to have an important impact on disease dynamics within the entire locality under the influence of similar parameters (intensity of cropping, cultural practices used, cultivars grown, rate of crop turn-over, meteorological and other environmental factors, virulence of prevailing virus strains, vector thrips population dynamics). Such a tool would need to be designed in such a way as to allow improvements and adjustments over time and would need validation in different potato growing areas in Australia

7. Technological transfer of key management strategies and revised certification tolerance levels

Any achievement accrued from efforts undertaken to address the above issues will have to flow effectively to potato growers, processors and consumers. Current certification guidelines will have to be revised to reflect gained knowledge on potato cultivar reaction to TSWV. Concerns from industry with regard to the flow of benefits from research will have to be addressed (Eccles 2003).

The questions above raise complex, and in some instances contentious, issues that require more detailed consideration and research effort than is feasible in this thesis alone. There are currently no answers to most of the above issues in literature, a situation that complicates the development of novel control strategies in potato. Nevertheless, it is clear that much could be achieved by applying existing knowledge, despite the need for increased research on the ecology and epidemiology of TSWV. While the issues are not insurmountable, they are robust and dynamic in nature and require considerable time and effort to solve. Current and future efforts are expected to build up on prior research and contribute to the continuing effort of seeking answers to the above issue in Australia. To this end, work was carried out on some aspects of the above areas and the findings are presented in the chapters that follow.

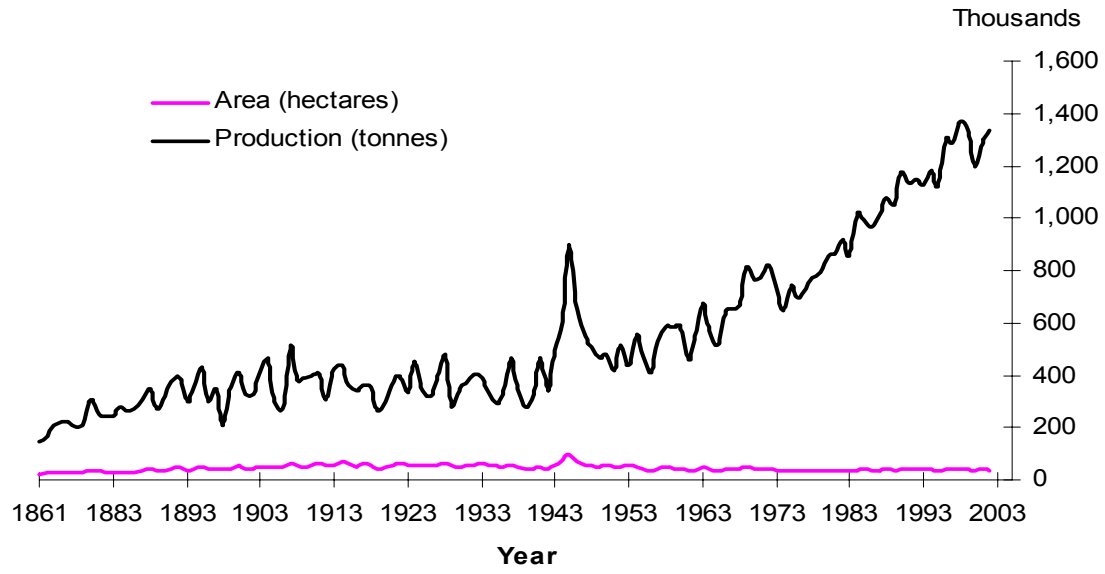
Chapter 1

Literature Review

Potato Production in Australia

The Australian horticultural industry is characterized by a great diversity of annual crops of high commercial value, among which potato is particularly important. The history of potato production in Australia starts with the first European settlements in Tasmania in 1803, expanding northwards to the mainland with regular trade in the 1870s (Taylor, 2001, 2003). From the small patches of land grown by the early settlers, the area planted to potato in Australia has remained fairly constant over the past hundred years after the initial expansion, but yields per hectare have substantially increased (Figure 1). Most of the potato grown in Australia now comes from the southern, south-eastern and western states of the country (Anon, 2003a). Estimates for 2001/2002 growing season indicated that slightly over 2000 growers produced 1,333,200 metric tonnes of potato from 37,900 hectares (Anon, 2003a)(Figure 1). This represents a combined value exceeding $500m, which makes up about half of the gross value of vegetable production in the country. These productivity gains are mainly attributed to the use of machinery, better cultivation practices and management (Taylor, 2001, 2003).

Figure 1. Potato Production and Area (1861 to 2002), Australia



(Data Source:Year Book Australia: 2002, Australian Bureau of Statistics)

Disease constraints have constituted serious challenges to potato production in Australia, by causing a significant economic impact from losses in productivity, the cost of disease management and the economic penalty as a result of growing less profitable alternative crops (Khurana 1992; Chakraborty *et al*. 2002*;* Hijmans, 2003). While no formal collective crop loss assessments caused by potato diseases have been undertaken across the many different ecological zones in Australia, the pattern of their devastating impact is reflected in the estimates, based on plausible industry needs, by the potato industry advisory committee (IAC) and its two sub-committees (fresh and processing) of levy funded research and development allocations. During 2002/2003 funding rounds by Horticultural Australia Limited, 25% of funds were spent on pests, disease and weed management research (Oakeshott, 2003). The continuing challenge of diseases to the potato industry was most notable and further highlighted in the setting of research priorities for the following years. In particular, the soilborne diseases, potato viruses X, Y, S and Tomato Spotted Wilt Virus (TSWV) were singled out to be limiting factors to production, processing and competitiveness in a global market and given highest priority (Oakeshott, 2003; Gallagher, 2003).

Tomato Spotted Wilt Virus (TSWV): Historical Perspective and economic relevance.

The origin of *tomato spotted wilt virus* (TSWV) is likely to remain in doubt but the virus has a long history of causing disease in plants. The wide host range of this virus (Peters 1998) supports this hypothesis. Since the first observations in 1895 of "an obscure disease of greenhouse tomatoes at Ohio Agricultural Experimental Station, USA" (*c.* Brittlebank, 1919), there has been several reports of the disease worldwide on many annual, biennial and perennial horticultural crops, flowering ornamentals, native flora and weeds (Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b; Cho *et al*., 1986; German *et al.*, 1992; Johnson *et al.*, 1995; Dewey *et al.*,1996; Mumford *et al.*,

1996a; Mertelík *et al.*, 1996; Hill & Moran, 1996; Latham & Jones, 1996, 1997; Daughtrey *et al.*, 1997; Al-Shahwan *et al.*, 1997; Peters, 1998; Gitaitis *et al.*, 1998; Ochoa *et al.*,1999; Gracia *et al.*, 1999; Clift *et al.*, 1999; Wilson, 1998, 2001; Hristova *et al.*, 2001; Wangai *et al.*, 2001; Golnaraghi *et al.*, 2001; Williams *et al.*, 2001; Jericho & Wilson, 2002, 2003; Abad *et al.*, 2003a).

In 1905, a serious wilt disease of tobacco, locally known as "Kromnek" or "Kat River wilt" was described in the Kat River Valley (Eastern Cape, South Africa)(Moore 1933; Van der Plank & Anderssen, 1944). A similar disease in tomatoes was described in the Australian southern state of Victoria by Brittlebank (1919), who also proposed to name it "Spotted Wilt". The disease was, subsequently shown to be caused by a virus, naturally transmitted by thrips (Pittman, 1927; Samuel *et al.*, 1930; Sakimura, 1939). These discoveries were followed by many incidence reports of the disease in several countries on different hosts, resulting in a wide variation in nomenclature (Sakimura, 1963a). Since then, several aspects of the disease have become known and reviewed extensively (German *et al.*, 1992; Mumford *et al.,* 1996a; Ullman *et al.,* 1997) including its biology (Elliot 1990; German *et al.*, 1992; Goldbach & Peters, 1996; Mumford *et al.,* 1996a), vector transmission (German *et al.*, 1992; Ullman *et al.,* 1997; Nagata & Peters, 2001; Nagata *et al.*, 2002), host range (Peters, 1998), virus-host interactions (Garg *et al.*, 1999; Soellick *et al*., 1999; Bucher *et al*., 2003; Kainz *et al*., 2004; Gunasinghe & Buck 2003; Aramburu & Martí 2003; Schwach *et al*., 2004) and management (Cho *et al.*,1989, 1998; Thompson & van Zijl, 1996; Latham & Jones, 1996; Yudin *et al.,* 1990; Riley and Pappu, 2000; Reitz *et al.,* 2003). A number of studies have also shown that there are distinct levels of host specificity (Wijkamp *et al*., 1995; Nagata *et al.*, 2002), host influence and virus acquisition on transmission efficiencies of tospoviruses by thrips vectors (Sakimura, 1963a, 1963b; Chatzivassiliou *et al*., 1999, 2001; Inoue *et al.*, 2002) and that host preferences and reproductive strategies play a role in this diversity (De Kogel; 2002; Chatzivassiliou *et al*., 2002).

Even with this refined and deepened fundamental knowledge of the biology and epidemiology of the disease, TSWV and its vectors have been and continue to be responsible for substantial and sometimes devastating losses in a number of commercial crops. Much of this has been attributed to the rapid expansion in geographic distribution and host range of *Frankliniella occidentalis* (Pergande)(Western Flower Thrips), a more efficient vector of the virus (Wijkamp *et al.,* 1995; Ullman *et al.,* 1997). The thrips vectors, and the tospoviruses they transmit, are now responsible for significant crop losses in many areas of the world with tropical, subtropical, arid, and Mediterranean climates. Over the past decade, severe outbreaks of TSWV have become more frequent and infected crops have suffered major losses, with yield reductions ranging from 17.5% in flue-cured tobacco in Georgia, USA (McPherson *et al.,* 1999), 90% in chrysanthemum, (*Dendranthema grandiflora*) in Mexico (Ochoa *et al.,* 1999) to 100% in pepper and tomato in Georgia (Gitaitis *et al.,* 1998). Tomato (*Lycopersicon esculentum* Mill.), Lettuce (*Lactuca sativa* L.), and pepper (*Capsicum annuum* L.) in Argentina have been decimated since 1994 (Gracia *et al.,* 1999) and in Tasmania, TSWV causes severe disease in lettuce and in recent years epidemics have resulted in 5-60% losses in autumn harvested crops (Wilson 1998). In recent times the potato crop in North Carolina, USA (Abad *et al.,* 2003a) and in the southern states of Australia has also been threatened by TSWV (Wilson, 2001; Clift & Tesoriero, 2002; Medhurst *et al.,* 1993-2003). In Portugal, Greece, Carpathian Basin of Eastern Europe, Kenya, South Africa and New Zealand and in many other parts of the world, lettuce, pepper, tomato, stonefruit, potato, tobacco, groundnuts, stone fruits and many other crops have been subjected to losses as a result of thrips feeding and TSWV (Klesser 1966; Louro 1996; Roselló *et al.,* 1996; Thompson & van Zijl, 1996; Chatzivassiliou *et al.,* 1996; Teulon *et al.,* 1996; Wangai *et al.*, 2001; Jenser *et al.*, 2003). In addition to field crops, thrips are a serious pest problem in protected environment, which enable it to survive during the winter in temperate climates (Daughtrey *et al.*, 1997; Chatzivassiliou *et al*., 2001). It is increasingly becoming clear that TSWV is a growing problem (German, 2003)

The above are but some of the deleterious examples of TSWV and its thrips vectors, indicating the magnitude of the problem and the level of research that require attention. Granted, some practical problems still remain unsolved, and there is still a great dearth of knowledge on many aspects of the disease in many crops including potato and much remains to be done in understanding the molecular, biology, ecology and epidemiology of TSWV.

TSWV in Australian Potato crops: Distribution and the magnitude of the problem

There are numerous viral diseases of potato in Australia, but that caused by TSWV is by far one of the most important, sporadic and difficult to control. Numerous and detailed studies of TSWV has been done in many horticultural crops in many parts of the world, including Australia. However, very little is known about the epidemiology and control of the virus in potato. In particular, ecological studies are currently neglected because of the increasing preoccupation of virologists with the biochemical features of viruses. This is reflected in the dearth of published accounts on the subject on potato. Part of this is due to the near absence of TSWV epidemics in potato in many parts of the world. The lack of adequate ecological information is a serious obstacle in developing effective virus disease control measures as discussed here in relation to TSWV.

TSWV has been known in Australia since 1915, and after the initial discovery and description of symptoms on tomato in Victoria (Brittlebank, 1919), other states confirmed its presence (Best 1968). There has now been nearly some 70 years of effort in quantitative analysis and research seeking practical goals of controlling the disease. In the early years following the first record, most of the work was restricted to a few pioneers (Pittman, 1927; Samuel *et al.*, 1930; Bald & Samuel, 1931; Bald, 1937; Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b). Much of the subsequent work and most early records of the disease were on infections in potato crops (*Solanum*

tuberosum L.), the first being in 1935 (Magee, 1936). In the summer seasons of 1945 – 1948, major outbreaks of the disease were recorded in potato crops in coastal areas and the main growing regions on the Central and Southern Tablelands of New South Wales and Victoria, resulting in significant crop losses and total crop failure in some cases (Conroy *et al*., 1949; Norris, 1951a, 1951b). These epidemics were largely sporadic and unpredictable, although the prevalence and severity of the disease was observed to coincide with summer seasons with less precipitation (Conroy *et al*., 1949). Except for a few reports such as that by Smith (1961) on the density dependence in the Australian thrips and Helms *et al.*, (1961) for the disease in peanuts in Queesland, there was a relative absence of accounts of TSWV in Australia in the intervening years between the 1950s and early 1980s, until the early 1990s when a surge of reports and interest in the disease and thrips vectors occurred. The interest was generated by the increasing frequency and severity of epidemics that were occurring in many crops and pastures (Moran *et al.*, 1994; Hill and Moran, 1996; Latham & Jones, 1996, 1997; Clift *et al.*, 1999; Wilson, 2001; Wilson, 1998; Jericho & Wilson, 2003). A similar decline in the occurrence of TSWV was observed after the Second World War in much of Western Europe and United States while it remained a serious problem in Eastern Europe, South America and South Africa (Goldbach & Peters, 1996). This decline in Western Europe and United States was attributed to the use of insecticides to control thrips in greenhouses (Peters *et al.*, 1996). During the early 1980s, TSWV re-emerged and reports of the disease in many parts of the world started accumulating. It was at this time that, Sampson & Walker (1982) recorded incidences of the disease in Tasmania. In the decade that followed, Clift *et al.*, (1999) in New South Wales observed varying incidences of the TSWV monitored through samples submitted to diagnostic laboratories in Sydney from the 1991/1992 season to 1998/1999. TSWV incidences ranging from 3-50% were observed in seed potato during 1992/1993 and 1993/1994 seasons in the Crookwell region of New South Wales. In Western Australia, sporadic infections in potato ware crops were reported in the Metropolitan regions of Perth (Latham & Jones, 1997). There were also reports of major outbreaks of TSWV in ware crops in South Australia during 1997/1998 and 1998/1999 season and sporadic occurrences of the disease in Tasmania (Wilson, 2001). The disease incidences in Tasmania varied from trace to 28%, resulting in 1000 tonnes of seed failing certification. In the summer seasons of 2001/2002 and 2002/2003, outbreaks of TSWV were observed in a number of potato crops during surveys in South Australia, Victoria and New South Wales (Jericho & Wilson, unpublished data), resulting in a collective 30% of the seed potato failing certification in Ballarat, Berrigan, Portland, Colac and Gippsland (D. Antrobus, unpublished data). Industry estimates of a commercial dockage at 10% on a potato pivot circle were calculated at $40,000 - $50,000 loss to the grower per annum. And at the factory, a 1% cull led to $1m loss to the grower each year (Anon., 2003b). The above observations and the consensus view of stakeholders in the potato industry during 2002/2003 season (Anon., 2003b), showed clearly that TSWV had emerged as a serious threat to the economic viability of the Australian potato industry and related activities (e.g. employment in the processing industry). Losses caused by TSWV are not limited to reductions in yield and quality. The economic effect of this virus is felt at many levels and may also include the direct costs of virus control measures that are applied routinely in commercial potato production. This is more apparent by the situation that prevailed in Ballarat during 2002/2003 season where a statutory field tolerance of TSWV at 1% based on foliar assessments in seed crops led to an increase in rouguing cost to $720 per hectare (Anon, 2003b). Epidemics of TSWV are particularly detrimental to commercial fields of processing potato where once plants are infected and the virus causes internal necrosis or dark discolouration of the tubers they become unmarketable and unsuitable for processing by which time most of the production costs will have already been incurred. TSWV is now a striking example of a disease that has become prevalent every year in Australia. The disease has now been recorded in all states except Northern Territory (Table 1).

As in many cropping systems where resurgence of virus epidemics has been reported, the factors responsible for these epidemics are thought to be many and complex (Gray & Banerjee, 1999; Damsteegt 1999 and references therein), and largely not well understood. In Australia, like in many other parts of the world, the marked spread and subsequent introduction of the polyphagous Western flower thrips *F. occidentalis* (Malipatil *et al*., 1993) was thought to be a factor in exacerbating the TSWV epidemics, given the propensity and transmission efficiency of this vector in other crops (Ullman *et al.,* 1997). Four thrips species known to vector TSWV have been recorded in Australia; onion thrips, *Thrips tabaci* Lindeman; tomato thrips, *Frankliniella schultzei* Trybom; the western flower thrips, *F. occidentalis* (Pergande); and the melon thrips, *Thrips palmi* Karney (Malipatil *et al*., 1993; Mound, 1996; 2004; Austin *et al*., 2004). But only three are thought to be driving TSWV epidemics, singularly or in tandem. Early outbreaks of TSWV in potatoes and tomatoes in South Australia, New South Wales and Victoria, were attributed to *T. tabaci* and *F. schutzei* (Pittman 1927; Samuel *et al.*, 1930; Magee, 1936; Norris & Bald 1943; Conroy *et al.*, 1949; Norris 1951a & 1951b). And indeed, vector transmission of TSWV by *T. tabaci* was first established by Pittman (1927) in Australia. Decades later, in 1993, *F. occidentalis* was recorded for the first time in both Western and Eastern Australia (Malipatil *et al.*, 1993). And sporadic infections in potato ware crops reported in the Metropolitan regions of Perth in Western Australia were associated with *F. occidentalis* (Latham & Jones, 1997). *T. tabaci* and *F. schutzei* were also recorded in the potato growing areas in the south-western part of Western Australia (Thomas & Jones 2000). An increase in TSWV epidemics in various vegetable and ornamental crops in some regions within Australia prompted research on *F. occidentalis.* Subsequently, this led to the formation of the national strategy for the management of western Flower Thrips and tomato spotted wilt virus in 1994, as it was feared that epidemics of TSWV in many crops could be exacerbated by the more efficient vector. However, only limited anecdotal evidence of trapping of this species within potato crops was available in Australia. In South Australia, and Victoria *T. tabaci* and *F. schutzei* continued to be trapped within potato fieds (Jericho & Wilson, 2003). In New South Wales, *tabaci, F. schutzei* and *F. occidentalis* were found (Clift & Tesoriero 2002). In Tasmania, *T. tabaci* was the only known vector trapped in open cultivation (Wilson,

1998; Jericho and Wilson, 2002), with low level infestation of *F. occidentalis* under quarantine reported in protected cultivation by two cut flower growers and in three of the state's four major wholesale nurseries since 1995 (Hill, 2003). *T. palmi* was reported in the warmer parts of New South Wales and Queensland (Mound 1996), but is yet to be recorded within potato crops. There has been no experimental investigation to confirm the competence of these thrips species in the transmission of TSWV in potato.

Table 1 Reports of TSWV in Australia

Location	Year	Crop/weeds	Reference
Victoria	1917-1919	Tomato	Brittlebank, 1919
New South Wales	1927	Tomato	Pittman 1927
South Australia	1930	Tomato	Samuel *et al.*, 1930
South Australia	1931	Tomato	Bald & Samuel, 1931
New South Wales	1935	Lettuce and Potatoes	Magee, 1936
New South Wales	1937	Tomato	Bald 1937
New South Wales	1941	Potato	Norris & Bald 1943
New South Wales	1946-1947	Potato	Conroy *et al.*, 1949
New South Wales (including ACT) & Victoria	1945-1947	Potato	Norris 1951a &1951b
Queensland	1961	Peanuts	Helms *et al.*, 1961
Australia wide	1994	Potato	Moran *et al.*, 1994
Western Australia	1996	Capsicum, Tomato & Dalia	Latham & Jones 1996
Western Australia	1993-1996	Broad bean, Capsicum, Celery, Chilli, eggplant, globe artichoke, Lettuce, paprika, potato, tomato, Native flora & weeds.	Latham & Jones, 1997
Tasmania	1994-1995	Lettuce & weeds	Wilson, 1998
New South Wales, Victoria, Tasmania, Queensland	1992-1999	Potato, Tomato, Lettuce, & Pepper	Clift *et al.*, 1999,
Australia wide	1998-2000	Potato	Horne & Wilson 2000
Tasmania, Victoria, South New South Wales	2001	Potato	Wilson, 2001
Australia wide	1994-2001	Various crops	Clift & Tesoriero, 2002
Tasmania, Victoria, South Australia & New South Wales	2001-2002	Potato	Jericho & Wilson, 2002
Tasmania, Victoria, South Australia & New South Wales	2001-2003	Potato	Jericho & Wilson, 2003
Tasmania, Victoria, South Australia & New South Wales	2003	Potato	Wilson & Jericho, 2003
Western Australia	2003	Pepper	Thomas-Carroll & Jones 2003
Australia-wide	1993- 2003	Various crops	Western Flower Thrips Newsletters (No. 1-30) Medhurst et al.(eds.)

Despite the many concerted efforts in the past to understand and manage the vectors and the disease, the debilitating impact of TSWV epidemics continues in potato and various other vegetable and ornamental crops because the crucial ecological and epidemiological factors underpinning these outbreaks in Australia are not well understood. Recurring and unpredictable outbreaks of TSWV in the potato industry not only pose enormous concerns and challenges to growers and scientists (Norris, 1951a, 1951b; Wilson, 1998; Herron and Cook 2002; Jericho & Wilson, 2003), but hampers production and expansion and cause the loss of income and substantially changes cropping patterns in some areas when they strike. This was more evident in the Derwent Valley of Tasmania (C. Wilson, pers. Comm), northeastern Spain (Moriones *et al*., 1998) where tomato production had to be abandoned due to outbreaks of TSWV, and in Pennsylvania, USA, where huge losses due to tospovirus infections caused growers to abandon gloxinia (*Sinningia speciosa* Lodd) and exacum (*Exacum affine* Balf.) production (Daughtrey *et al.*, 1997).

One of the central challenges over the years has been to control and manage the disease. As in many other plant diseases caused by arthropod-vectored viruses (Plumb & Thresh, 1983; Gray & Banerjee, 1999), TSWV epidemics result from interrelationships that are often obligatory and specific, a consequence of complex and intricate evolutionary interaction of factors involving close biological relationships between the virus, thrips vectors and plant hosts under the influence of environmental and human interferences (Duffus, 1971; Kranz, 1974, 1990; Plumb & Thresh, 1983; Ullman *et al*., 1997). In most of these systems, the underlying factors regulating the initiation of epidemics represents a level of complexity beyond that of the classic disease tetragon of efficient vector, viable and abundant inoculum, susceptible host and favourable environment. The existence of the virus within potato crops from the onset poses a particular problem. It may be originating from propagation of infected seed tuber stocks (Norris & Bald 1943; Conroy *et al*., 1949; Norris 1951b; Shepherd 1972), regenerated infected plants from previous crops (Norris & Bald 1943; Horne & Wilson, 2000), hibernating viruliferous thrips (Groves *et al*., 2001; Jenser *et al*., 2003), distant or nearby infected horticultural crops or from annual or perennial weeds (Norris & Bald 1943; Duffus, 1971; Thresh, 1974). The epidemiological impacts of each of these factors in, either, initiating, perpetuating or increasing the amount of inoculum and thus intensifying epidemics requires a careful evaluation and determination (Duffus, 1971; Kranz, 1974, 1990; Thresh 1974; Plumb & Thresh, 1983; Gray & Banerjee, 1999) from representative

sites over sufficient multiple seasons (Thresh, 1974; Barnett 1986). This requires the understanding of dynamics of the complex matrix of interactions among multiple populations of hosts, vector species, the virus strains, and between these populations and the environmental factors in triggering, establishing and sustaining or advancing the epidemics (Kranz, 1990; Jericho & Wilson, 2003). The acquisition and use of such necessary data is essential to understand the ecology and epidemiology of the virus and would indicate an understanding of the main factors contributing to the epidemics. Results from such studies would also assist in the evaluation, refinement and submission of adjusted national seed certification guidelines appropriate to and reflecting real risks of TSWV in potatoes. Success in such an endeavour would help to explain the sporadic nature of the epidemics and, therefore, facilitate the development of risk assessment models (Nutter, 1997; Madden *et al*., 1990; Madden and Campbell, 1986; Kranz, 1988, 1990; Madden and Hughes, 1995), and ultimately, lead to the development of an early warning system for potato growers, processors and farm advisers, and consequently, better decisions regarding cropping patterns and sequences and disease control strategies in space and time (Thresh, 1974).

TSWV: Foliage and tuber symptoms in potato

A marked feature of symptoms induced by tospoviruses in many plants is that they are highly variable (German *et al.*, 1992; Goldbach & Peters, 1996; Latham & Jones, 1996; Roselló *et al.*, 1996), even in the same genotype under different environmental conditions (Llamas-Llamas *et al.*, 1998). A critical determinant of symptom severity is the inherent sensitivity of the host. TSWV symptoms differ in severity and extent and range from local lesions with chlorosis and necrosis in some instances in non-systemic hosts to irregular chlorotic and necrotic areas, ring spots, line patterns, stunting, mottling and wilting in systemic hosts. Some genotypes develop conspicuous symptoms and are severely damaged, whereas others under similar conditions develop only inconspicuous symptoms that are restricted to a few leaves or shoots (Daughtrey *et al.*, 1997). Norris & Bald (1943) and Norris (1951a, 1951b) gave a detailed description of symptoms of TSWV infection in potato plants arising from infected tubers. Symptoms of the disease in potato vary according to cultivars (Wilson 2001) and may be mistaken for those caused by the common early blight fungal pathogen *Alternaria solani* (Norris (1951a), therefore, lead to an underestimation of the disease, and consequently, inappropriate control responses. On shoots in susceptible cultivars like Factor (Up-To-Date) (Norris (1951a) and Shepody (Wilson 2001), Riverina Russets (Jericho & Wilson, 2003), conspicuous brown blotches and ring spots may appear which in time coalesce leading to early death of the leaves. In some cultivars there is general leaf chlorosis, distortion and severe stunting of the whole plant. In plants infected through thrips feeding, local lesions or necrotic lesions may appear at the point of piercing. Brown streaks may also be evident on petioles, veins and stems (Norris (1951a; Costa & Hooker 1981). Infections in tubers also vary and may include scattered internal dark brown necrotic patches (Wilson 2001), which render them unsuitable for processing or consumption and all except the least profitable of processed products. In moderately resistant cultivars or those that translocate the virus poorly to tubers (like Russet Burbank), there may be no visible effects on the tubers, particularly if the tuber is infected late. Occasionally, internal spots and flecks may appear. Cracked, pitted or distorted tubers may also be present (Norris 1951a, 1951b) although Wilson (2001) observed that malformed tubers and secondary growth found following foliar TSWV infection is not necessarily associated with tuber infection. A common feature in most susceptible potato cultivars is that symptoms of TSWV tend to become less conspicuous as plants mature (Norris, 1951a). This has been confirmed in studies by Wilson (2001).

Causal agent: Virus structure and classification

Tomato spotted wilt disease is caused by a tospovirus, a plant-infecting group within the otherwise animal-infecting *Bunyaviridae* (Elliot 1990; Francki *et al.*, 1991). The virus has been well characterized (Mohamed *et al.,* 1973; Tas *et al.,* 1977; De Haan *et al.*, 1989b, 1990, 1991; Kormelink *et al.*, 1992; German *et al.*, 1992; de Ávila *et al.*, 1993; Goldbach & Peters, 1996; Mumford *et al.,* 1996a), being a spherical lipid membrane-bound particle *c.* 80 x 110 nm in dimension covered with surface projections consisting of two glycoproteins (Mohamed *et al.,* 1973; Tas *et al.,* 1977) and a genome containing three single-strand RNA segments, small (S), medium (M) and large (L), that are c. 2.9 kb, 5.0 kb and 8.9kb, respectively. The S and M RNAs are ambisense in their genome organisation, while the L RNA is in negative polarity. The S segment produces nucleoproteins. The M segment produces glycoproteins designated as G1 and G2. The L segment produces a protein, which is a polymerase. The nucleocapsid protein (N) is thought to contribute to the viral replication cycle in a structural and perhaps, regulatory manner via its role in the formation of ribonucleoproteins (RNPs) which are structural features of TSWV (Kainz *et al.*, 2004). Each of the RNA segments together with the nucleocapsid protein (N), form pseudocircular nucleocapsid complexes (Mohamed *et al.,* 1981; De Haan *et al.*, 1989b; 1991) and ecodes two envelope glycoproteins GI and G2 that are expressed from the common precursor gene (De Haan *et al.*, 1990; Kormelink *et al.*, 1992). The role of these glycoproteins in plant and thrips infections or in viral replication is not well understood (Bandla *et al.*, 1998; Nagata *et al.*, 2000a; Assis Filho *et al.*, 2002) and is a subject of further investigations (Naidu *et al.*, 2003). But RNPs are thought to be central to the infection cycle of TSWV and other bunyaviruses because they, and not naked viral genomic RNA, serve as templates for both viral gene transcription and genomic replication (Elliot 1996; Schmaljohn 1996). The accumulation of N protein and NSs has also been convincingly demonstrated in midgut epithelial cells by electron microscopy (Ullman *et al.*, 1993) and in the salivary gland cells of *F. occidentalis,* leading to suggestions that they may be involved in the uptake by and replication within the thrips vectors and essential for systemic infection of

plants (Wijkamp *et al.*, 1993; Ullman *et al.*, 1997; Bandla *et al.*, 1998; Kikkert *et al.*, 1998; Griep *et al.*, 2000; Nagata *et al.*, 2000a; Ohnishi *et al.*, 2001; Assis Filho *et al.*, 2002; Bucher *et al.*, 2003). However, regulatory sites for TSWV movement necessary to establish systemic infection have not been determined. But recent reports seem to confirm the role of NSm protein in the systemic movement of TSWV within infected plants (Gunasinghe & Buck, 2003). Deletions in the envelope glycoproteins and accumulation of defective interfering RNA impede thrips transmission (Nagata *et al.*, 2000a; Sin *et al.*, 2003).

As more isolates of TSWV are gathered and compared, considerable evidence is emerging that support a great variation in populations corresponding to geographic source of isolates, genetic differentiation between subpopulations, high Intraspecific polymorphism, and decreased diversity within subpopulations (Moyer *et al.*, 2003a). The evolving diversity within the highly heterogeneous natural TSWV populations can be expected to increase substantially in the next few years. The situation may eventually resemble that which prevails in the genus begomovirus (family: Geminiviridae) where many distinct viruses and different isolates have been identified and characterised (Brown, 1994; Jericho 1999; Varma & Malathi 2003; Jones, 2003). This trend is becoming evident from recent literature (Moyer *et al.*, 2003a, 2003b; Adkins, 2003)

Virus-vector-host plant interactions and virus transmission

TSWV host range: e host range of TSWV has been reviewed extensively (German *et al.*, 1992; Mumford *et al.,* 1996a; Ullman *et al.,* 1997). Natural populations of TSWV isolates are highly heterogeneous with a great capacity for genetic variation (Moyer *et al.*, 2003a), and, therefore, an ability to infect many hosts. Many field studies have identified plant species that serve as natural hosts of TSWV through vector thrips transmissions (Cho *et al*., 1986; Stobbs *et al*., 1992; Hobbs *et al*., 1993; Kaminska & Korbin 1994; Johnson *et al*., 1995; Mertelík *et al.*, 1996; Latham & Jones 1997; Chatzivassiliou *et al*., 2000a, 2000b, 2001; Groves *et al.,* 2001, 2002). Additionally, many experimental hosts have also been identified through laboratory inoculations (Stobbs *et al*., 1992; Bautista *et al*., 1995; Mertelík *et al.*, 1996; Adkins & Rosskopf, 2002) and a great many of these from multiple plant genera have proven to be useful indicator hosts (Adkins & Rosskopf, 2002; Wijkamp & Peters 1993). Peters (1998) recorded many plant species (>1,050), a composite of monocots as well as dicots, in over 92 distinct botanical families, as hosts of tospoviruses worldwide, with more than 900 as hosts of TSWV. Susceptible hosts include many important agricultural crops such as lettuce (Cho *et al.,* 1987; Wlson 1998), potato (Norris 1951a; Al-Shahwan *et al.*, 1997; Wilson 2001), tomato (Aramburu *et al.,* 1997; Gitaitis *et al.,* 1998; Williams *et al.,* 2001; Wangai *et al.,* 2001), peanuts (Camann *et al.,* 1995; Hoffmann *et al.,* 1998; Mandal *et al.,* 2001), pepper (Hobbs *et al*., 1993; Gitaitis *et al.,* 1998), papaya (Gonsalves & Trujillo, 1986), Soybean (Golnaraghi *et al.,* 2001), tobacco (Chatzivassiliou *et al*., 1998, 2001; McPherson *et al.,* 1999), ornamentals (Ochoa *et al.,* 1999; Chatzivassiliou *et al*., 2000b) and many weeds (Cho *et al.,* 1986; Latham & Jones 1997; Stobbs *et al*., 1992; Hobbs *et al*., 1993; Groves *et al.,* 2002). Many of these species are annual or biennial although a few such as *Sambucus nigra* (Mertelík *et al.*, 1996), *Plantago rugelii* and *rumex crispus* (Groves *et al.,* 2002) are perennial. Where such perennial species are locally abundant, they may serve as important and long lasting TSWV inoculum sources or breeding hosts for vector thrips. Perennial plants, *Plantago rugelii* and *rumex crispus* in North Carolina, USA, were observed to remain TSWV infected for 2 years in a small plot field test (Groves *et al.,* 2002). Large breeding populations of vector thrips have also been observed on *Crataegus monogyna* (Hawthorne) although TSWV has not been detected in these plants in Australia (Jericho, unpublished data). Such plants may serve as good breeding hosts for thrips vectors, but they are not epidemiologically important in TSWV transmission since plants serving as a source for spread of the virus must be susceptible to systemic infections and the virus has to be acquired by the immobile first and second instar larvae to facilitate vector transmission competence (Sakimura, 1963a; Van de Wetering *et al.,* 1996; Ullman *et al*., 1993). One main factor contributing to the wide host range of TSWV is the tenacity and extreme polyphagy of its vectors. The broad host range suggests that TSWV is only naturally constrained by the ecological success or epidemiological competence of its thrips vectors other than that of its losses in fitness due to the accumulation of deleterious mutations through successive host passages (Resende *et al.,* 1991; Nagata *et al*., 2000a; Nagata & Peters 2001).

Thrips host range, preference and performance: e host range of thrips have been studied primarily in relation to their epidemiological significance in diseases caused by tospoviruses (Roselló *et al*., 1996; Ullman *et al*., 1997) than by the direct injury they cause through feeding (Van de Wetering *et al.,* 1998; Herrin & Warnock, 2002) or as predators (Agrawal & Colfer 2000) and to explain the seasonal survival of vectors (Groves *et al*., 2001; Jenser *et al.,* 2003).

Thrips host preference of plants that are susceptible to the virus influence thrips transmission efficiency (Allen & Broadbent, 1986; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 2002) because the virus must be acquired by the immobile larvae (Lindorf, 1931, 1932; Sakimura, 1963a; Van de Wetering *et al.,* 1996), which must be able to complete its development on the host selected by the adult thrips (Hobbs *et al.,* 1993; Bautista & Mau 1994; Terry 1997). Therefore, host preference by vector adult thrips among plants susceptible to the virus becomes a critical aspect of epidemiology of virus diseases (Bautista & Mau 1994; Gray and Banerjee 1999). Thrips are opportunistic species that exploit temporary or intermittently occurring environments (Mound & Teulon 1995; Mound 1997). They have extreme polyphagy and the ability to reproduce on a broad range of host plants (Terry, 1997; Ullman *et al*., 1997; Groves *et al.,* 2002). This exacerbates the rate of spread and distribution of plant diseases caused by tospoviruses which they vector, particularly where the patterns of plant distributions in agroecosystems are dominated by large patches of genetically homogenous hosts as is the case in modern agriculture (Duffus 1971;

Thresh 1974, 1982). Conversely, it has been argued that natural non-cropped ecosystems are usually less prone to rapid and severe epidemics because host genotypes are more diverse than in agroecosystems and are distributed in small patches (Kennedy *et al.,* 2002; Ye *et al.,* 2003). However, the existence of many vector thrips species that have a very broad host range widens the ecological success and epidemiological competence of tospoviruses such as TSWV that they transmit. This factor is central to any consideration of control strategies in Australia where more than one vector thrips species are thought to be involved in the epidemics (Jericho & Wilson, 2003). The catalogues of plants recorded as hosts (Cho *et al*., 1986; Stobbs *et al*., 1992; Hobbs *et al*., 1993; Wijkamp *et al*., 1995; Johnson *et al*., 1995; Mertelík *et al.*, 1996; Wilson 1998a; Ochoa *et al.,* 1999; Chatzivassiliou *et al*., 1996; 2000a, 2001; Groves *et al*., 2001, 2002; Adkins & Rosskopf, 2002) is largely dominated by species that act as reproductive hosts for thrips and reservoir hosts for tospoviruses (Yudin *et al*., 1986; Stobbs *et al*., 1992; German *et al.*, 1992; Bautista *et al*., 1995; Teulon *et al.,* 1993; ; Teulon & Penman*,* 1996; Mertelík. *et al*., 1996; Lewis *et al*., 1997c; Peters 1998; Ochoa *et al.*, 1999; Mound 2002). These include both annual and perennial crops and weeds, as well as susceptible transient hosts of polyphagous vector thrips species, which get infected with the virus during exploratory host suitability probing by thrips (Ullman, 1997). This large number of hosts serves as a testament to the growing importance of thrips as vectors of viral plant pathogens. The host range is certainly more diverse than published records indicate, as there are few data on non-cultivated ecologies.

In host range ecology and evolution, there are two important components determining adaptation, host preference and subsequent performance on the plant (Terry 1997; Ullman, 1997). Adaptation and performance of thrips on the diverse host range is driven by complex dispersal patterns and host utilization (Terry 1997).

The dispersal patterns of thrips to new hosts or within the hosts vary from some species being sedentary, having several generations on one host to others being highly mobile from host to host during the day or dispersal within the host. *T. tabaci* has a dispersal pattern occurring daily within the onion plant, while *F. occidentalis* exhibits a nocturnal pattern of random dispersion followed by aggregation on apical half of leaves during afternoons (Sites *et al*., 1992) and dispersion among cotton plants (Matteson & Terry 1992). Long distance movement is mainly through human transport that breaches isolation in commercial agriculture (Mound 1983). Although weak fliers, adult trips may travel long distances between fields (Lewis 1997b), with greatest dispersal activity taking place during the warmest part of the season (Teulon & Penman 1996). They may also get carried on air currents over great distances in seasonal or prevailing winds. Under these conditions, individual thrips have no control over their flight path and destination (Lewis 1997b). This pattern of migration is of special significance to Australia in view of the sporadic nature of TSWV epidemics. High-altitude and long-distance migration of aphid vectors of viruses in Australia has been recognized for some time (Gutierrez *et al*., 1971, 1974).

Utilisation patterns vary from strictly being sporophagous, mycophagous or herbivorous and others being omnivorous or primary predators (Mound & Teulon 1995; Mound 1997). Within these categories, some maybe more host-specialised species or brachypterous or apterous species. The diversity resistance hypothesis, which argues that diverse communities of plants are highly competitive and readily resist invasion and consequently infection, is supported by both theory (Thresh 1974, 1982; Holmgren & Getz 2000; Ye *et al.,* 2003) and experimental studies (Yudin *et al.,* 1988; Ochoa *et al.*, 1999; Herrin & Warnok, 2002). Theory also predicts that polyphagy in herbivorous arthropods is restricted by trade-offs in performance on different hosts (Fox & Morrow 1981; Futuyma and Moreno 1988; Jaenike 1990; Thompson 1988, 1996; Terry 1997). Results consistent with this view have been obtained in ecological studies of thrips preferences, associations and performance, providing insight into the potential constraints and selection pressures on the evolution of host range (Kirk 1985; Teulon 1993; Bautista & Mau 1994; Chatzivassiliou *et al*., 1999, 2001; Agrawal & Colfer 2000). Such studies in crops like lettuce have provided evidence that there are distinct levels of host preference by thrips, particularly *F. occidentalis* (Yudin *et al*., 1988). Further studies have also provided information on feeding and oviposition preferences (Bautista *et al*., 1995; Chatzivassiliou *et al*., 2002) and suitability of some TSWV host plants for the development of *F. occidentalis* (Bautista & Mau 1994). In transmission tests, Wijkamp *et al*., (1995) found out that there are distinct levels of specificity in thrips transmission of tospoviruses with *F. occidentalis* appearing to be the most efficient vector for TSWV, Impatiens necrotic spot virus (INSV), tomato chlorotic spot virus (TCSV) and groundnut ring spot virus (GRSV).

Host range limitations due to trade-offs in fitness on alternative hosts (Fox & Morrow 1981; Terry 1997) are important in determining the abundance and distribution of thrips in natural and managed ecosystems. Understanding the complexity of this phenomenon in thrips has grown tremendously since Jones (1959) provided evidence of the failure of thrips to transmit an isolate of TSWV. The observation was subsequently confirmed decades later in Canada by Paliwal (1974, 1976) and in Hawaii (Sakimura, 1963b). There is now additional laboratory evidence suggesting that host-range limitation maybe due to trade-offs in fitness on alternative hosts (Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 1999, 2001), and this has further questioned the competency and role of some thrips species in the field spread of tospoviruses in different plant hosts and ecosystems. For example, while it was believed for decades that *T. tabaci* was the main vector of TSWV even when other vectors were already known, its ambiguity to transmit some isolates of TSWV, particularly in some parts of Europe during the last decade, has continued to raise doubts about its vector status (Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 1999, 2001). The failure of *T. tabaci* to transmit *Chrysanthemum stem necrosis virus* reinforces this argument (Nagata & de Ávila 2000b). This has lead to several hypotheses such as evolution of either the virus or *T. tabaci* or both, making them incompatible. Selective displacement of TSWV isolates transmitted by *T. tabaci*, with those transmitted by the more tenacious *F. occidentalis* has also been suggested (Ullman *et al.,* 1997; Nagata & Peters 2001) but still need to be proven. During the last decade, and with the identification of more tospovirus vectors, it has emerged that vector preferences, fitness and

trade-offs on host plants is not limited to *T. tabaci* but rather a wider and complex phenomenon than previously surmised. A growing body of evidence of this has been provided by observations on *F. occidentalis*, a well known vector of TSWV and INSV in controlled environments in the horticultural and floral industries, but which has been observed to play a limited role in tospovirus epidemics in large field crops of tobacco (Gloves *et al*., 2002; Chatzivassiliou *et al*., 2001), sweet pepper and tomato (Nagata & Peters 2001) and potato (Jericho & Wilson 2003). However such studies are few and limited to a small number of plants and thrips species, especially when considered in relation to the great importance of this feature in tospovirus epidemiology and control. The mechanisms causing the vector preferences and trade-offs in different hosts and ecosystems are still largely unknown, but visual ecological cues (colour, host shape and size) and plant chemistry have been argued to play a central role in thrips specialization (Terry 1997). However, factors other than chemistry and visual ecological cues may be more important in specialization (Bernays & Graham 1988; Joshi & Thompson 1995; Fry 1996). Selection experiments provide a unique tool to study these traits and are the only way of establishing the preferences, performances and trade-offs in different crops and ecologies. Although it has long been suggested that *F. occidentalis* infests potato crops in Australia, the theory and evidence have not been well matched and the assertions of transmission competency of TSWV in potato have never been experimentally assessed in laboratory or field experiments. The topic is an important one for further study, because there is a need to determine the magnitude of the threat posed by this species in potato under different environments.

Virus transmission: Virus-thrips vector relationships in the transmission of TSWV have been reviewed comprehensively (Sakimura 1963a; German *et al.,* 1992; Goldbach & Peters 1996; Roselló *et al.,* 1996; Ullman *et al.,* 1997). TSWV is, so far, known to be efficiently transmitted in a propagative manner by eight thrips species belonging to the genera *Thrips* and *Frankliniella* (Mound 1996; German *et al.*, 1992; Mumford *et al.,* 1996a; Ullman *et al.,* 1997; Nagata & Ávila, 2000; Nagata & Peters 2001). Four of these species are found in Australia; onion thrips, *Thrips tabaci* Lindeman; tomato thrips, *Frankliniella schultzei* Trybom; the western flower thrips, *F. occidentalis* (Pergande); and the melon thrips, *Thrips palmi* Karney (Malipatil *et al*., 1993; Mound, 1996). TSWV can also be transmitted from plant to plant under laboratory conditions through mechanical inoculations of crude sap (Norris 1946) and buffered solutions (Kumar *et al.,* 1993; Mertelík *et al.,* 1996; Latham & Jones 1997; Hristova *et al.,* 2001; Mandal *et al.,* 2001, 2002; Adkins & Rosskopf, 2002). Transmission of TSWV by mechanical means through leaf or stem contact or seed and pollen has not been reported, although Reddy *et al.,* (1983) recovered infective virus from the testa of immature and freshly harvested mature seed and non from the cotyledons or embryos. However, some of the vectors of TSWV, viz, *T. tabaci* and *F. occidentalis* have been implicated in the transmission of pollen-borne viruses belonging to ilarvirus, sobemovirus and carmovirus groups (Ullman *et al.,* 1997 and references therein). And in a recent study (Milne & Walter 2003), it was demonstrated that *Prunus necrotic ringspot virus* (PNRSV) (family *Bromoviridae*) can be readily transmitted when *T. tabaci, Thrips imaginis* and *Thrips Australis* and virus-bearing pollen are placed together onto test plants.

Although observed and suggested many decades ago that TSWV could only be transmitted by adult thrips, when the virus was acquired by larvae (Bald & Samuel 1931; Linford 1932; Sakimura 1963a), the theory and evidence have only been matched in the last decade. Through detailed experiments using a biotype of *F. occidentalis* from the Netherlands, it was demonstrated that the ability to transmit the virus is related to the peculiar characteristic of the thrips to acquire the virus as first instar larvae (Van de Wetering *et al.,* 1996) and transmitted by second instar larvae and adults after a latent period during which the virus replicates within the thrips (Sakimura 1963a; Ullman *et al.,* 1993; Wijkamp *et al.,* 1993). However, comparative studies using biotypes from other countries indicated that the ability to acquire the virus only up to the first instar stage was limited to the biotype used and that other biotypes investigated were able to acquire TSWV even during the second instar stages (Ullman *et al.,* 1997; Van de Wetering *et al.,* 1999a).

The route of TSWV through the thrips body in relation to transmission has been a subject of many extensive studies (Ullman *et al.,* 1993, 1995, 1997; Bandla *et al.,* 1997; Kikkert *et al.,* 1997; Assis Filho *et al.,* 2002; Kritzman *et al.,* 2002), and excellent and comprehensive reviews (Ullman *et al.,* 1997; Nagata & Peters, 2001). And some of these investigations have elucidated the possible underlying mechanisms involved in virus acquisition and general pathway through the arthropod to reach the salivary glands and consequently, transmission. Essentially, the virus is imbibed along with the plant sap. It then attaches to and infects midgut cells, usually reaching high titers in these tissues (Tsuda *et al.,* 1996; Nagata *et al.,* 1999; Assis Filho *et al.,* 2002). It is then released into the hemocoel and secondarily infects other tissues. Horizontal transmission to other plant hosts occurs following infection of salivary tissues and subsequent release of the virus in the salivary secretions that are egested into the host during feeding (Ullman *et al.,* 1993, 1995; 1997). This hypothesis was first tested in *F. occidentalis.* However, in later studies TSWV infection of the midgut and later the salivary glands was shown in *T. setosus* (Tsuda *et al.,* 1996), *F. fusca* (Pappu *et al.,* 1998) and in more recent studies, comparative circulation and replication in both *F. occidentalis* and *F. fusca* has been reported (Assis Filho *et al.,* 2002). Acquisition competency is lost when the thrips becomes an adult due to a midgut barrier (Ullman *et al.,* 1992 Ohnishi *et al.,* 2001). The virus is transtadially passed and is not lost during acdysis. As a result the thrips remains infective for life (Wijkamp *et al.,* 1996b), which may last 20 to 40 days depending on the environmental conditions (Goldbach & Peters 1996). This makes thrips to act as hosts and virus reservoirs as indicated by the role of overwintering *F. fusca* (Johnson *et al.,* 1995; Groves *et al.,* 2001, 2002) and the significance of hibernated *T. tabaci* adults (Jenser *et al.*, 2003) in the epidemics of TSWV. No transovarial passage of the virus to the progeny occurs (Sakimura 1963a; Wijkamp *et al.,* 1996b).

After acquisition of the virus by vector thrips there is a latent period before transmission. During this time, the virus multiplies within the vector. In earlier studies, the minimum latent periods for *T. tabaci, F. occidentalis* and *F. schultzei* were reported to vary between 4 and 18 days (Sakimura, 1963a). But following a series of classical experiments in the transmission of TSWV and INSV, Wijkamp & Peters (1993) calculated that the mean latent period (LP_{50}) in viruliferous second instar *F. occidentalis* larvae previously given a 24-hr acquisition period was 80-170 hours at 27°C or 20°C, respectively. The minimum acquisition access periods (AAP_{min}) for *T. tabaci* larvae fed on *Datura stramonium* can be as short as 5-15 minutes (see references in Sakimura, 1963a). The median acquisition access periods (AAP_{50}) of *F. occidentalis* larvae fed on TSWV infected *Impatiens* plants was found to be 106 minutes and transmission was possible after a mean inoculation access period (IAP_{50}) of 58 minutes on *petunia* and 137 minutes on *D. stramonium* leaf disks (Wijkamp *et al*., 1996a). TSWV transmission competencies are temperature-dependent, at least in larvae of *T. tabaci* (Chatzivassiliou *et al.,* 2002) and *F. occidentalis* (Wijkamp & Peters 1993). Brief periods of 5 minutes characterize successful acquisition or inoculation periods suggesting that the virus is ingested from superficial plant cells and small amounts of ingested virus suffice to initiate an infection in thrips (Nagata & Peters 2001).

Thrips species show distinct patterns of virus accumulation, vector and host specificity in the transmission of TSWV and other tospoviruses (Paliwal 1976; Wijkamp *et al.,* 1995; Inoue *et al.,* 2002; Nagata *et al.,* 2002; Sakurai *et al.,* 2002). The specificity is thought to be governed by factors such as thrips vector preferences and performances on TSWV accumulation hosts (Allen & Broadbent, 1986; de Kogel, 2002; Chatzivassiliou *et al*., 2001), thrips development stage (Moritz 2002; Inoue *et al.,* 2002), temperature (Wijkamp & Peters 1993; Chatzivassiliou *et al.,* 2002), and vector sex (Wijkamp *et al.,* 1995; Van de Wetering *et al.,* 1998; Sakurai *et al.,* 1998, 2002).

Since Pittman (1927) demonstrated that *T. tabaci* could transmit TSWV, the occurrence of virus transmitters and non -transmitters within vector populations has been reported (Jones 1959; Sakimura 1963a; Paliwal 1974, 1976; Wijkamp *et al.,* 1995; Jenser *et al*., 2002). Many populations of *T. tabaci* have been observed to transmit TSWV poorly or not at all in some parts of North America and Western Europe (Jones 1959, Paliwal 1974, 1976; Wijkamp *et al.,* 1995; McPherson *et al.,* 1999; Chatzivassiliou *et al*., 1998a, 1999, 2001), South America (Nagata *et al.,* 2002), and Hawaii in recent years (Mound, pers.comm.). Explanations of possible causes of this incompetence to transmit the virus has been attributed to incompatibilities among thrips populations and the TSWV isolates used (German *et al.,* 1992; Jenser *et al.,* 2002) and correlated with the absence of males in *T. tabaci* populations (Wijkamp *et al.,* 1995; Chatzivassiliou *et al*., 1998a), Others have attributed the differences in transmission efficiencies in *T. tabaci* to trade-offs and performance on some hosts such as leek, tobacco, *Datura stramonium* and *Petunia hybrida* (Chatzivassiliou *et al*., 1999, 2002) and tomato (Nagata *et al.,* 2002). Significant differences in colour and body size of *T. tabaci* under different temperature regimes have been observed (Murai & Toda 2002). But whether these biotype differences and any other also translate to transmission competence or lack of it is still unclear and remains a topic of continuing investigations. However, *T. tabaci* has been implicated in the spread of TSWV in California (Sakimura 1961), potato and lettuce fields in southern Tasmania (Wilson 1998a, 1998b, 2001) and tobacco fields in Eastern Europe when other vector species were not present (Sakimura 1963a; Zawirska 1976 - references in Nagata & Peters 2001 and Chatzivassiliou *et al.,* 1998a, 1999; Jenser *et al*., 2002). *T. tabaci* is not considered a vector of TCSV and GRSV (Wijkamp *et al.,* 1995). This diversity in the competence of *T. tabaci* to transmit TSWV and other tospoviruses and the present lack of plausible and detailed knowledge on the mechanisms involved points to gaps in the present available knowledge on vector-virus-host interactions and the need to address them. The vector status of *T. tabaci* is still unfolding and may eventually mirror that of whiteflies and geminiviruses interactions in crops such as cassava, cotton, grain legumes and vegetables in which differences in plant-virus transmission capabilities between biotypes have been recognized (Bedford *et al.,* 1994; Brown, 1994; Jericho, 1999; Varma & Malathi 2003; Jones, 2003). *F. schultzei* is considered the main vector in tobacco in United States (McPherson *et al.,* 1999) while *F. occidentalis* has been observed to play a limited or no role in the spread of tospoviruses in large fields (Gloves *et al*., 2002; Chatzivassiliou *et al*., 2001; Nagata & Peters 2001; Jericho & Wilson 2003). Certainly, there is plenty to learn from this hypothesis in Australia where both *F. schultzei* and *T. tabaci* have been trapped in potato field in which sporadic and sometimes severe epidemics have occurred, suggesting their role in these epidemics in the absence of other vector species (Jericho & Wilson 2003). There are no significant molecular differences observed among TSWV strains in Australia (Talty & Dietzgen, 2001; Dietzgen, 2003), although resistance-breaking strains have been reported (Latham & Jones 1998). And there is no information on the existence of *T. tabaci* biotypes or having occurred in the past. But such a proposition cannot be discounted completely, as investigations on this aspect of *T. tabaci* biology are yet to be done.

The ability to accumulate and transmit TSWV is also related to the stage of development in the genera *Thrips* and *Frankliniella* (Inoue *et al.,* 2002). Accumulation of TSWV from the second larval to adult stage as measured by TAS-ELISA titers of the virus N protein were almost the same or increased in *F. occidentalis* and *F. intonsa,* but decreased significantly in *T. tabaci, T. setosus* and *T. hawaiiensis.* These differences in virus accumulation may explain some of the differences observed in the transmission efficiencies between the species, particularly in different hosts.

Distinct inter- and Intraspecific variations in susceptibility and the competences to transmit TSWV has also been related to vector preferences and performances on many hosts (Bautista & Mau 1994; Bautista *et al.,* 1995; Wijkamp *et al.,* 1995; Chatzivassiliou *et al.,* 1998b, 1999, 2001, 2002; Sakurai *et al.,* 2002; Maris *et al.,* 2003a) and geographic regions (Sakimura 1963b; Roselló *et al.,* 1996). Behavioral responses of vectors and virus transmission

competence of each thrips species varies considerably according to the host species (Sakimura 1963b) and phenological stages in each of the species (Inoue *et al.,* 2002). Comparative studies of virus transmission efficiency by *T. tabaci* and *F. fusca* in Hawaii (Sakimura 1963b) demonstrated that TSWV was transmitted more efficiently by the two vectors from *Emilia sonchifolia* L when used for acquisition and inoculation. Transmission was reduced for inoculations involving acquisition from *Emilia sonchifolia* and inoculation to Aster or vice versa. Wijkamp (1995) observed that larvae of *F. occidentalis* and dark form of *F. schutzei* survived well to adult stage on *Nicotiana benthamiana, Nicotiana clevelandii* and *Emilia sonchifolia.* However, mortality of *T. tabaci* and *F. intonsa* juveniles reached 90 to 100% on these species. *F. occidentalis, F. intonsa* and *T. tabaci* survived and were able to acquire the virus from *D. stramonium* and *Impatiens sp*. Transmission of TSWV, TCSV, GRSV and INSV to petunia leaf disks was more efficiently done by *F. occidentalis* than any other vector thrips species. *Petunia* leaves are good indicators for TSWV infection, but are not generally good hosts for *F. occidentalis* (Ullman *et al.,* 1997). In the same study, the transmission efficiency of TSWV by *F. schultzei* was lower than those of TCSV and GRSV. The light form of *F. schutzei, F. intonsa* and arrhenotokous populations of *T. tabaci* could also transmit TSWV, albeit with low efficiency. However, *T. tabaci* populations in which males were absent could not transmit the virus. Zawirska (1976, reference in Nagata & Peters 2001) argues that *T. tabaci* comprises two taxonomically identical "types" from among which the populations on tobacco (*Nicotiana tabacum*) are considered as *T. tabaci* subsp. *tabaci* and those living on *Galinsoga parviflora*, potato and other hosts as *T. tabaci* subsp. *communis*. Populations of *T. tabaci* subsp. *communis* on different plant populations, mainly on onion, propagate parthenogenetically and not considered virus vectors. Parthenogenetic and arrhenotokous populations reared on beans (Wijkamp *et al.,* 1995) and leek (Chatzivassiliou *et al*., 1999), were unable to transmit TSWV isolates. These observations have also been made by Jenser *et al*., (2002), and appears to be a common phenomena on many host plants. In other studies it has been found that *F. occidentalis* does not thrive well on leaves of chrysanthemum, peanuts and some cotton cultivars, and by implication, unable to acquire and transmit TSWV (Terry, 1997 and references therein). In contrast, studies by Maris *et al.,* (2003a) on the effect of TSWV source on virus acquisition and transmission by *F. occidentalis* in resistant pepper demonstrated that both virus acquisition from and inoculation into the TSWV-resistant cultivars were not impaired compared with susceptible varieties and that inoculation efficiency was not affected in short periods but significantly lower during longer periods. From the above studies it is clear that the existence and prevalence of plant hosts which influence vector performance in TSWV transmission has epidemiological significance for managing and controlling the spread of the disease.

As in many vector species transmitting viruses, such as aphids in potato (Radcliffe & Ragsdale 2002), the feeding behaviour of thrips in relation to their ability to transmit TSWV has been studied (Harrewijn *et al*., 1996; Van de Wetering *et al*., 1998; Van de Wetering, 1999b). Differences in the feeding behaviour of male and female *F. occidentalis* on petunia leaf disks have been observed (Van de Wetering *et al*., 1998). Using electrical penetration graph (EPG) analysis, female thrips were observed to feed more frequently and intensively than males. However, males transmitted TSWV more efficiently than females. Independent studies in Japan by Sakurai *et al.,* (1998, 2002) using *F. occidentalis* confirmed this phenomenon. Using a petunia leaf disk assay, TSWV transmission varied for nine populations from 6.1 to 29.2%, with male *F. occidentalis* populations transmitting the virus more efficiently than females. Transmission competencies were also observed to vary between sexes based on TSWV accumulation thresholds in their bodies. Males transmitted the virus at lower thresholds than females.

The molecular and physiological bases of TSWV-vector interactions that regulate transmission are not well understood (Ullman *et al.,* 1997; Nagata & Peters 2001; Assis Filho *et al.,* 2002). But there is a growing body of knowledge that genetic elements within both the virus and the vector ultimately decide the transmission competency of thrips species during different phenological stages (Ullman *et al.,* 1993, 1995; Kikkert *et al.,* 1998; Bandla *et al.,* 1998; Nagata *et al.,* 2000; Sin *et al.,* 2003). Some TSWV stains are not transmissible or are poorly transmitted by some vector thrips (Jones 1959; Sakimura 1963a; Paliwal 1974, 1976; Wijkamp *et al.,* 1995; Jenser *et al*., 2002). These differences in transmissibility are probably not only attributable to differences in the lectin-binding properties and glycosidase sensitivities of G1 and G2 glycoproteins (Naidu *et al.,* 2003), but also to specific segments of the genome (Sin *et al.,* 2003), whose altered structure through deletions in the lipid envelope membranes (G1/G2) and accumulation of defective interfering RNAs may affect protein/protein or RNA/protein interactions with a dramatic impact on particle stability. Complete loss or reduced rate of vector thrips transmissibility may result (Nagata *et al.,* 2000a), which is not in the interest of the virus. The results of these studies are beginning to clear up some of the central dogmas of TSWV-vector interactions and transmission competency.

Systemic movement of TSWV in plants is mediated by the viral NSm protein (Gunasinghe & Buck 2003) and is influenced by plant species (Llamas-Llamas *et al*., 1998; Garg & Khurana, 1999; Kikkert *et al*., 1999), variety (Wilson, 2001; Aramburu & Martí, 2003) and growth stage (Soler *et al*., 1998). The rates of virus movement may be greater in sensitive compared with tolerant varieties (Moury *et al*., 1997; Soler *et al*., 1998, 1999; Wilson, 2001; Maris *et al*., 2003b). Furthermore, systemic movement is conditioned by temperature (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998) and water stress (Córdoba *et al*., 1991). Changes in virus titer have the potential to alter the chances of a vector acquiring and transmitting a virus and, thus, may influence disease epidemics (Van de Wetering *et al*., 1998)

Virus detection and Diagnosis

Identification and characterisation of viruses is a prerequisite for developing effective management of viral diseases (Hamilton *et al.*1981). Management and control of diseases caused by tospoviruses can be accomplished by establishing new plantings from virus-tested plants. Fast, reliable, inexpensive detection methods for tospoviruses are important in the implementation of programs to establish, maintain and disseminate virus-free seed stocks, and are crucial to the enforcement of quarantine and certification, as well as the establishment of therapy protocols. Disease diagnosis based on established methods provides quantitative and qualitative information required in the study of pathogen ecology, epidemiology and control.

Various methods for detection and diagnosis of viruses have been reviewed (Mathews, 1993). Several relatively reliable detection and diagnosis methods developed for other viruses are now used for tospoviruses (German *et al.,* 1992), and may be considered central to the currently detailed understanding of the virus. Variables relating to sampling methods and the effects of several factors on test variability have been described (Barnett 1986; German *et al.,* 1992; Nutter & Schultz 1995; Goldbach & Peters, 1996; Nutter 1997). The techniques have been used for detection and diagnosis of the virus in systemically infected plant parts (Norris 1946; Gonsalves & Trujillo 1986) and vector thrips (Paliwal 1976; Cho *et al.*1988; Tsuda *et al.* 1994; Bandla *et al.* 1994). Refinement of these methods has also made them to be now applicable to epidemiological problems (Gaunt 1995), particularly for studying pathogen ecology and population dynamics. For example, visual and biological (Brittlebank, 1919;Norris 1946; Norris, 1951a, 1951b; Latham & Jones 1996), serological (Gonsalves & Trujillo, 1986; Cho *et al.* 1988; Wang & Gonsalves 1990; de Ávila *et al.*1990; Bandla *et al.* 1994) and molecular techniques (Tsuda *et al.* 1994; Weekes *et al.* 1996; Dewey *et al.* 1996; Mumford *et al.* 1996b; Jain *et al.* 1998; Roberts *et al.* 2000; Boonham *et al.* 2002) have all been adapted and applied successfully in the diagnosis of TSWV to (i) ascribe field isolates to different serotypes; (ii) identify TSWV as the cause of specific plant diseases; (iii) detection of the virus in thrips for infectivity-based forecasting systems (iv) achieve timely virus detection for quarantine and certification purposes; and (v) study virus population dynamics. The use of detection and diagnosis tools in the above investigations has varied widely and continue to be driven by refinements of and breakthroughs in new technologies and changing demands for information and new areas of investigations. Some of the commonly used methods for detecting tospoviruses are discussed here.

Visual Assessments and bioassays: Detection of TSWV based on symptom expression is fundamental to epidemic analysis and can relatively be simple provided one has extensive experience with disease diagnosis. Visual detection of virus-infected plants depends on the virus isolates present (De Ávila *et al.*, 1990; Latham & Jones 1997), the susceptibility and sensitivity of the cultivars (Norris 1951a; Wilson 2001; Jericho & Wilson, 2003) and the effects of growing conditions (Llamas-Llamas *et al.*, 1998; Daughtrey *et al.*, 1997). Visual inspection of growing crops has long been the method used to determine disease incidence and the basis for the rejection thresholds in routine regulatory certification of potato seed stocks. The method has been used in the past to distinguishing TSWV isolates on tomatoes (Norris 1946) and potatoes (Norris 1951a) and is still a vital diagnostic technique used in supplementary and confirmatory assays. The method is also suitable in epidemiological investigations covering large areas as it offers fewer limitations in terms of sample size and including the potential for use in combination with other assays. Several experimental indicator hosts of TSWV have been recorded (Mumford *et al.*, 1996; Adkins & Rosskopf, 2002) However, the diversity of symptoms caused by TSWV in many plant genotypes (German *et al.*, 1992; Goldbach & Peters, 1996; Latham & Jones, 1996; Roselló *et al.*, 1996) and the asymptomatic characteristic of some infected plants (Matteoni & Allen 1989; Stobbs *et al.*, 1992; Latham & Jones, 1997; Chatzivassiliou *et al*., 2001) often leads to underestimation of virus incidence because symptoms may be masked or infection latent. Also, symptoms of TSWV in some plant genotypes vary according to cultivars and may be mistaken for the common early blight fungal pathogen *Alternaria solani* infections in potato (Norris 1951a; Wilson 2001) or peanut mottle potyvirus infections in peanuts (Sreenivasulu *et al.*, 1988; Hoffmann *et al.*, 1998) or Impatiens necrotic spot virus (INSV) in both gladiolus and iris (Derks & Lemmers 1996) and therefore, lead to an underestimation of the disease, and consequently, inappropriate control responses. This precludes the use of the technique as the main indicator of virus presence in plants and consequently the easy selection and removal (rouging) of diseased plants in the field. Visual assessments also have other numerous disadvantages including a considerable measurement error (James 1974; Nutter *et al.*, 1993, 1995; Nutter, 2002), longer time required to confirm the symptoms, greater labour costs, high levels of skill required, lack of detection sensitivity relative to the quality required and lack of correlation with the effect of virus translocation within the plants being assessed (Norris 1951b; Wilson 2001).

Bio-assays to detect viruses in plants and vectors, accomplished by inoculating indicator plants in the field or in the greenhouse by mechanical means or using vectors are still the only methods available to detect viruses that are not well characterised (Spiegel *et al*. 1993; Mathews 1993). Host range information is not only valuable for virus characterization, but also has significant implications for the epidemiology and management of TSWV. Detection of TSWV in plants (Norris 1946; Stobbs *et al.*, 1992; Mertelík *et al.*, 1986; Resende *et al.*, 1996) and thrips (Allen & Matteoni 1991; Hobbs *et al.*, 1993; Bandla *et al.*, 1994; Gloves *et al.*, 2003) has been generally achieved by bioassays on host plants. Biological characterisation has been conducted, mainly in greenhouses, to establish taxonomic distinctions of TSWV isolates (Best & Gallus 1955; De Ávila *et al.*, 1990; Chatzivassiliou *et al*., 2000). It is now recognised that significant host range differences exist among TSWV isolates (De Ávila *et al.*,

1990; Latham & Jones, 1997; Chatzivassiliou *et al*., 2000). Different symptoms in different host plants ranging from mild to severe chlorotic local lesions to systemic leaf curling and leaf distortion are induced by TSWV infection (Latham & Jones 1996). However, the use of host-range procedures in routine detection of viruses is not only labour intensive, often requiring several weeks to obtain results, significant amounts of greenhouse and field space, and extensive retesting for reliable results, but also impractical for large numbers of samples. In addition, the reliability of such indexing programs can suffer from technical difficulties commonly encountered when transmitting viruses from vegetatively propagated plants to new hosts (Mathews 1993). Host-range studies that use host response may also not always be reliable to classify tospovirus isolates because of drastically reduced virulence due to mechanical passage and even inability to infect original hosts as observed by Resende *et al*., (1991, 1996).

The inherent limitations in the use of the visual and biological tests and the combined need for speed and accuracy in routine testing and diagnosis of pathogen populations and sub-populations in asysmptomatic plant parts, as well as the discrimination of closely related types consequently led to the development of other methods to detect TSWV so as to decrease initial virus incidence in the stocks by appropriate measures.

Serological assays: Various serological detection methods for plant viruses have been reviewed (Hampton *et al*. 1990). The use of serological assays such as the enzyme-linked immunosorbent assay (ELISA)(Clark &Adams, 1977; Gonsalves & Trujillo, 1986), tissue blots (Whitfield *et al.*, 2003), and immunosorbent electron microscopy (ISEM) (De Ávila *et al.*, 1990; Kitajima *et al.*, 1992) has increased dramatically in recent times with the proliferation and modifications of the assays aimed at reducing host antigen background reactions in ELISA. Though developed for phylogenetic studies and diagnostics, these immunological techniques are used increasingly in epidemiological studies of TSWV (Cho *et al*., 1986, 1987; Stobbs *et al*., 1992; Bautista *et al*., 1995; Camann *et al*., 1995; Johnson *et al*., 1995; Mertelík *et al*., 1996; Gracia *et al*., 1999; Hobbs *et al*., 1993; Aramburu *et al*., 1997; Latham & Jones 1997; Wilson 1998; Gitaitis *et al*., 1998; Ochoa *et al*., 1999; McPherson *et al*., 1999; Chatzivassiliou *et al*., 2000, 2001; Williams *et al*., 2001; Groves *et al*., 2001, 2002, 2003; Hristova *et al*., 2001; Jenser *et al*., 2003). Since TSWV has very similar properties to members of the Family *Bunyaviridae:* genus *Tospovirus* (De Haan *et al.* 1989a, 1989b; Elliot 1990; Francki *et al.*, 1991), the group-specific antigenic determinants occurring in the nucleocapsid protein of virus (De Ávila *et al.*, 1990; German *et al.*, 1992; De Ávila *et al.*, 1993; Goldbach & Peters, 1996; Mumford *et al.,* 1996a) makes it possible for monoclonal (Sherwood *et al.*, 1989; Huguenot *et al.*, 1990; De Ávila *et al.*, 1990) and polyclonal antibodies (Gonsalves & Trujillo, 1986; De Ávila *et al.*, 1990; Chatzivassiliou *et al*., 2000) to be used in ELISA and ISEM to detect heterologous viruses.

Monoclonal and polyclonal antisera are now widely used in many plant diagnostic laboratories where they provide a compromise of speed and sensitivity in routine virus tests and epidemiological surveys in plants (Stobbs *et al.*, 1992; Mertelík *et al.*, 1986; Chatzivassiliou *et al*., 2000) and thrips (Cho *et al.*, 1988; Allen & Matteoni 1991; Hobbs *et al.*, 1993; Bandla *et al.*, 1994). Serological detection of TSWV in thrips was demonstrated by Paliwal (1976) using homogenates of 50 first-generation adults of *Frankliniella fusca* that were reared on newly infected plants. Cho *et al.*, (1988) also detected TSWV in individual thrips by means of ELISA. Monoclonal antibody to non-structural proteins (NSs) encoded by the small RNA of TSWV and fluorescence microscopy have been used to identify virus replication within the thrips alimentary canal (Ullman *et al*., 1993; Nagata & Peters 2001; Assis Filho *et al*., 2002). The capability of serological antigen detection in individual thrips has considerably been enhanced and broadened by adapting and using immunocapture-polymerase chain reaction (IC-PCR) (Sano *et al.* 1992; Weekes *et al.*, 1996; Jain *et al.*, 1998). IC-PCR is an important method for the detection of low-titer, RNA-containing viruses and generally has been found to be more sensitive than many other nucleic acid-based assays (Wetzel *et al*. 1992). Appropriate precautions are, however, necessary in the use of these techniques to avoid technical problems with false positives and negative results (Sutula *et al.*, 1986; Chatzivassiliou *et al*., 2001). Refinements of these enzyme immunoassay techniques and their use in epidemiological studies of tospoviruses based on the serological relationships of the N protein has now led to an increase in the number of recognised serotypes from four (De Ávila *et al.*, 1993) to ten, representing possibly 13 species, with new serogroups continuing to be established (Law & Moyer 1990; De Ávila *et al.*, 1992; Satyanarayana *et al.*, 1996; Resende *et al.*, 1996; Dewey *et al*., 1996; Pappu *et al*., 1996; Ullman *et al.,* 1997; Cortês *et al.*, 1998; Pozzer *et al.*, 1999; Griep *et al.* 2000; Nagata & Peters 2001; Chu *et al.*, 1997, 2001; Adkins, 2003; Abad *et al*., 2003b). Although this has greatly enhanced studies of virus population and disease dynamics in epidemics, it also leads to confusion in diagnostics, since the phenotypes of these novel viruses can vary greatly (Mumford *et al.*, 1996; Adkins, 2003) due to reasortments of the genomes (Qiu & Moyer 1999). And since monoclonal antisera are highly specific, only those species against which they are raised will be detected. This problem has recently been addressed by the adaptation and use of recombinant antibody techniques in combination with phase display to produce antibodies that widens detection of viruses (Griep *et al.* 2000). In addition, the use of tissue blot immunoassay (Whitfield *et al.*, 2003) has increased options available for the serological detection of TSWV and quantifying the amount of pathogen present. However, these techniques have not yet been conventionally adopted in epidemiological investigations.

Whilst these developments of serological detection techniques provide another example of improvements in virus detection, they have numerous disadvantages. Complications in serological detection of TSWV can arise with genotypes that do not react typically (Chatzivassiliou *et al*., 2001; Whitfield *et al.*, 2003), with plants that are infected with a complex of two or more viruses (Hoffmann *et al.*, 1998), or that have chimaeric abnormalities resembling TSWV symptoms (Norris 1951a; Sreenivasulu *et al.*, 1988). Serological methods such as most types of ELISA also lack the sensitivity required for the detection of tospoviruses with the certainty required for quarantine

due to the erratic distribution of the virus within potato tubers as observed in the cultivar Russet Burbank (Wilson 2001) and in tubers of the ornamental plant *Ranunculus asiaticus* L. (Whitfield *et al.*, 2003). These tubers, despite giving negative results in ELISA tests, subsequently produced shoots bearing TSWV symptoms. Lack of sensitivity has also been shown by the non-reaction of symptom-bearing peanut samples in studies by Kresta *et al.* (1995) although these results were later contrasted by those of Hoffmann *et al.*, (1998) who found no correlation between symptom severity and ELISA readings. Non-reaction in ELISA tests of symptom-bearing samples has also been observed by Ogbe *et al.* (1996) during studies on cassava mosaic begomovirus, although this was attributed to the use of highly specific monoclonals that do not detect other isolates arising from transecapsidation. The conclusions based on negative results from detection of poorly characterised, unchacteracterised, or unknown viruses are reliable only when testing includes alternative methods utilizing different virus properties for detection (Spiegel *et al.*, 1993).

Electron Microscopy: Tospoviruses can be directly identified by electron microscopy using leaf-dip preparations (Black *et al.*, 1963; Milne, 1970; Ie, 1971; Kitajima, 1965) and using immunosorbent electron microscopy (ISEM) (De Ávila *et al.*, 1990; Kitajima *et al.*, 1992). Investigations on the replication of TSWV in vector thrips have been done using laser scanning fluorescence microscopy and immunolabeling with monoclonal antibody to NSs (Ullman *et al*., 1993; Nagata & Peters 2001; Assis Filho *et al*., 2002). The use of electron microscopy for routine detection is limited by initial set up costs, time consuming sample preparations and the level of skill required for such examination and analysis.

DNA-based assays: For over a decade now there has been increasing interest in the use of DNA-based methods for phylogenetic studies and diagnostics of tospoviruses and these are beginning to be used extensively in epidemiology and population biology (Tsuda *et al.* 1994; Weekes *et al.* 1996; Dewey *et al.* 1996; Mumford *et al.* 1996b; Jain *et al.* 1998; Chatzivassiliou *et al*., 2001; Roberts *et al.* 2000; Talty & Dietzgen, 2001; Boonham *et al.* 2002; Schaad & Frederick 2002; Dietzgen, 2003). Since control of TSWV must be largely preventative, it should benefit from the availability of reliable and rapid methods of virus detection and diagnosis. DNA-based assays offer these benefits. Sequence homologues that exist between the genomes of TSWV have allowed dot blot hybridisation tests with cloned cDNA probes to be used in detecting heterologous viruses in plants (Ronco *et al.*, 1989; Wang & Gonsalves, 1990) and individual *F. occidentalis* (Rice *et al.*, 1990), and nucleic acid hybridisation tests with radioactive riboprobes (Huguenot *et al.*, 1990). Although these detection methods are undoubtedly effective and highly sensitive in the detection and diagnosis of tospoviruses, the availability of cDNA probes and the short half-life, cost, and hazard of radioisotopes present drawbacks to routine and large-scale use of these diagnostic tools (German *et al.* 1992; Spiegel *et al*. 1993; Schaad & Frederick 2002).

In recent times, considerable attention has been focused on relating assessments to detection and discrimination of closely related pathogen populations. Such emphasis is especially pertinent in relation to understanding epidemic development and prediction. The development of polymerase chain reaction (PCR) in 1984 (Mullis 1987), and its subsequent use in plant pathology (Innis *et al.* 1990; Henson & French 1993; Thomson & Dietzgen 1995; Hadidi *et al.* 1995; Talty & Dietzgen, 2001) have contributed to solving some of these problems. The specificity of PCR is based on its ability to amplify a specific nucleic acid segment flanked by a set of oligonucleotide primers that are complimentary to the regions to be amplified (Innis *et al.* 1990) and therefore helps to overcome some difficulties inherent with serological methods such as low titre of antigen, cross-reaction of antibodies with heterologous antigens, and developmental or environmental regulation of antigen production (Rojas *et al.*1993). Depending on the choice of primers, a PCR can provide both narrow and broad specificities for various isolates or strains of pathogens (Henson and French 1993; Hadidi *et al.* 1995). PCR is an extremely sensitive alternative to ELISA, providing a means for detecting viruses in plants throughout the year. The extremely high specificity of PCR for a target sequence defined by a set of primers also avoids the generation of false positives and negatives from the sample (Sano *et al.* 1992; Balfe 1992). This makes it an appropriate assay for mixed infections as those reported in peanuts (Hoffmann *et al.,* 1998). In the last decade, classical PCR has been used by Mumford *et al.*, (1994, 1996b), to detect and discriminated TSWV, INSV and other tospoviruses using specific and universal primers. Several adaptations and modifications have also been made to classical PCR to facilitate detection of tospoviruses in plants and thrips vectors. For example, ddetection of tospovirus species from different crops and geographical areas of Argentina was achieved by RT-PCR of the N-gene and restriction enzyme digestion of products (Dewey *et al.* 1996). RT-PCR was applied to determine whether TSWV-S RNA was present in individual *Thrips setosus* throughout their lives (Tsuda *et al.* 1994). Detection and differentiation of TSWV and INSV has been made in IC-PCR using primers corresponding to segments of the L and S genomic RNA (Weekes *et al.* 1996). IC-PCR has also been used to overcome the difficulties associated with prolonged extraction procedures and inhibitory effects of polyphenols present in peanut tissues during assays to detect and diagnose TSWV (Jain *et al.* 1998). More recently, real-time PCR based on TaqMan® assays has been used to detect TSWV in thrips vectors (Boonham *et al.* 2002) and in plants (Roberts *et al.* 2000; Talty & Dietzgen, 2001; Dietzgen, 2003). The use of techniques such as real-time PCR (Roberts *et al.* 2000; Talty & Dietzgen, 2001; Dietzgen, 2003), which can discriminate isolates while at the same time handle large sample sizes may revolutionize the manner in which plant pathogens are identified and diseases are diagnosed (Schaad & Frederick, 2002). The application of this technique is likely to be widened for routine use as the potential benefits are perceived to justify the cost involved in setting up and familiarisation with the technique. Other enhancements to the sensitivity of PCR have also been made

through the coupling of the calorimetric assay for the detection of viruses in woody plants (Rowhani *et al.* 1998), use of molecular beacons (Eun & Wong, 2000) and fluorescent resonance energy transfer (Schaad & Frederick, 2002). The refinement and use of these techniques over the next decade will influence progress in tospovirus epidemiology and control.

However, while the use of PCR offers many benefits, the design of degenerate primers requires the full knowledge of genomic sequences of the viruses or strains to be detected. Furthermore, the designed primer sequences may require revision in the advent of evolved strains of TSWV (Qiu & Moyer 1999). The conventional PCR requires manipulation of each sample prior to amplification and electrophoresis or blot hybridisation of each PCR product.

The relative suitability of the above tests for routine detection of viruses depend greatly on the concentrations of viral particles in infected tissue and on the range of variations that occurs among the viruses. There is a need, therefore, to develop new or modify currently used methods of detecting tospoviruses to make them more sensitive for routine detection purposes.

Components of TSWV epidemics

Epidemics of TSWV have been attributed to the effects of different interacting factors. In any one epidemic, a number of favourable circumstances must occur for the epidemic to get initiated and sustained. The emergence of TSWV epidemics combines two elements: the introduction of the virus into the crop population and its subsequent spread and maintenance within the population. Ecological factors can influence both of these elements, and consequently ecology has been recognized to have an important role in the emergence of TSWV epidemics (Smith 1961; Kirk 1997; Lewis 1997a, 1997b; Funderburk 2002). Different interacting ecological factors such as changes in host density or susceptibility can increase the initial virus incidence, as can genetic changes in the virus population or in the population of weed hosts. Genetic changes in the virus can arise either through 'coincidental' processes such as neutral drift or coevolution of the pathogen and its reservoir host (Buddenhagen 1983; Ye *et al.,* 2003), or through adaptive evolution of the pathogen during chains of transmission in host plants (Qiu *et al.,* 1998; Latham & Jones 1998; Qiu & Moyer 1999; Thomas-Carroll & Jones 2003; Moyer *et al.,* 2003a; Bucher *et al*., 2003).

Initial virus source incidence: The vegetative propagation of potato forms an important source for dispersal of TSWV and is, therefore, a crucial factor and has marked impact on the epidemiology of the virus. The importance of infected propagating material in establishing TSWV epidemics has long been recognised, and careful precautions are taken to produce healthy propagating material through certification programs. Epidemics can result from planting infected seed tubers from the previous season (Norris & Bald 1943; Conroy *et al*., 1949; Norris 1951a, 1951b; Shepherd 1972). Plants that emerge from such tubers have been observed to show symptoms in potato cultivars like Shepody and Russet Burbank (Wilson, 1998b; 2001). Infected tubers left in the ground after harvest can also produce plants that may serve as reservoirs of both vectors and the virus (Norris & Bald 1943; Horne & Wilson, 2000). The epidemiological significance of these volunteer plants depends on whether they can support breeding thrips populations since the virus has to be acquired by thrips larvae to be transmitted by adults. Even when there is little or no spread by thrips vectors, introduction of TSWV to new areas is possible through short- or long-distance movement of infected seed material, in the absence of a stringent certification and quarantine schemes. Under practical circumstances, even rigorous certification schemes cannot guarantee 100% freedom from TSWV in seed stocks. This is because certification is based on foliar infections and it is not possible to test every tuber that is to be planted and low rates of virus infection in seed lots may be under detectable levels. Under these conditions, sampling error is much more important than test sensitivity and, therefore, a certain threshold (eg 1%) of tubers with the virus in seed stocks is permitted. And if efficient vectors are present and favourable conditions exist, spread of the virus and epidemics may occur. The significance of this component is revealed by the TSWV epidemic during 1945/1946 and 1946/1947 season when high rates of infections were reported throughout New South Wales and Victoria in crops grown from certified seed (Norris, 1951b, 1951b). Spread of the virus from infected propagating material to weeds may also occur and this widens the variety of hosts and, therefore, increases the possibility of overwintering (Dinnor 1974). Thus the initial level of inoculum in seed stock plays a crucial role in virus spread and epidemics.

Susceptibility of cultivars: Even where the initial virus source is limited or non-existent, the cultivar characteristics influence both vector and virus population dynamics and the incidence and severity of the disease. Once TSWV is established in commercial cultivars that are susceptible, spread within the crop depends on whether the cultivars can support breeding colonies. Further spread of the virus to epidemic proportions may occur, particularly if vector intensity and propensity, and sufficient inoculum exist within the environment (Duffus 1971; Thresh 1974; Irwin & Ruesink 1986). There is published evidence that differences exist in cultivar preferences by vector thrips (Herrin & Warnock, 2002; Maris *et al*., 2003a), and incidence of virus is less in TSWV-resistant cultivars than in susceptible ones (Maris *et al*., 2003b Culbreath *et al.* 1992; Camann *et al.* 1995; Yang *et al*., 2004).

Planting of host crops in succession and cropping intensity: Increased intensity of planting genetically homogeneous crop genotypes that are hosts to TSWV and its vectors and consequent decrease in mean separation between plantings provides a continuity of congenial host plant populations, which inevitably lead to epidemics. The mode of overseasoning of vector thrips and the sources for the recurrence of TSWV following unfavourable climatic conditions have been studied in different areas (Chatzivassiliou *et al.,* 2000a, 2001 Groves *et al.,* 2001, 2002; Jenser *et al.,* 2003). Where susceptible homogeneous plant populations are grown in succession, TSWV outbreaks are recurring and contemporary problems. Such epidemics had occurred in Hawaii on lettuce farms (Cho *et al.,* 1987a), potatoes and paprika in the Northern Cape and North-Western Provinces of South Africa during the summer seasons of 1997 and 1998 (Cloete, 2001). In many agroecosystems, the problem is exacerbated by an increased reliance on a limited number of cultivars such as Shepody and Russet Burbank in Australia, often grown in large, almost continuous monocultures. Shepody is susceptible to TSWV and epidemics in regions where it is grown have been reported (Wilson 2001). Genetically homogeneous crop genotypes that are hosts to TSWV may also provide suitable breeding ecologies for vector thrips and generation of larger amounts of inoculum for further spread of the virus.

Presence of weeds serving as vector thrips and virus reservoirs: Many plants have been documented as hosts for vector thrips, TSWV or both (Latham & Jones 1997; Peters, 1998; Chatzivassiliou *et al*., 2000a, 2000b, 2001; Groves *et al.,* 2001, 2002; Adkins & Rosskopf, 2002; Herrin & Warnock, 2002). In many reported outbreaks, weeds play a key role in determining epidemics of TSWV that were maintained in weeds (Cho *et al*., 1986; Stobbs *et al*., 1992; Hobbs *et al*., 1993; Kaminska & Korbin 1994; Johnson *et al*., 1995; Mertelík *et al.*, 1996; Latham & Jones 1997; Wilson 1998; Ochoa *et al*., 1999; Chatzivassiliou *et al*., 2000a, 2000b, 2001; Groves *et al.,* 2001, 2002). This occurs mainly through overwintering of the virus and vector thrips in weeds, which ensures survival in both space and time, thus constituting a constant potential danger. For example, in drier areas of Australia, many weed species that are hosts to vector thrips and TSWV persist along the banks of many creeks. These weeds are where vector thrips and TSWV survive, and from which infections of TSWV in nearby potato fields may be initiated. Overwintering hosts may also increase the amount of inoculum or genetically screen inoculum at an early stage in the epidemic through genome reasortment (Qiu *et al.,* 1998; Qiu & Moyer 1999) and selective transmission by vector thrips (Nagata *et al.,* 2000a; Naidu *et al.,* 2003; Sin *et al.,* 2003). The species composition, distribution and abundance of weed hosts influence the relative importance of vector thrips and TSWV (Groves *et al.,* 2001) and consequently epidemics. And TSWV hosts, which support vector thrips breeding, are considerably more important in epidemics than those that do not (Sakimura 1963a). Some work has also shown that breeding hosts can also serve as hibernating hosts for viruliferous thrips (Jenser 2003).

Plant age at infection*:* The age at which plants are infected with TSWV is often an important factor in determining the rate of spread of the virus. When TSWV infection occurs early in the cropping season, infected plants can act as sources for further spread (Thresh 1974). This may explain the greater spread of TSWV in susceptible crops that are infected soon after emergence compared with crops colonized later by vector thrips. Plant age at the time of TSWV infection also influences the translocation of the virus from the shoot to fruits and tubers. Plants tend to become decreasingly vulnerable to infection with age in tomato (Moriones *et al.,* 1998) and potato (Norris 1951a, 1951b; Wilson 2001) even though they come into more contact with neighbouring plants and present an increasing catchment area to thrips. As in aphid-transmitted viruses (Beemster 1976, 1987; Sigvald 1985), the older the plants at the time of TSWV inoculation, the less virus is translocated downwards to the tubers (Norris 1951a, 1951b; Wilson 2001); a process Beemster (1987) termed 'mature plant resistance'. Mature plant resistance to TSWV is also cultivar specific and virus translocation was more pronounced in the potato cultivars Atlantic and Shepody, but less evident in the cultivars Russet Burbank and Coliban (Wilson 2001). The risk of TSWV epidemics, therefore, depends on when and to what extent the plants develop mature plant resistance in relation to the age of the crop. If the migration of viruliferous vector thrips during the growing season is early, when the plants are young and still vulnerable, the risks of TSWV epidemics increase.

Thrips species, development and population dynamics: The rapid geographic spread of TSWV in many crop systems during the last decade has been attributed to the efficient vectoring capacity of *F. occidentalis* (German *et al.,* 1992; Wijkamp *et al.,* 1995; Goldbach & Peters 1996; Roselló *et al.,* 1996; Ullman *et al.,* 1997). However, specificity in transmission of the virus has also been identified, thus making other vector thrips more important in some regions (Wilson 1998, 2001; Groves *et al.,* 2001, 2002). The biological diversity, structure, growth and development of vector thrips (Lewis 1973, 1997; German *et al.,* 1992; Goldbach & Peters; Moritz 1997; Mound 1997) have profound effects on the initiation of epidemics. Chatzivassiliou *et al*., (2002) found that the efficiency by which *T. tabaci* populations transmit TSWV depends on their host preference and reproductive strategy. Arrehenotokous adult populations of *T. tabaci* collected on infected tobacco plants were more efficient TSWV transmitters and several generations survived well on this host than similar populations from leek. Field populations of most vector thrips are bisexual but females often predominate (Lewis 1973). Inter- and Intraspecific variations in vector thrips survival and performance (Wijkamp, 1995), vector sex (Wijkamp *et al.,* 1995; Van de Wetering *et al.,* 1998; Sakurai *et al.,* 1998, 2002), feeding behaviour (Harrewijn *et al*., 1996; Van de Wetering *et al*., 1998; Van de Wetering, 1999b; Sakurai *et al.,* 2002), and virus accumulation during different stages of development (Inoue *et al.,* 2002) in relation to transmission fitness on different hosts, all influence TSWV epidemics. Vector thrip population

dynamics as influenced by vector intensity (Irwin & Ruesink, 1986) usually determine if and when a crop will become initially infected. Hibernating viruliferous thrips have been shown to posses the capacity of transmitting TSWV (Jenser *et al*., 2003).

Diversity of TSWV strains and vector species: TSWV exists in nature as a highly heterogenous population with genetic competency for adaptation (De Avila *et al.,* 1990; Moyer *et al*., 2003a; Adkins 2003). Various strains of TSWV have been identified based on the kind and severity of symptoms they cause on potato and other indicator plants (Norris 1951a; Best & Gallus 1955), and pepper (Thomas-Carroll & Jones 2003) and on serological or molecular properties (Pappu *et al.,* 1996; Qiu *et al.,* 1998; Qiu & Moyer 1999; Aramburu & Martí 2003; Bucher *et al*., 2003). The existence of many vectors and TSWV strains that has a broad host range suggests that the virus is less constrained in its ecological success or epidemiological competency than by other environmental factors. In tobacco, specific genome reasortment by TSWV generates new variants to counterbalance losses in fitness, which enables the virus to easily overcome nearly all host resistance genes (Qiu *et al.,* 1998; Qiu & Moyer 1999), and consequently lead to epidemics (Moury *et al*., 1997; Roggero *et al*., 2002; Bucher *et al*., 2003). The new reasortants may possess selective advantages that may be further enhanced albeit inadvertently by cropping practices introduced to improve productivity. Conversely, genome reasortment may lower pathogenic fitness (Fraser, 1990), leading to radical recognition changes and loss of fitness in vector thrips transmission (Nagata *et al.,* 2000a;Naidu *et al.,* 2003; Sin *et al.,* 2003). This may explain the loss of fitness in vector thrips transmission by *T. tabaci*, in Western Europe (Jones 1959, Paliwal 1974, 1976; Wijkamp *et al.,* 1995; McPherson *et al.,* 1999; Chatzivassiliou *et al*., 1998a, 1999, 2001), and South America (Nagata *et al.,* 2002). The selective spread of different TSWV isolates and rate of transmission is facilitated and affected by the distinct transmission specificity between (Wijkamp *et al.,* 1995; Nagata *et al.,* 2002) and within vector species (Van de Wetering *et al.,* 1999; Sakurai *et al.,* 2002), and by quality of recipient and donor plants (Norris 1951a; Sakimura 1963b; Wijkamp *et al.,* 1995; Chatzivassiliou *et al*., 1999). As postulated several decades ago, there are biological differences between TSWV strains in such features as symptom expression in tomato (Norris 1946), potato and other hosts (Norris 1951a; Best & Gallus 1955). Based on these differences, a strain of TSWV was attributed to the epidemics of 1945/1946 and 1946/1947 seasons in potato crops throughout New South Wales and Victoria (Norris 1951a).

Location of production area. Geographical and topographical conditions influence the dispersal and host selection behaviour of thrips vectors (Lewis 1997b; Terry 1997), which inadvertently has a variable impact on the dispersal and incidence of TSWV. In Argentina, Williams *et al*., 2001 found that there were differences in the geographical distribution of tospoviruses, which was related to the dispersal of *F. schultzei,* a more efficient vector in some regions. In Hawaii Cho *et al.,* (1987) observed that TSWV disease incidence and thrips numbers were greatest at low elevation compared to areas at a higher elevation.

Climatic conditions: TSWV has been reported from a wide range of agro-ecological conditions and in very diverse cropping systems, varying in temperature, rainfall and length of growing season. These environmental conditions have marked effects on vector thrips populations (Andrewartha & Birch 1954; Smith 1961; Boissot *et al*., 1998; Kirk 1997) and species composition (Andrewartha & Birch 1954; Kirk 1997; Mound 1997), and consequently, big differences between cropping systems and regions in the epidemiology of TSWV. Climatic conditions strongly influence the distribution of thrips species and populations, with warm and moist conditions favouring thrips breeding and survival of host plants, which then act as inoculum sources (Lewis, 1997a). In Hawaii, epidemics were particularly devastating in the summer months when losses were reaching 50-90% (Cho *et al*., 1987; Yudin *et al*., 1990). Studies on Reunion Island (Boissot *et al*., 1998) established that although *F. occidentalis* was present all year round on the site of investigation population densities started to increase when temperatures reached 15^{o}C. Heavy rainfall favouring the abundance of the entomopathogen, *Orius* sp. was attributed to the decrease in populations. Temperature was also observed to influence the survival of both larvae and adult thrips with populations significantly getting reduced at colder temperatures and increasing at higher regimes (Bautista & Mau 1994). In regions with cold winters, the overwintering habits of vector thrips and the plants on which they hibernate play a key role in the epidemiology. Such phenomena has been reported by Jenser *et al*., (2003), who observed that *T. tabaci* was capable of transmitting TSWV after hibernating for up to six months during winter. Generally, any environmental stress that weakens plants makes them more susceptible to infestation by thrips. This was the observation made in Queesland, Australia, where unthrifty onions carried more *T. tabaci* than vigorous ones (Passlow, 1957).

Management of TSWV epidemics in Potato

Although research on TSWV has been going on in potato and many other vegetable and ornamental cropping systems in different parts of the world for many years, successful management of this virus has not been achieved in most cases. The lack of successful management options in potato is in part due to the limited knowledge available on the epidemiology of the disease in the crop. This is partly due to the fact that epidemics in potato are rare, particularly outside Australia, and they are sporadic in nature, a factor that complicates planning of control strategies. And adaptation of control strategies from other crop systems is limited by differences in vector-host relationships, cropping systems and environmental factors.

The successful and sustainable management of TSWV in potato with the current level of knowledge implies two distinct processes. Firstly, consolidating and furthering the current knowledge on the agro-ecosystem, the virus epidemiology and vector thrips ecology, and the environmental factors influencing these parameters. Secondly, defining the most appropriate strategy using results of the first diagnostic assessment stage to tailor prevention and/or control tactics in a multi-disciplinary effort. The management of TSWV and its vectors in potato should be directed toward the analysis of constraints in the system, and determination of which manipulations are firstly, adoptable and secondly, necessary to bring about changes which are in harmony with the environment and which will produce optimum and sustainable yields and quality. In this context, it can be anticipated that the cornerstones of the management strategies in potato will need to be multi-component in nature as suggested in other crops (Cho *et al.,* 1989,1998; Plasencia & Sánchez 1999) and based on a thorough understanding of the pathosystem involved in the diverse agro-ecological zones in which TSWV epidemics frequently occur. In tandem with this effort, it is worth emphasizing the use of resistant/tolerant potato varieties through selection and breeding for host plant and vector resistance, together with other integrated disease management options, which are of crucial importance in avoiding rapid virus re-infection in the field.

Selection and breeding for host plant resistance: The use of potato varieties with high levels of resistance or tolerance to TSWV presents the best option in view of the role played by vector thrips carrying the virus from external sources thereby rendering chemical control less effective. However, rational selection and breeding programs depend on a thorough knowledge of a targeted pathogen and epidemiology of the disease. Thus, the understanding of TSWV epidemiology is a critical first step to setting up effective breeding programs for the disease. Sources of resistance to TSWV in some potato cultivars have been identified (Hooker 1981; Wilson 2001). These sources of resistance to TSWV could be exploited by incorporating them into other potato cultivars. Resistance to TSWV is not immunity and seems to be overcome under certain conditions (Thompson & van Zijl 1996; Qiu *et al.,* 1998; Qiu & Moyer 1999; Roggero *et al.,* 2002; Aramburu & Martí 2003; Thomas-Carroll & Jones 2003), thus underlining the importance of selecting and breeding for both glasshouse and field resistance. Deployment of resistant potato genotypes will have to be accompanied by vector management schemes.

In addition to conventional potato breeding efforts (Kirkham *et al.,* 2001; Isenegger *et al.,* 2001; Dawson *et al.,* 2002; Williams *et al.,* 2003; Jansky & Rouse 2003), biotechnological approaches as those utilized for aphid-transmitted virus (Rodoni, 2003), should be considered for TSWV to incorporate pathogen-derived resistance. Constructs based on the coat protein gene of TSWV have been developed and used in other crops (Goldbach & Peters 1996; Cho *et al.,* 1998; Yang *et al.,* 2004) and a new strategy for engineering virus-resistant plants by transgenic expression of a dominant interfering peptide has been reported (Rudolph *et al.,* 2003). These achievements are very encouraging and it would be advantageous to advance from there to exploit a combination of virus resistance genes and genes for vector-resistance in order to broaden the genetic base of resistance to TSWV, and possibly other viruses infecting potato, and enhance its durability. However, progress in this area will have to be balance by the ongoing public debate about genetically modified crops in order to provide acceptable benefits to consumers in any new potato cultivars as highlighted by Eccles (2003).

Selection and breeding for vector resistance: The use of potato cultivars with resistance or tolerance to vector thrips could be expected to reduce both damage to the crop through feeding and consequential transmission of TSWV. Plants that are not reproductive hosts of vector thrips, even though hosts to TSWV, are not epidemiologically important because the virus must be acquired by immobile larvae to be transmissible by adult thrips (Sakimura 1963a; Bautista & Mau 1994). Resistance to thrips feeding has been has been observed in pepper (Maris *et al.* 2003a, 2003b), impatiens germplasm (Herrin & Warnock 2002) and potato (Jericho & Wilson 2003), suggesting that genetic resistance to thrips feeding may be improved through selection and breeding. But whether resistance to thrips in potato can be overcome under certain conditions is currently unknown and requires investigation. Screening for host-plant resistance and subsequent breeding programs to introduce genetic resistance to vector thrips, should also aim at maintaining other desirable qualities for yield, processing quality and appearance in order to be of any benefit to potato growers, processors and consumers.

Integrated disease management: As observed by many other researchers (Cho *et al.,* 1989,1998; Ullman *et al.,* 1997; Plasencia & Sánchez 1999) the nature of TSWV epidemics, which involves the interaction of variable factors, makes it difficult to control by means of a single strategy. This makes the use of a integrated disease management strategy, which advocates a holistic approach to prevent TSWV infections from occurring in the first place or solving the problem in the framework of the agro-ecosystem in question more appropriate. In this strategy, the use of resistant potato cultivars is likely to be the key element of TSWV management, but there is an urgent need to clarify the role of phytosanitation (rouging), set up thresholds and monitoring of incoming viruliferous thrips (Aramburu *et al.,* 1997), decoy crops, border crops, planting time, insecticides, weed control and a reliable early warning system. Further progress in sustainable TSWV management in potato will require a better and more basic understanding of the agro-ecosystem and the biotic, abiotic and socio-economic factors influencing it.

In consideration of the existence of more than one vector thrips species involved in epidemics and evolution of TSWV strains infecting potato than those currently known, there is an urgent need to develop appropriate conceptual framework for the TSWV pathosystem in potato like those proposed for other crops by Latham & Jones (1996) and in peanuts in the USA (Culbreath *et al*., 2003). These would include the selection

(short term), breeding (medium to long term) and then sequential and systematic deployment of resistant potato genotypes to counter any possible progressive degeneration of released potato cultivars in the field due to evolution of resistance-breaking TSWV strains, in line with market needs and preferences. These measures will have to be in tandem with vector management schemes involving the use of other appropriate components of the integrated disease management strategy such as phytosanitation (rouging), decoy crops, border crops, planting time, insecticides, weed control and a reliable early warning system. Such measures will have to be designed for easy adoption, sustainability and economic efficiency in each of the agrosystems involved. One argument is that the use of certified potato seed stocks, selection and rouging are unnecessary if the cultivars grown are sufficiently resistant to infection. The alternative view is that such measures complement the use of resistant cultivars and render them even more effective. The paradox is that phytosanitation is only feasible when resistant potato cultivars are used and yet phytosanitation may be unnecessary if such cultivars are used. It is probable that only through this multi-component approach long proposed by others in many other crops (Cho *et al.,* 1989, 1998; Plasencia & Sánchez 1999), such as the use of host-plant resistance, increasing farmers' awareness of TSWV and benefits of phytosanitation, testing of tuber seed stocks, strict quarantine and germplasm exchange, will ecologically sustainable and economically sound plant protection approaches be developed for the successful management of TSWV across the whole of the diverse range of agro-ecological environments in which potatoes are grown.

The above strategies should be based on knowledge of TSWV/potato cultivars/grower interrelationships contrary to many proposed interventions found in literature, which although very promising, remain mostly theoretical in view of the need for effective implementation in the field. The approaches are in contrast to crop protection, where the word 'protection' already implies an intervention -oriented control strategies, often narrowed down to the application of pesticides.

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Chapter 2

QUANTIFYING COMPONENTS CONTRIBUTING TO TOMATO SPOTTED WILT VIRUS EPIDEMICS ON POTATO CROPS IN AUSTRALIA AND DEVELOPMENT OF RISK ASSESSMENT MODELS

Close to 100 years after the discovery and description of tomato spotted wilt virus (TSWV) on tomato in the southern state of Victoria, Australia (Brittlebank, 1919), and notwithstanding many concerted efforts to understand the factors associated with TSWV epidemics (Pittman, 1927; Samuel *et al.*, 1930; Bald & Samuel, 1931; Magee, 1936; Bald, 1937; Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b; Helms *et al.*, 1961; Smith 1961; Hill & Moran,1996 Latham & Jones, 1996, 1997; Clift *et al.*, 1999; Wilson, 1998, 2001; Clift & Tesoriero, 2002; Medhurst *et al.,* 1993-2003; Herron & Cook, 2002) and improve disease management (Jones 2004), TSWV is still difficult to control in many crop systems. Despite the availability of informative reports of the disease on potato for many decades (Magee 1936; Bald, 1937; Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b; Wilson, 2001; Jones 2004), it has been difficult, over the years, to determine the underlying ecological and epidemiological factors regulating either the initiation, perpetuation or increase of the amount of inoculum and thus intensifying epidemics. Part of the problem stems from the sporadic nature of the disease, which makes it hard to find a consistent data set for analysis, at the desired level of aggregation, both for disease incidence and intensity as the variables to be explained and for explanatory variables (Getz & Pickering, 1983; Kranz, 1990).

A plethora of data on climate and weather are available from the records of the Australian Bureau of Meteorology dating back to the earliest years of European settlement in Australia. However, datasets of agronomic, vector thrips and other relevant variables are patchy, and in many cases, unavailable. This is especially true for the human factor, which is often overlooked in the study of plant disease epidemics (Zadoks & Schein, 1979). For example, crop management affecting the host (e.g. choice of cultivar, application of fertilizers); the environment (crop microclimate, control measures); and the pathogen (virus strain reasortment due to selection pressure; use of virus-free seed tubers, negligence in refuse piles elimination). The many hosts of TSWV and vector thrips, virus strains and environment in the disease tetragon are unknown. In many of the sporadic TSWV epidemics, the source of inoculum has been the first question to confront; Is the virus originating from propagation of infected seed tuber stocks (Norris & Bald 1943; Conroy *et al*., 1949; Norris 1951b; Shepherd 1972)? Is it from regenerated infected plants from previous crops (Norris & Bald 1943)? Or hibernating viruliferous thrips (Groves *et al*., 2001; Jenser *et al*., 2003)? Is it from distant or nearby infected horticultural crops or from annual or perennial weeds (Norris & Bald 1943; Duffus, 1971; Thresh, 1974; Groves *et al*., 2002)? In such situations, the difficulty for implementing any disease control strategy has been compounded even further by the existence of more than one known vector species in some parts of Australia (Malipatil *et al*., 1993; Latham & Jones, 1996, 1997; Mound, 1996; 2004; Wilson 2001; Austin *et al*., 2004). Early outbreaks of TSWV in potatoes and tomatoes in South Australia, New South Wales and Victoria, were attributed to *T. tabaci* and *F. schultzei* (Pittman 1927; Samuel *et al.*, 1930; Magee, 1936; Norris & Bald 1943; Conroy *et al.*, 1949; Norris 1951a & 1951b). Decades later, in 1993, the polyphagous Western flower thrips *F. occidentalis* (Pergande) was recorded for the first time in both Western and Eastern Australia (Malipatil *et al.*, 1993). As in many other parts of the world, the marked introduction and subsequent spread of *F. occidentalis* was thought to be a factor in exacerbating the TSWV epidemics, given the propensity and transmission efficiency of this vector in other crops (Ullman *et al.,* 1997). During the past decade, some of the vector thrips species have been implicated in TSWV epidemics in the country. In the Metropolitan regions of Perth in Western Australia, sporadic infections in potato ware crops were associated with *F. occidentalis* (Latham & Jones, 1997). In the southwestern part of Western Australia *T. tabaci* and *F. schutzei* were also recorded in the potato growing areas (Thomas & Jones 2000). The melon thrips, *Thrips palmi* Karney has also been recorded in New South Wales and Queensland (Malipatil *et al*., 1993; Mound, 1996; 2004; Austin *et al*., 2004). Consequently, using resistance to a single vector as a control strategy may not provide a full solution to compound vector species. TSWV control might become even more difficult under some scenarios of climate change, such as decreased precipitation. This was recognized even during the 1940s, with observations that although TSWV outbreaks were largely sporadic and unpredictable, increased prevalence and severity of the disease was coinciding with summer seasons with less precipitation (Conroy *et al*., 1949). The increased frequency of TSWV epidemics during the last decade (Wilson 2001; Clift & Tesoriero, 2002; Medhurst *et al.,* 1993-2003), when there has been droughts in much of the potato growing areas (Lindesay, 2003), appears to confirm this hypothesis.

Two models for the management of TSWV in lettuce (Yudin *et al.,* 1990) and peanuts (Culbreath *et al*., 1992, 2003) have been developed in the USA. The model for TSWV in lettuce is based on conditional probability and linear regression and is devised for predicting TSWV incidence at harvest as a function of early disease incidence and cumulative vector thrips abundance. However, this model has not been adopted and used widely elsewhere. Part of the reason could be due to the absence of local data to use in such a model. The model for the disease in peanuts uses risk assessments, which incorporate data from individual risk factors to formulate recommendations for management of TSWV. The models provide prediction of disease potential and are useful from the perspective of awareness and tactical management of disease in individual fields in areas where all growers in the neighbourhood comply and use the tool. However, their usefulness for strategic management in whole localities is limited in the absence of total compliance in the use of the tool, which would allow for pockets of reservoirs of both vector thrips and TSWV to remain in the environment, posing a potential danger for epidemics. A better understanding and use of an area-wide approach would likely be more effective.

Studies of TSWV disease as well as relevant agronomic variables from representative sites over sufficient multiple seasons (Duffus, 1971; Kranz, 1974, 1990; Thresh, 1974; Barnett 1986; Plumb & Thresh, 1983; Gray & Banerjee, 1999) can help to infer key factors which trigger outbreaks or perpetuates the pathogen between seasons. The results of such studies involving short-term surveys could place the results of long-term studies in context and provide information, which can deepen insights into the ecology and epidemiology of the virus and its vector thrips and how they interact into functional complexes, which cause epidemics in potato crops and thus help to explain the sporadic nature of the epidemics (Kranz, 1990). Such understanding would assist in the evaluation, refinement and submission of adjusted national seed certification guidelines appropriate to and reflecting real risks of TSWV in potatoes and, therefore, facilitate the development of risk assessment models (Nutter, 1997; Madden, 1990; Madden and Campbell, 1986; Kranz, 1988, 1990; Madden and Hughes, 1995). And because of the wide host range of both vector thrips and TSWV (Peters 1998) and the sporadic nature of epidemics in potato, such risk assessment models should respond to the formulation of recommendations for management that do not focus on individual fields but rather consider the dynamics of the disease in a whole locality. Consequently, the formulation of such a predictive model should respond to the postulated flexibility in parameter fixation for the computation of interactions between management variables with a minimum number of parameters, all of which should have a clear biological interpretation in different potato growing areas in Australia. This kind of analysis allows consideration and inclusion of all factors likely to have an important impact on disease dynamics within the entire locality under the influence of similar parameters (intensity of cropping, cultural practices used, cultivars grown, rate of crop turn-over, meteorological and other environmental factors, virulence of prevailing virus strains, vector thrips population dynamics). Success in such an endeavor would ultimately, lead to the development of an early warning system for potato growers, processors and farm advisers, and consequently, better decisions regarding cropping patterns and sequences and effective management of the virus and its vectors in space and time (Thresh, 1974).

As part of a continuing effort to understand the epidemiological features of the TSWV pathosystem in potato, baseline surveys were conducted to establish the role of each of the perceived components of the epidemics. The objective of this study was to obtain quantitative and qualitative information on:

(a) the sources of TSWV; with emphasis on seed health and weeds
(b) key vector thrips species associated with TSWV epidemics in potatoes
(c) the population dynamics of vector thrips within potato crops as influenced by cultivar and weather parameters?
(d) virus levels and patterns of infection in crops over time
(e) potato cultivar assessment for TSWV infection and translocation.

While data was collected on all the above five focus areas during the surveys, the last one received further detailed investigations in a series of glasshouse and field experiments. Results from these investigations are reported separately in subsequent chapters.

Materials and Methods

Study areas and sampling periods

Most of the potato grown in Australia comes from the southern, south- eastern and western states of the country (Anonymous, 2003). Since TSWV causes sporadic epidemics in potato crops in Australia, areas with a known history of the disease were selected. The baseline surveys were conducted from June 2001 through March 2004 in commercial potato fields in south-eastern part of South Australia (near Penola), South-west Victoria (Ballarat), Tasmania and the western part of New South Wales (Berrigan)(Figure 1).

During the 2001/2002 season, four potato ware crops were surveyed in South Australia, two ware crops in Victoria, and 41 seed crops in Tasmania. For the 2002/2003 season, five ware crops were surveyed in South Australia, 12 crops, a mixture of seed and ware crops in Victoria, 41 seed crops in Tasmania and one ware crop in New South Wales. The 2003/2004 survey was a much scaled down one. All crops were grown from virus-free certified potato seed stocks. For the purpose of the survey, the sampling periods were defined as indicated in Table 2.1

TSWV monitoring, sample collection and identification

Unrestricted random sampling of plant materials (Madden & Campbell, 1986; Barnett 1986; Hughes *et al*., 1996; Nutter, 1997) was carried out during winter, two weeks before planting, middle and end of the growing season. During the winter period, only weeds were sampled. Two weeks prior to planting, weeds and seed tubers were sampled and tested. In mid-season, weeds and potato leaf samples were tested. At the end of the season, potato tubers and weeds were sampled for testing. Potato seed tubers and leaves, leaf and flower samples of weeds and native flora growing within the cropped and 10m area surrounding the fields were collected at random while walking in a "Z" pattern across the field. These were then placed in zip-lock plastic bags and sent to the laboratory for virus testing. This sampling procedure eliminated the possibility of leaving out TSWV-infected symptomless weeds (Chatzivassiliou *et al*., 2001). Before testing, potato cultivars were specified and the weeds identified to species using keys by Hyde-Wyatt *et al*., (1975), Auld and Medd, (1987) and Wilding *et al*., (1998).

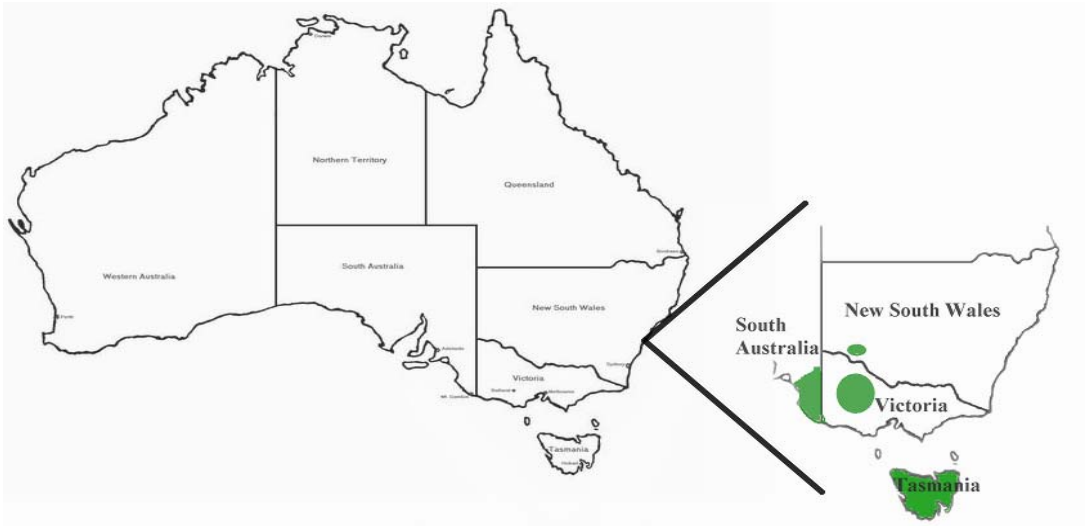


Fig 2.1. Map of Australia showing the location of sampling sites during the surveys from 2001 to 2004.

Table 2.1 Sampling periods during surveys of commercial potato fields (2001 –2003)

State	Sampling Period			
	Winter	Before Planting	Mid-season	End of Season
Tasmania	1 June – 30 August	1 September – 15 November	16 November – 15 January	16 January – 31 March
Victoria	1 June – 30 August	1 September – 15 November	16 November – 15 January	16 January – 31 March
South Australia	1 June – 30 August	1 September – 30 October	1 November – 15 January	16 January – 31 March
New South Wales	1 June – 30 August	1 September – 30 October	1 November – 15 January	16 January – 31 March

TSWV testing in potato, weeds and native flora

Virus diagnosis was done using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) protocol (Clark & Adams, 1977) with antisera to TSWV lettuce strain (Agdia, IN, USA) at a monoclonal antibody dilution ratio of 1:400 for both capture and detection. Extracts were prepared by crushing ~0.1g (w/v) leaf samples in phosphate buffered saline (pH 7.4) containing 2ml litre^{-1} Tween 20 and 20g litre^{-1} polyvinyl pyrrolidone using a leaf press. Expressed sap (100μl) was tested for TSWV in duplicate wells of a flat bottom microtitre plate (NUNC, maxisorb) in a crisscross plate layout. Bulk samples of the same species were tested as compound samples containing 10 leaves per well. Positive compound samples were retested to obtain actual incidence values. Positive and negative controls were included on each plate. Absorbances (A_{405}nm) were measured with a photometer (Titertek) 30 and 45 minutes after addition of 0.5mg ml^{-1} p-nitrophenyl phosphate in 10 ml litre^{-1} diethanolamine, pH 9.6 as substrate. The absorbance values were corrected for blank values consisting of only extraction buffer in the sample incubation step. Samples were considered positive if they had absorbencies greater than three standard deviations of the mean of the negative controls (Sutula *et al*., 1986). Percentage infection of composite samples was calculated using binomial theorem (Gibbs & Gower, 1960). The confirmation of the presence of the virus in the positive samples was done by mechanically inoculating sap from these samples onto tomato (*Lycopersicon esculentum* Mill. 'Grosse Lisse') and *Datura stramonium* L. plants and then observe the typical necrotic leaf symptoms of TSWV. The plants were maintained in the glasshouse (18–20^{o}C and 45-55% relative humidity) and symptoms confirmed after 3 weeks.

Thrips monitoring

Thrips trapping was done concurrently with monitoring of TSWV in potato, weeds and native flora using five 20 X 25cm yellow sticky trapps (Seabright Laboratories, Emeryville, CA 94608, USA) suspended just above the plant canopy and continuously adjusted with plant growth. Five traps were place around each field in a "Z" pattern, with four traps placed evenly around and close to the edges of the crop and one in the center to allow for directional monitoring of incoming thrips. The traps were changed weekly, and in some cases daily, from June 2001 to March 2003, and the caught thrips were counted and identified under the microscope to species.

Weather data collection

Weather data that included daily maximum temperature (°C), minimum temperature (°C), terrestrial minimum (°C), maximum wind gust (km/h), maximum gust direction, evaporation (mm), bright sunshine (hours), precipitation (mm), temperature at 9am (°C), relative humidity at 9am (%), cloud cover at 9am (oktas), wind speed at 9am (km/h), wind

direction at 9am, temperature at 3pm (°C), relative humidity at 3pm (%), cloud cover at 3pm (oktas), wind speed at 3pm (km/h) and wind direction at 3pm was obtained from the Australian Bureau of Meteorology.

Variable selection and development of quantitative models

To determine the most relevant variables for inclusion in the models from the epidemiological point of view, all variables were plotted and correlated with the response variables to investigate the data structure. No single weather variable was highly correlated with disease incidences in either potato crops or weeds. Weather variables which included daily maximum temperature (°C), minimum temperature (°C), precipitation (mm) and relative humidity at 3pm (%), correlated with thrips populations. Given that the "contact" between healthy potato plants and infected weeds is by means of a thrips vector population, flights by viruliferous thrips and the factors that influence such flights, determines if and when external inoculum will become available for infection, and consequently the initiation, sustenance and spread of an epidemic. Currently, no data are available that describe the relationship(s) between population dynamics of known vector thrips species and weather variables. Specifically, the optimum weather conditions that favor thrips flights and the extreme conditions or bounds that limit these flights are not known. Therefore, the objective was to quantitatively describe thrips population dynamics in Tasmania, Victoria and South Australia over the range of weather parameters that had shown correlations with vector thrips populations in exploratory analyses.

The relevant number of weather variables for inclusion in the models was then selected using the following procedure. Daily weather variables were reduced to fortnightly and grouped (i.e weather readings taken at 9am were put in one group and those recorded at 3pm were put in another group). These were correlated in pairs and only one variable of each highly correlated subgroup was taken for further analysis.

Data collation, statistical analysis, and parameter estimates for the relationship between population dynamics of known vector thrips species and weather variables

The relationships between the selected weather variables and the response variable (thrips populations) were determined using regression analyses. For the selection of the best regression models, the threshold criteria for a model plant disease was taken into consideration (Jeger & van den Bosch 1994; Kranz, 1988, 1990). For the selection of the best linear regression model, two variable selection techniques called "Cp statistic" and Schwartz's Bayesian information criterion (BIC) were used (Ramsey & Schafer 1997; Mila & Carriquiry 2004). These techniques assign the lowest value to those regression models that best explain the response variable with the least number of independent variables. The Multiple linear regression model which had the lower Cp and BIC statistic values was selected for analysis. To describe the non-linear (asymmetric) relationships between the selected weather variables and the thrips populations, three curves often associated with the growth of biological subjects and populations (Madden and Campbell, 1986; Kranz, 1974, 1988, 1990; Madden, 1990; Madden and Hughes, 1995; Nutter, 1997; Segarra *et al*., 2001) were used. These included the Gompertz, Logistic and Exponential curves.

Multiple linear regression analysis : Variables were screened for fulfillment of the regression assumptions (Neter *et al*.,1990; Ramsey & Schafer 1997; Garret *et al*., 2004). Correlation coefficients were calculated to check for high correlation between any two variables. Normality assumption of all the variables together was tested using a principle component analysis and analyzing histograms of the principal components. Variables (including the response variable) showing departures from normality were transformed to logarithmic scale ($\log_{10}$). Exploratory regression analyses were carried out and scatterplots of the residuals were constructed to detect possible non-linearity and non-constant variance. All these assumptions were met by the variables selected. Data was then fitted to the multiple linear regression equation:

$Y = A + B_1X_1 + B_2X_2 + \ldots + B_nX_n$

Where, Y, was the expected thrips population, A, as a constant, X_1 as the mean daily maximum air temperature (°C), X_2, as the mean daily minimum air temperature (°C), X_3, as the mean daily precipitation (mm), X_4 as the mean daily relative humidity at 3pm (%), and $B_{1\ldots n}$ as coefficients of $X_{1\ldots n}$.

Non-Linear Regression analysis : Data was fitted to the following equations to explore the non-linear relationships between the selected weather variables and the thrips populations.

1. Gompertz curve $Y = A + Ce^{-e^{-B(X-M)}}$

2. Logistic curve: $Y = A + C/(1 + e^{-B(X-M)})$

3. Exponential curve: $Y = A + BR^X$

Where, Y, was the expected thrips population, A, as a constant.

The Goodness-of-fit of model equations was evaluated by the examination of the percentage variance (r^2), *F* test, variance ratios and *t* probabilities of the parameter estimates. To make both regression results comparable, variables transformed to logarithmic scale ($\log_{10}$) were kept as such also for both linear and non-linear analyses. A probability level (under null hypothesis) of 0.05 was used for all tests to determine significance.

Validation of Models

A statistical test by Freese (Freese 1960), was used on the 2003/2004 data previously set aside to judge the predictive capability of the model and to compare the models. Freese's X^2 helps to determine whether a model achieves a certain degree of accuracy when a validating data set is used. Accuracy is chosen a priori and depends on the maximum

tolerated departure from the observed values, and the desired probability level. In this case, a maximum departure of 5 thrips and a 0.05 probability level were chosen. All data was collated using MS-Excel (Microsoft Corporation) and then analyzed using Genstat software ver 7.2.0.208 (Lawes Agricultural Trust, 2002).

Mathematical models defining regional response of dependent thrips populations to independent interacting weather effects were developed. Such basic descriptive information on the weather factors influencing thrips flights will be useful for understanding the epidemiology of TSWV in potato crops, particularly with respect to primary inoculum from external sources during the growing season.

Results

Quantifying sources of TSWV

Data on TSWV incidences across seasons (2001/2002 and 2002/2003) and sampling times (before planting, mid-season and at the end of the season) in Tasmania, Victoria, South Australia and New South Wales are given for weeds in Table 2.2 & 2.3 and potato in Table 2.4. Between the two seasons and sampling times, the incidence of TSWV in weeds and potato varied considerably. A total of 4038 weed samples from 82 species in 27 families were collected and assayed for TSWV; 1824 samples belonging to 50 species from 18 families in South Australia, 342 samples belonging to 15 species from 11 families in Victoria, 1829 samples belonging to 48 species from 20 families in Tasmania, and 43 samples belonging to 14 species from 11 families in New South Wales (Table 2.2). Eleven plant species from seven families were found positive for TSWV (Table 2.3). The highest number (8) of weed species that were positive for TSWV was found in South Australia. Most of the plants susceptible to TSWV were found in the Asteraceae and Solanaceae families.

In Victoria, no infections were observed in weed and potato samples during 2001/2002 season and none before planting and in mid-season in 2002/2003. A large migration of vector thrips (*T. tabaci* and *F. schultzei*) in mid- to end of season during 2002/2003 caused infections that were observed in both potato and weeds at the end of the season (Tables 2.2, 2.4 & 2.5). In South Australia, the 50% prevalence of TSWV observed in potato before planting during the 2001/2002 season was attributed to infected Kennebec and Shepody seed tubers. The virus was also detected in the weeds Marshmallow (*Malva parviflora,* Malvaceae) and Hare's foot clover (*Trifolium arvense,* Fabaceae). No infection was detected in Shepody and Russet Burbank before planting or in weeds in mid-season (Tables 2.3 & 2.4). However, the influx of vector thrips into potato crops in mid-season during 2001/2002 season, caused heavy infections (14.3% - 47.4%) depending on the potato cultivar. The potato crops were harvested before TSWV incidence could be determined at the end of the season, but the number of weeds that remained within and adjacent to the potato fields had the virus detected in them. In Fat Hen (*Chenopodium album,* Chenopodiaceae), 33.3 % of the samples tested had TSWV, 25% of Wild melon/Bitter melon (*Citrullus lanatus,* Cucurbitaceae), 33.3% of Wire weed (*Polygonum avicutare,* Polygonaceae), 4.8% of Blackberry nightshade (*Solanum nigrum,* Polygonaceae) and 33.3% of Common sowthistle (*Sonchus oleraceus,* Asteraceae) (Table 2.3). In Tasmania, there was generally low virus incidence in potato crops (0.3 –1.3%) during 2001/2002, which remained constant into the mid season. Virus incidences were not recorded at the end of the season as the crops got harvested before it could be done. No virus was detected in all weed samples tested.

During 2002/2003 season, TSWV was not detected across all sampling sites in both potato and weeds before planting (Table 2.2 & 2.4). Incidences of the virus were also low or absent in potato crops in mid-season except in New South Wales where there was a sporadic outbreak at one property with virus incidences in samples of some potato cultivars reaching 100%. A few but high incidences of TSWV were also recorded in weeds during mid-season in South Australia (*Malva parviflora* – 24%) and New South Wales (Treeflower nightshade *Solanum triflorum* – 77.3%, Salvation Jane/Paterson's curse *(Echium plantagineum/ E. lycopsis* –34%). In Tasmania the incidence was low with only 1.4% recorded in *Chenopodium album* during the mid-season. However the incidences of the virus in both potato and weeds at the end of the season changed with sporadic outbreaks of TSWV occurring in Victoria, South Australia and New South Wales. In Victoria incidences of the virus were 1.4% in cv. Shepody to 72% in generation 2 of Riverina Russet, 30.4% in Cape weed (*Arctotheca calendula*, Asteraceae) and 17.9% in *S. nigrum.* In South Australia, incidences of the virus in potato ranged from 20% in cv. Russet Burbank to 83.3% in cv. McCains 1, and in weeds from 12.5% in *S. nigrum*, 25% in *C. album* to 100% in Clammy goosefoot (*Chenopodium pumilio*). The incidence of TSWV in potato was low in Tasmania with only 1.4% detected in cv. Russet Burbank from one property in the Derwent Valley, but 28.6% of the weed *A. calendula*, was found with the virus (Table 2.2 & 2.4). Most TSWV infections in a number of weeds species were detected at the end of the season (Fig.2.5), indicating that weeds within and 10m around the potato fields were as much victims of infection as potato crops. Inoculum source(s) in such circumstances is thought to have been external to the sampled areas. There is also strong evidence to suggest that dispersing macropterous adult thrips were making short distance dispersal flights away from the maturing, overwintering wild plant species occurring along field margins or in non-cultivated areas near crops (Figure.2.2)

Table 2.2 List of weeds tested for TSWV within and adjacent to potato field crops during 2001/2002 and 2002/2003 season in Australia

Region[1]	Family	Species and Common Name[2]	Region[1]	Family	Species and Common Name[2]
TAS	Asteraceae	*Hypochoeris radicata (flatweed dandelion)*	**SA**	Amaranthaceae	*Gomphrena celosioides* (Gomphrena)
		Leontodon taraxacoides (hairy hawkbit)		Asteraceae	*Arctotheca calendula* (Cape weed)
		Picnomon acarna (Soldier thistle)			*Hypochoeris radicata* (Flatweed dandelion)
		Silybum marianum (Variagated thistle)			*Leontodon taraxacoides* (hairy hawkbit)
		Arctotheca calendula (Cape weed)			*Sonchus asper* (Prickly sowthistle)
		Carduus tenuiflorus (Slender thistle)			*Sonchus oleraceus* (Common sowthistle)
		Cirsium vulgare (Spear thistle)			*Lactuca serriola* (Prickly Lettuce)
		Hypochoeris radicata (Catsear/dandelion)		Boraginaceae	*Heliotropium europaeum* (Heliotroph)
		Leontodon taraxacoides (hairy hawkbit)		Brassicaceae	*Raphanus raphanistrum* (Wild radish)
		Sonchus asper (Prickly sowthistle)			*Sisymbrium irio* (London rocket)
		Sonchus oleraceus (Milk thistle)			*Brassica tournefortii* (Wild turnip)
		Taraxacum officinale (Dandelion)		Chenopodiaceae	*Chenopodium album* (Fat hen)
	Boraginaceae	*Borago officinalis* (Borage)			*Chenopodium pumilio* (Clammy goosefoot)
	Brassicaceae	*Hirschfeldia incana* (Buchan weed/Hairy brassica)		Cruciferae	*Sisymbrium orientale* (Indian Hedge Mustard)
		Raphanus raphanistrum (Wild radish)		Cucurbitaceae	*Citrullus lanatus* (Wild melon/Bitter melon)
		Sisymbrium irio (London rocket)		Dennstaedtiaceae	*Pteridium esculentum* (Bracken fern)
		Raphanus raphanistrum (Wild radish)		Geraniaceae	*Erodium cicutarium* (Common crowfoot)
	Caryophyllaceae	*Cerstium glomeratum* (Mouse ear/Chickweed)			*Geranium dissectum* (Cutleaf cranesbill)
	Chenopodiaceae	*Chenopodium album* (Fat hen)		Fabaceae	*Vicia hirsuta* (Hairy Tare)
	Convolvulaceae	*Ipomoea cairo* (Morning glory)			*Vicia sativa* L.spp *sativa* (Common Vetch)
	Dennstaedtiaceae	*Pteridium esculentum* (Bracken fern)			*Melilotus indica* (Sweet melilot)
	Geraniaceae	*Erodium botrys* (Long storksbill)			*Swainsona swainsonoides* (Downy swainsona)
		Erodium cicutarium (Common crowfoot)			*Medicago polymorpha* (Burr medic)
		Erodium moschatum (musky crowfoot)			*Medicago sativa* (Lucerne/Alfalfa)
	Fabaceae	*Medicago minima* (Woolly burr medic)			*Trifolium arvense* (Hare's foot clover)
		Medicago sativa (Lucerne)			*Trifolium glomeratum* (Cluster clover)
		Pisum sativum(Garden pea)			*Trifolium repens* (White clover)
		Trifolium arvense (Hare's foot clover)			*Trifolium subterraneum* (subterraneum clover)
		Trifolium glomeratum (cluster clover)			*Trifolium tomentosum* (wooly clover)
		Trifolium repens (White clover)		Lythraceae	*Lythrum hyssopifolia* (Hyssop loosestrife)
	Solanaceae	*Lycopersicon esculentum* (Tomato)		Malvaceae	*Malva parviflora* (Marshmallow)
		Solanum nigrum (Blackberry nightshade)		Onagraceae	*Oenothera stricta* (Common evening primrose)
	Urticaceae	*Urtica urens* (Small nettle)			*Oenothere indecora* ssp *bonariensis* (Evening Prirose)
		Trifolium subterraneum (subterraneum clover)		Papaveraceae	*Papaver hybridum* (Rough poppy/Wild poppy
	Malvaceae	*Malva parviflora* (Marshmallow)		Poaceae	*Avena fatua* (Wild oat)
	Onagraceae	*Epilobium billardierlanum* (Willow herb)			*Holcus lanatus* (Common velvetgrass)
	Oxalidaceae	*Oxalis pes-caprae* (Soursob)			*Hordeum leporinum* (Barley Grass)
	Papaveraceae	*Papaver somniferum* (Poppy)			*Lolium rigidum* (Wimmera rye grass/Annual rye grass)
	Plantaginaceae	*Plantago lanceolata* (Ribwort)			*Phalaris aquatica* (Phalaris Toowoomba canary grass)
	Poaceae	*Hordeum leporinum (barley Grass)*			*Phalaris paradoxa* (Phalaris)
	Polygonaceae	*Polygonum arenastrum* (Wire weed)			*Poa annua* (Winter grass, Annual poa)
		Polygonum avicutare (Wire weed)			*Bromus diandrus* (Brome grass)
		Rumex acetosella (Sorrel)			*Dactylis glomerata* (Orchardgrass Cocks Foot)
		Rumex crispus (Curled Dock)			*Panicum maximum* var *maximum* (Guinea grass)
TAS cont.	Primulaceae	*Anagalis arvensis* (pimpernel)	**SA cont.**	Polygonaceae	*Polygonum arenastrum* (Wire weed)
	Rosaceae	*Crataegus monogyna* (Hawthorn)			*Polygonum avicutare* (Wire weed)

		Rubus fruticosus (Blackberries brambles)			*Rumex acetosella* (Sorrel)
		Rubus parvifolius (Blackberries brambles)			*Rumex crispus* (Curled Dock)
				Rosaceae	*Rubus fruticosus* (Black berries)
VIC	Asteraceae	*Arctotheca calendula* (Cape weed)		Solanaceae	*Solanum nigrum* (Blackberry nightshade)
		Onopordium acanthium (Scotch Thistle)			
	Brassicaceae	*Raphanus raphanistrum* (Wild radish)	**NSW**	Asteraceae	*Arctotheca calendula* (Cape weed)
	Chenopodiaceae	*Chenopodium album* (Fat hen)			*Hypochoeris radicata* (Flatweed dandelion/Catsear)
	Gramineae	Zea mays (Maize)		Boraginaceae	*Heliotropium amplexicaule* (Blue heliotrope)
	Fabaceae	*Medicago sativa* (Lucerne)			*Heliotropium europaeum* (common heliotrope)
		Trifolium repens (White clover)		Brassicaceae	*Rapistrum rugosum* (Turnip weed/Wild turnip)
	Malvaceae	*Malva parviflora* (Marshmallow)		Chenopodiaceae	*Chenopodim album* (Fat Hen)
	Papilionaceae	*Ulex europaeus* (Gorse)		Cucurbitaceae	*Citrullus lanatus* (Wild Melon/Bitter melon)
	Poaceae	*Avena fatua* (Wild oat)		Gramineae	*Triticum sati*vum (Wheat)
		Phalaris paradoxa (Paradoxa grass/Canary grass)		Malvaceae	*Malva parviflora* (Marshmallow)
	Polygonaceae	*Polygonum avicutare* (Wire weed)		Plantaginaceae	*Plantago lanceolata* (Ribwort/Common plantain)
		Rumex acetosella (Sorrel)		Poaceae	*Poa annua* (Winter grass/Annual poa)
	Rosaceae	*Crataegus monogyna* (Hawthorne Hedge)		Polygonaceae	*Polygonum avicutare* (Wire weed)
	Solanaceae	*Solanum nigrum* (Blackberry nightshade)		Solanaceae	*Solanum nigrum* (Blackberry nightshade)
				Solanaceae	*Solanum triflorum* (Treeflower nightshade)
					Echium plantagineum/ E. lycopsis (Salvation Jane/Paterson's curse)

[2] Source of Common names is Hyde-Wyatt *et al*., 1975; Wilding *et al*., 1998; Auld and Medd, 1987

Table 2.3 TSWV incidence in weeds found within and adjacent to potato field crops in Australia

Region	Family	Species and Common Name	Life form, size lifespan[1]	TSWV incidence (%) [2]					
				2001/2002			2002/2003		
				BP	MS	ES	BP	MS	ES
Victoria	Asteraceae	*Arctotheca calendula* (Cape weed)	AWGA	0	0	0	0	0	30.4
	Solanaceae	*Solanum nigrum* (Blackberry nightshade)	ASGA	0	0	0	0	0	17.9
South Australia	Chenopodiaceae	*Chenopodium album* (Fat hen)	SSGA	0	0	33.3	0	0	25.0
	Cucurbitaceae	*Citrullus lanatus* (Wild melon/Bitter melon)	SSGA	0	0	25.0	0	0	0
	Malvaceae	*Malva parviflora* (Marshmallow)	A/SLP	6.3	0	0	0	24.0	0
	Polygonaceae	*Polygonum avicutare* (Wire weed)	AWGA/B	0	0	33.3	0	0	0
	Solanaceae	*Solanum nigrum* (Blackberry nightshade)	ASGA	0	0	4.8	0	0	12.5
	Asteraceae	*Sonchus oleraceus* (Common sowthistle)	AWGA	0	0	33.3	0	0	0
	Fabaceae	*Trifolium arvense* (Hare's foot clover)	AWGA	8.3	0	0	0	0	0
	Chenopodiaceae	*Chenopodium pumilio* (Clammy goosefoot)	AAH	0	0	0	0	0	100
Tasmania	Asteraceae	*Arctotheca calendula* (Cape weed)	AWGA	0	0	0	0	0	28.6
	Chenopodiaceae	*Chenopodium album* (Fat hen)	SSGA	0	0	0	0	1.4	0
New South Wales	Solanaceae	*Solanum triflorum* (Treeflower nightshade)	SAH	0	0	0	0	77.3	0
		Salvation Jane/Paterson's curse *(Echium plantagineum/ E. lycopsis).*	AWGA	0	0	0	0	34	0

[1] AWGA = Autumn-Winter germinating annual; SSGA = Spring – Summer germinating annual; ASGA = Autumn-Spring germinating annual; WGA/B = Autumn-Winter germinating annual or biennial; AAH = Aromatic annual herb; A/SLP = Annual or short-lived perennial; SAH = Sprawling annual herb (Hyde-Wyatt *et al*., 1975; Wilding *et al*., 1998; Auld and Medd, 1987)

[2] BP = Before Planting, MS = Mid-Season, ES = End of Season

Table 2.4 Potato cultivars grown and incidence of TSWV during 2001/2002 and 2002/2003 season

		2001/2002		2002/2003	

State	Location	Potato Cultivar(s) grown	TSWV incidence (%)			Potato Cultivar(s) grown	TSWV incidence (%)		
			BP	MS	ES		BP	MS	ES
Victoria	Paddock # 1	Shepody	0	0	0	Shepody (G5)	0	0	4
	Paddock # 2	Shepody	0	0	0	Shepody (G5)	0	0	2
	Paddock # 3	-	-	-	-	Shepody (G5)	0	0	3.7
	Paddock # 4	-	-	-	-	Shepody (G5)	0	0	0
	Paddock # 5	-	-	-	-	Shepody (G5)	0	0	7.8
	Paddock # 6	-	-	-	-	Shepody (G5)	0	0	1.9
	Paddock # 7	-	-	-	-	Russet Burbank (G1)	0	0	23.1
	Paddock # 8	-	-	-	-	Shepody (G5)	0	0	3.6
	Paddock # 9	-	-	-	-	Ranger Russet (G1)	0	0	64
	Paddock # 10	-	-	-	-	Riverina Russet (G2)	0	0	72
	Paddock # 11	-	-	-	-	Shepody (G5)	0	0	4.1
	Paddock # 12	-	-	-	-	Riverina Russet (G2)	0	0	8
South Australia	Paddock # 1	Shepody	0	14.3	*	Shepody	0	0	*
	Paddock # 2	Russet Burbank	0	47.4	*	Shepody	0	0	*
	Paddock # 3	Kennebec	1.9	37.9	*	Russet Burbank	0	0	20
	Paddock # 4	Shepody	1.7	27.2	*	Shepody	0	0	*
	Paddock # 5	-	-	-	-	Shepody	0	1.6	*
	Paddock # 6	-	-	-	-	Shepody	*	*	40
		-	-	-	-	McCains 1	*	*	83.3
		-	-	-	-	Russet Burbank	*	*	45.2
Tasmania	Derwent Valley	Shepody (G4)	*	0	*	Shepody	0	0	0
		Russet Burbank	*	0	*	Russet Burbank	0	0.2	1.4
		Kennebec	*	0	*	Ranger Russet	0	0	0
		-	-	-	-	Kennebec	0	0	0
		-	-	-	-	Pink Eye	0	0	0
		-	-	-	-	Nooksack	0	0	0
	Southern Midlands	Russet Burbank (G4)	0.3	0.3	*	Ranger Russet	0	0	0
		-	-	-	-	Russet Burbank (G4)	0	0	0
		-	-	-	-	Shepody (G2)	0	0	0
		-	-	-	-	Pink Eye	0	0	0
	Northern Midlands	Russet Burbank (G4)	*	0	*	Ranger Russet	0	0	0
		-	-	-	-	Gem Russet	0	0	0
		-	-	-	-	Russet Burbank	0	0	0
	North East	Russet Burbank	1.3	1.3	*	Russet Burbank	0	0	0
	North West	R. Burbank/Shepody	*	0	*	Shepody	0	0	0
		-	-	-	-	Ranger Russet	0	0	0
	Paddock # 1	-	-	-	-	Atlantic (own seed)	*	5.3	10
New South Wales		-	-	-	-	Shepody (ex Ballarat)	*	0	54.5
		-	-	-	-	Ranger Russet (ex Technico)	*	5.9	71.4
		-	-	-	-	Riverina Russet (ex Technico)	*	10	71.4
		-	-	-	-	Riverina Russet (ex Scot)	*	100	54.5
		-	-	-	-	Ranger Russet (ex Scot)	*	100	40
		-	-	-	-	92-37-1	*	21.7	29.6
		-	-	-	-	95-109-6	*	50	30
		-	-	-	-	Ranger Russet (ex Ballarat)	*	73.3	54.5

BP = Before Planting, MS = Mid-Season, ES = End of Season. * = Data not collected

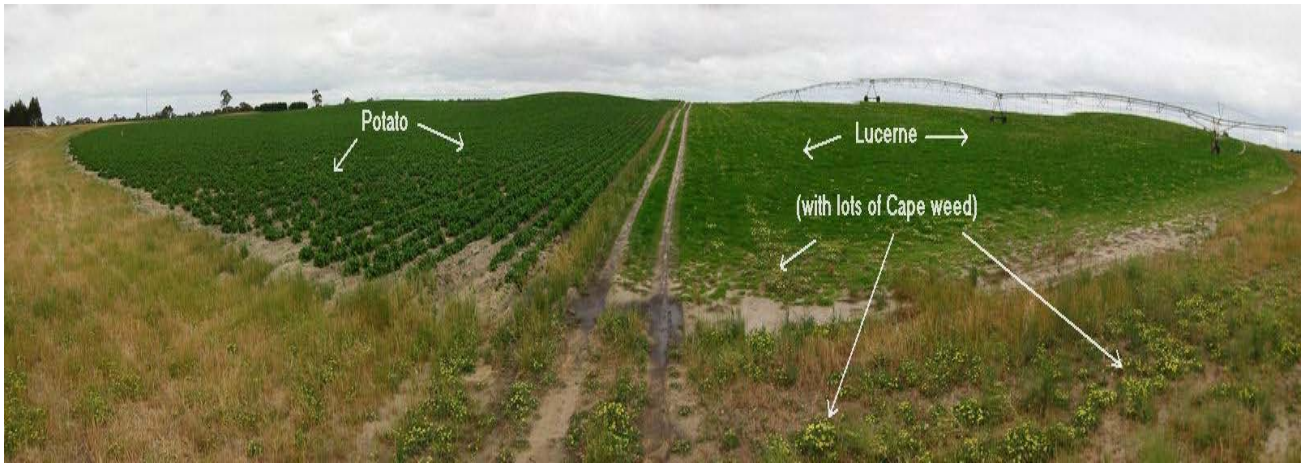


Figure 2.2 Centre Pivot covering a potato field (left) and Lucerne (right) in South Australia (2001/2002)

Figure 2.3 TSWV incidence and prevalence in weeds and potato (seed tubers and leaves) in Victoria during 2001/2002 and 2002/2003 seasons

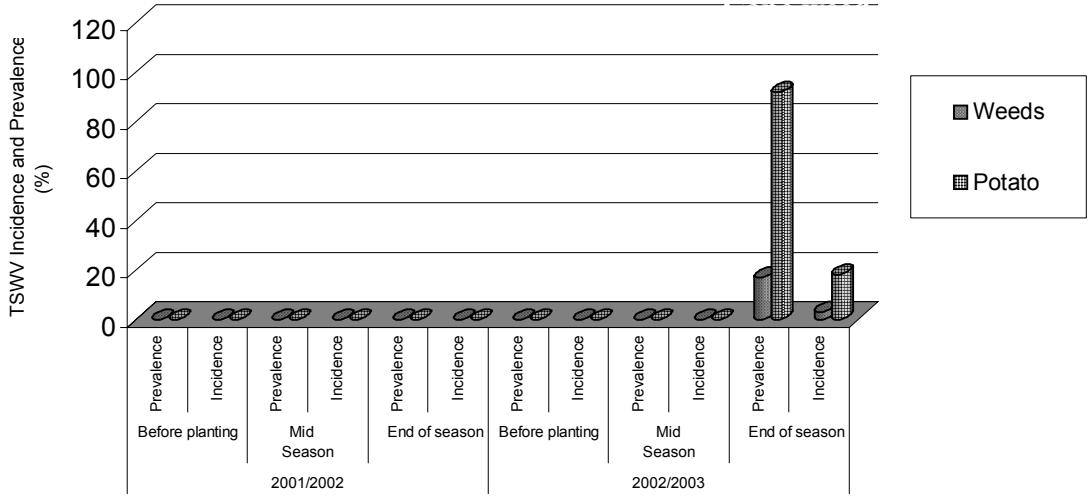


Figure 2.4 TSWV incidence and prevalence in weeds and potato (seed tubers and leaves) in South Australia during 2001/2002 and 2002/2003 seasons

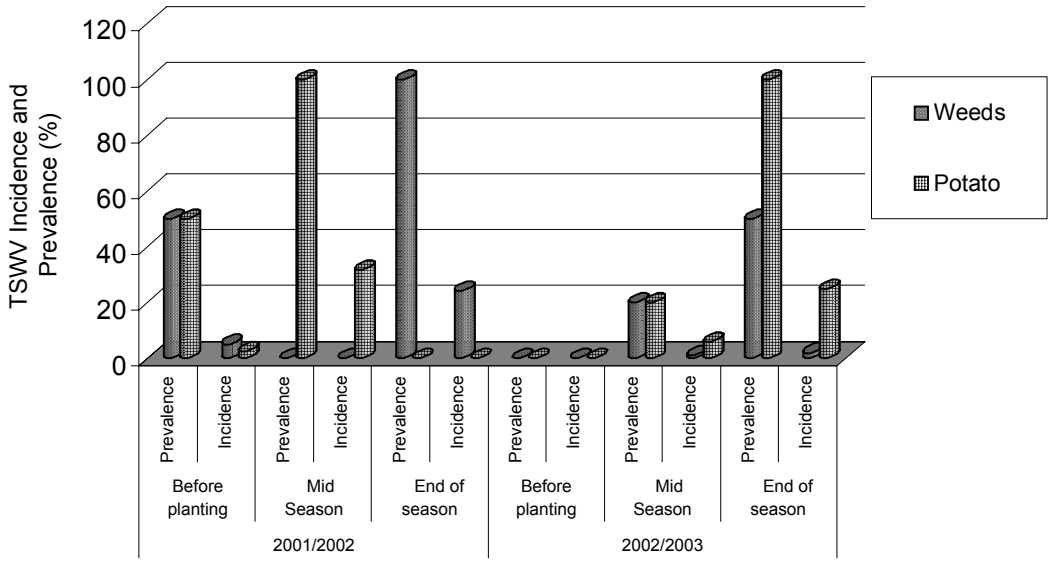


Figure 2.5 TSWV incidence and prevalence in weeds and potato (seed tubers and leaves) in Tasmania during 2001/2002 and 2002/2003 seasons

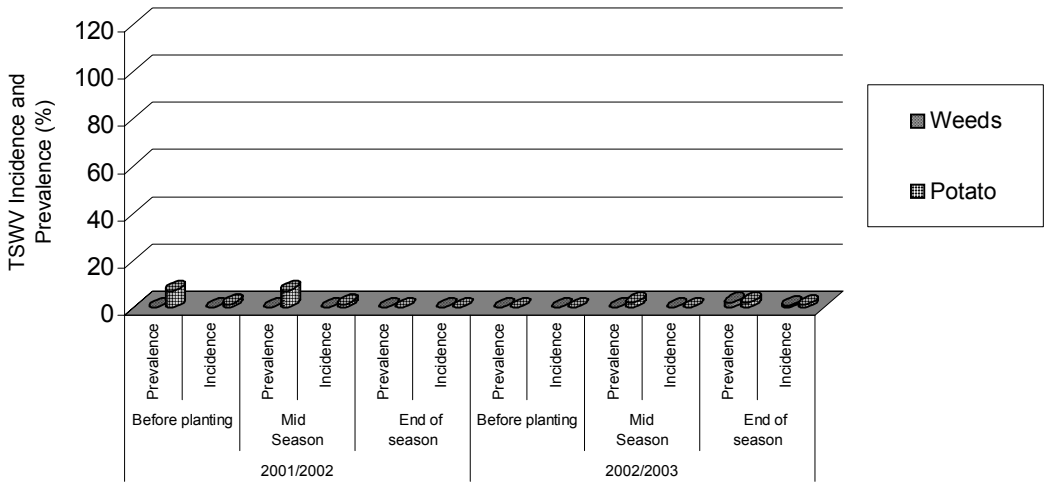
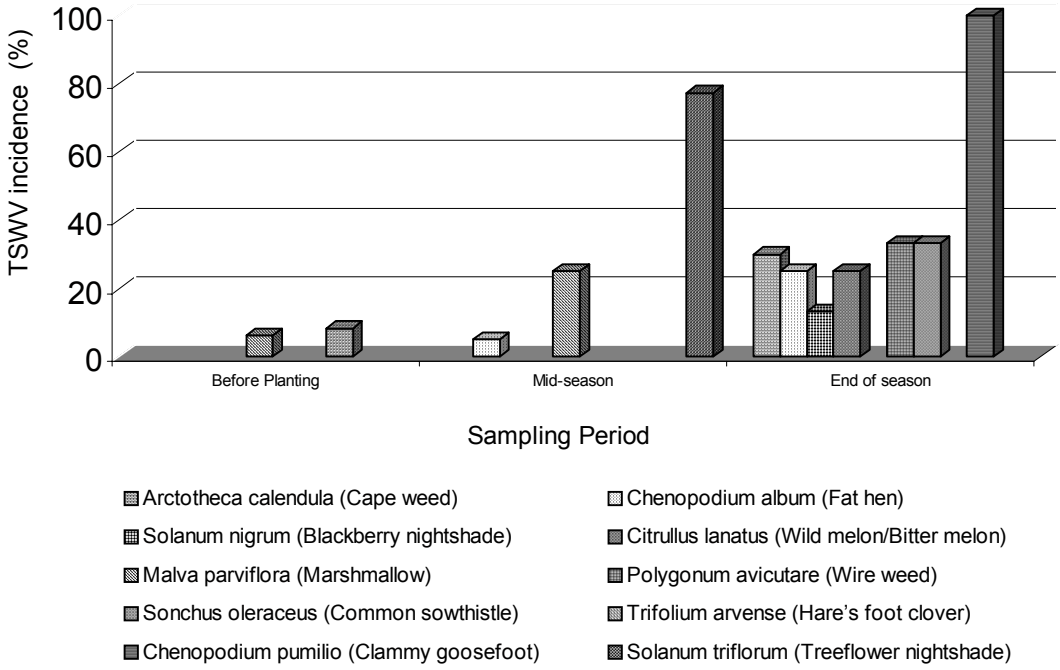


Figure 2.6 TSWV incidence (%) in weeds across the four states (Tasmania, Victoria, South Australia and New South Wales) during different periods of the 2001/2002 and 2002/2003 growing seasons



Quantifying thrips species and population dynamics in potato crops.

Data on the thrips species, time and population size into potato crops during 2001/2002 and 2002/2003 are presented in Table 2.5. No trapping was done in New South Wales although TSWV incidences in both weeds and potato were determined during 2002/2003. The mean maximum thrips populations differed between seasons, states, sampling sites and species. The lowest and highest thrips populations were observed in Victoria during 2002/2003.

While several thrips species were found in potato crops (Table 2.5), only onion thrips (*Thrips tabaci)* and tomato thrips (*Frankliniella shultzei)* are known vectors of TSWV and therefore, assumed to be responsible for the incidences of TSWV that were observed during the survey, both in weeds and potato. However, from the present data, it is difficult to evaluate the relative importance of each of these two species as vectors of TSWV within potato crops. The other species have not been proven as vectors of TSWV. Western flower thrips (*Frankliniella occidentalis*) was not observed on traps within and 10m adjacent to potato crops (Table 2.5).

Table 2.5 Thrips species caught at canopy height in different States of Australia before planting (BP), mid-season (MS) and end of season (ES) during surveys of commercial potato fields in 2001/2002 and 2002/2003 seasons

		State/time of thrips count [a]								
		Victoria			*South Australia*			**Tasmania**		
Season	Thrips species	BP	MS	ES	BP	MS	ES	BP	MS	ES
2001/2002	*Thrips tabaci*	8	86	8	1	212	630	1	5	61
	Thrips imaginis	412	65	81	5	43	84	0	3	32
	Thrips australis	5	0	0	0	0	0	0	0	0
	Frankliniella occidentalis	0	0	0	0	0	0	0	0	0
	Frankliniella schultzei	0	4	5	0	1	10	0	0	0
	Chirothrips manicatus	0	0	0	1	0	0	0	0	0
	Limothrips cerealium	1	0	0	1	27	99	4	12	22
	Limothrips angulicornis	0	0	0	0	2	8	0	2	8
	Tubuliferan spp.	0	0	0	0	5	30	0	0	0
	Tenothrips spp.	0	0	0	0	0	0	1	0	0
	Pseudanaphothrips achaetus	0	0	0	0	0	0	0	0	0
	Apterothrips apteris	0	0	0	0	0	0	0	0	0
	Others	53	257	0	2	135	0	2	0	0
	Total number	**479**	**412**	**94**	**10**	**425**	**861**	**8**	**22**	**123**
2002/2003	*Thrips tabaci*	0	904	4619	0	94	387	1	81	13
	Thrips imaginis	-	-	-	2	320	299	0	160	26
	Thrips australis	-	-	-	-	-	-	0	10	10
	Frankliniella occidentalis	0	0	0	0	0	0	0	0	0
	Frankliniella schultzei	0	260	322	0	38	202	0	0	0
	Chirothrips manicatus	-	-	-	-	-	-	0	0	0
	Limothrips cerealium	-	-	-	-	-	-	0	0	0
	Limothrips angulicornis	-	-	-	-	-	-	0	0	0
	Tubuliferan spp.	-	-	-	-	-	-	0	0	0
	Tenothrips spp.	-	-	-	-	-	-	2	7	7
	Pseudanaphothrips achaetus	-	-	-	-	-	-	0	0	0
	Apterothrips apteris	-	-	-	-	-	-	0	0	0
	Others	-	-	-	1	20	118	0	1	0
	Total number	**0**	**1164**	**4941**	**3**	**472**	**1006**	**3**	**259**	**56**

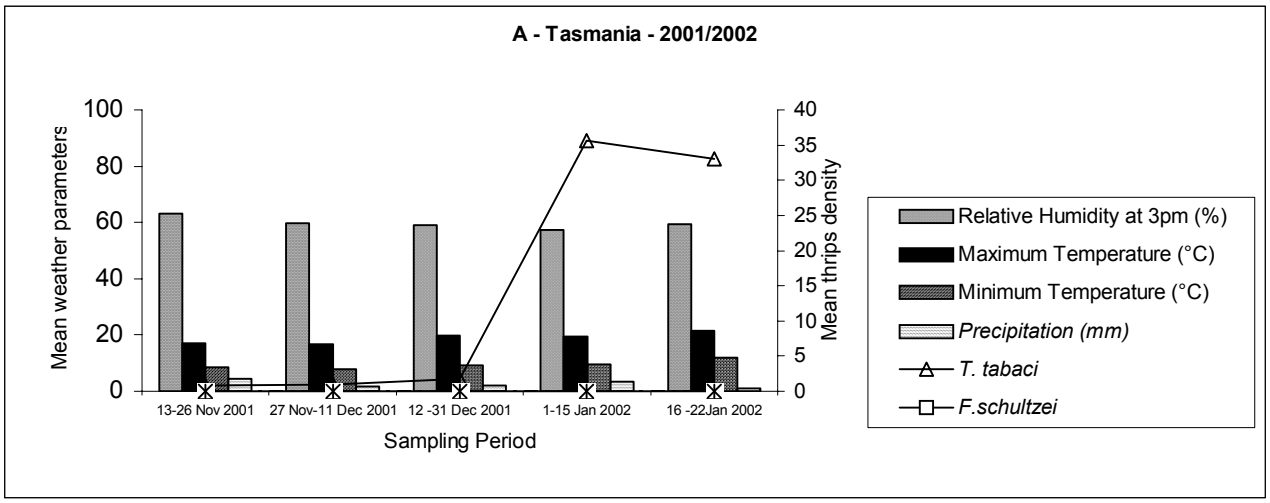
[a] BP = Before Planting, MS = Mid-Season, ES = End of Season

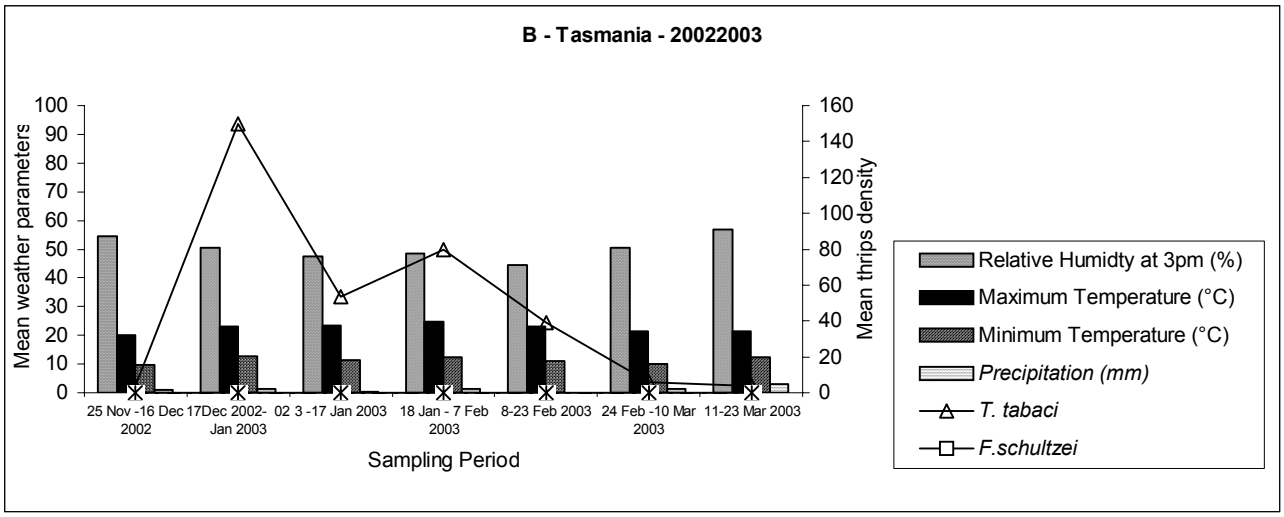
Development of quantitative models for describing the effects of weather parameters on thrips flights and population dynamics

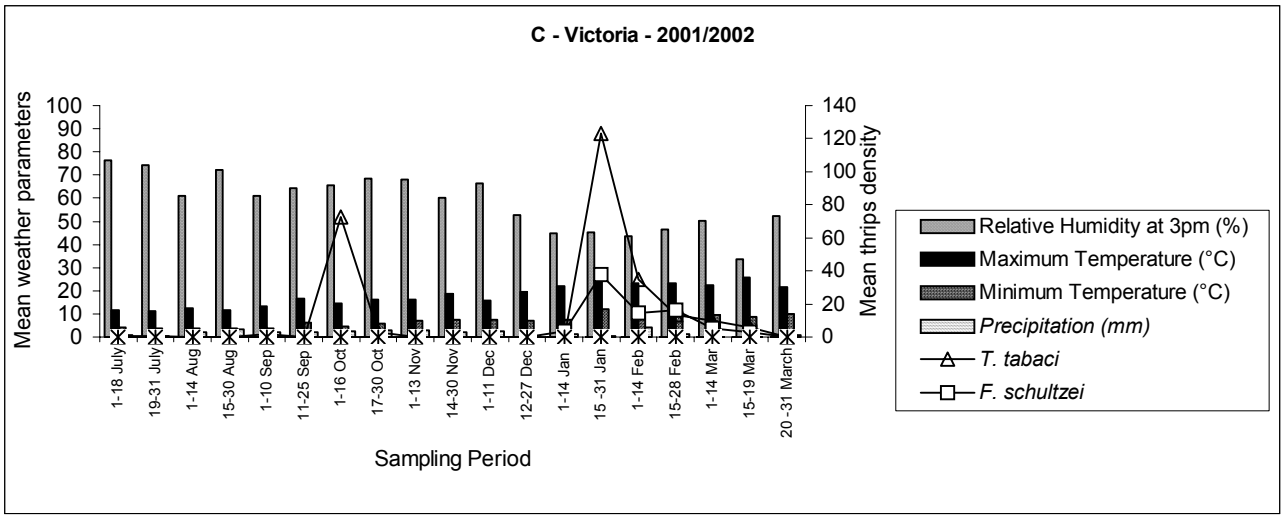
The regional population dynamics of *T. tabaci* and *F. schutzei* and weather parameters are presented in (Figure 2.7 A-F). The two seasons were different with respect to all the weather parameters used in the study, hence the differences observed in the thrips population dynamics. Multiple linear regression analysis indicated non significance (P=0.05) for all interactions, except in only one instance in Victoria during 2001/2002 season, when a fit to the model of *F. schutzei* population was obtained with a 67% certainty (Table 2.6). This indicates that the response of thrips population to the weather parameters analyzed could not adequately be described as linear. Therefore, non-linear relationships had to be explored using the Gompertz, Logistic and Exponential curves to obtain the best fitting equations (Table 2.7, Appendices 2, 3, & 4). Several regression models fitted to regional data sets of each dependent variable (thrips populations) had significant to highly significant *F* values and parameter estimates (Appendices 2, 3, & 4). Based on the percentage variance (r^2), *F* test, variance ratios and *t* probabilities of the parameter estimates, the models with best fits were selected to describe the response of vector thrips populations to different weather variables (Table 2.7). The Gompertz and exponential curves provided the best fit for 56% and 44% of all models fitted to the dependent variables, respectively. However, the r^2 for some of the significant regression models were small (Table 2.7), indicating a low correlation between thrips population response to weather parameters. Taking models with ≥50% variance (r^2) and significant *t* probabilities, the graphical comparisons of observed and predicted data indicated that in Tasmania, only the daily minimum air temperature had a significant effect on the flights and populations of *T. tabaci*, with the Gompertz curve providing the best fit (r^2 = 0.58) than other curves (Table 2.7, Figure 2.8 – A). Examination of predicted versus observed curves in Victoria, indicated good fits to the estimated rate parameters. The populations of *F. schutzei* responded more significantly ($P \leq 0.05$) to weather variables than *T. tabaci*. The daily maximum air temperature significantly affected the *F. schutzei* flights and populations more than the daily minimum air temperature and daily relative humidity as indicated by the high variance, 87%, 75% and 84% and variance ratios of 60.26, 41.04 and 47.37, respectively (Table 2.7), with good fits between the observed and expected (Figures 2.8, B - E). The relationship of individual thrips species to weather parameters in South Australia, followed a similar pattern to that observed in Victoria with populations of *F. schutzei* responding more significantly ($P \leq 0.05$) than those of *T. tabaci* to daily maximum air temperature than the daily minimum air temperature and daily relative humidity (Table 2.7). The predicted versus observed data revealed good to excellent fits to the estimated rate parameters of the Gompertz and Exponential

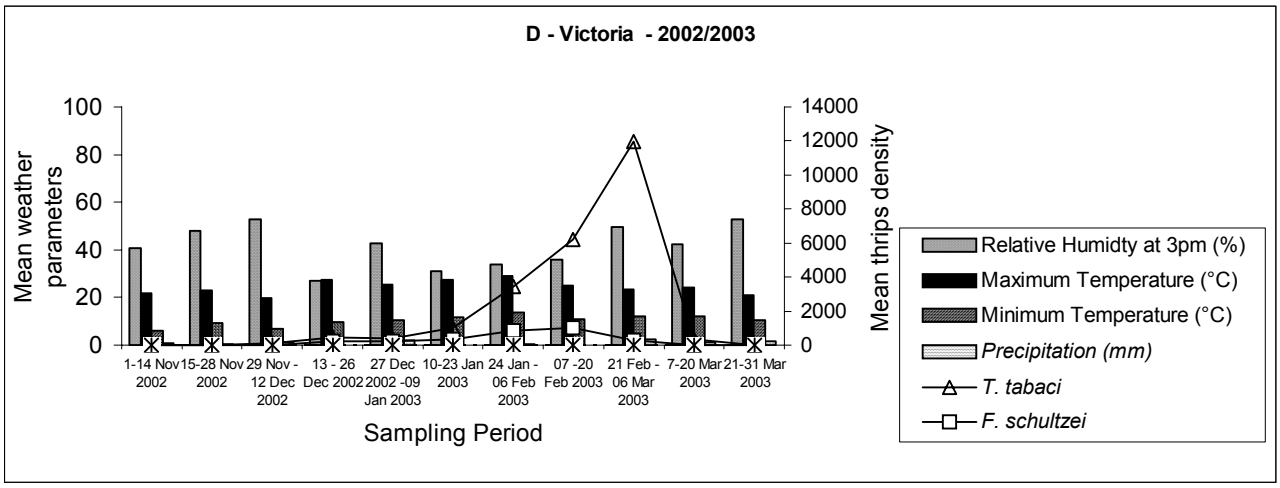
curves (Figures 2.8, F - J), with observed *F. schutzei* population densities in 2002/2003 indistinguishable from the expected distribution (Figures 2.8, I &J).

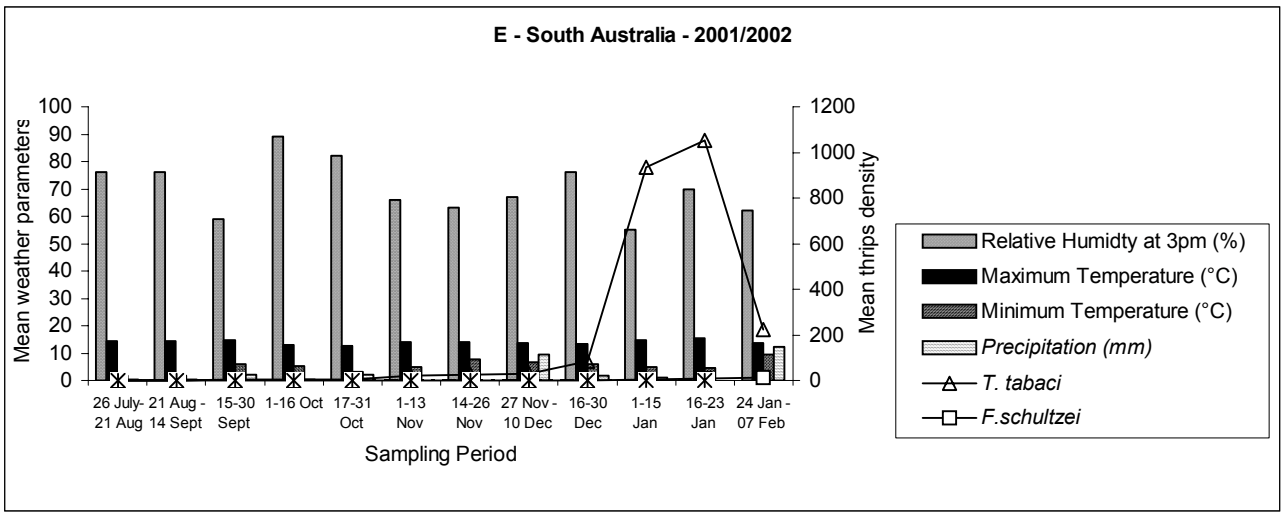
Figure 2.7. Relationship between seasonal population dynamics of known vector thrips and weather parameters in Tasmania, Victoria and South Australia (2001/2002 and 2002/2003)








D - Victoria - 2002/2003
Mean weather parameters
Mean thrips density
100
80
60
40
20
0
14000
12000
10000
8000
6000
4000
2000
0
1-14 Nov 2002
15-28 Nov 2002
29 Nov - 12 Dec 2002
13 - 26 Dec 2002
27 Dec 2002 -09 Jan 2003
10-23 Jan 2003
24 Jan - 06 Feb 2003
07 -20 Feb 2003
21 Feb - 06 Mar 2003
7-20 Mar 2003
21-31 Mar 2003
Sampling Period
Relative Humidty at 3pm (%)
Maximum Temperature (°C)
Minimum Temperature (°C)
Precipitation (mm)
T. tabaci
F. schultzei


E - South Australia - 2001/2002
Mean weather parameters
Mean thrips density
100
90
80
70
60
50
40
30
20
10
0
1200
1000
800
600
400
200
0
26 July- 21 Aug
21 Aug - 14 Sept
15-30 Sept
1-16 Oct
17-31 Oct
1-13 Nov
14-26 Nov
27 Nov - 10 Dec
16-30 Dec
1-15 Jan
16-23 Jan
24 Jan - 07 Feb
Sampling Period
Relative Humidty at 3pm (%)
Maximum Temperature (°C)
Minimum Temperature (°C)
Precipitation (mm)
T. tabaci
F.schultzei

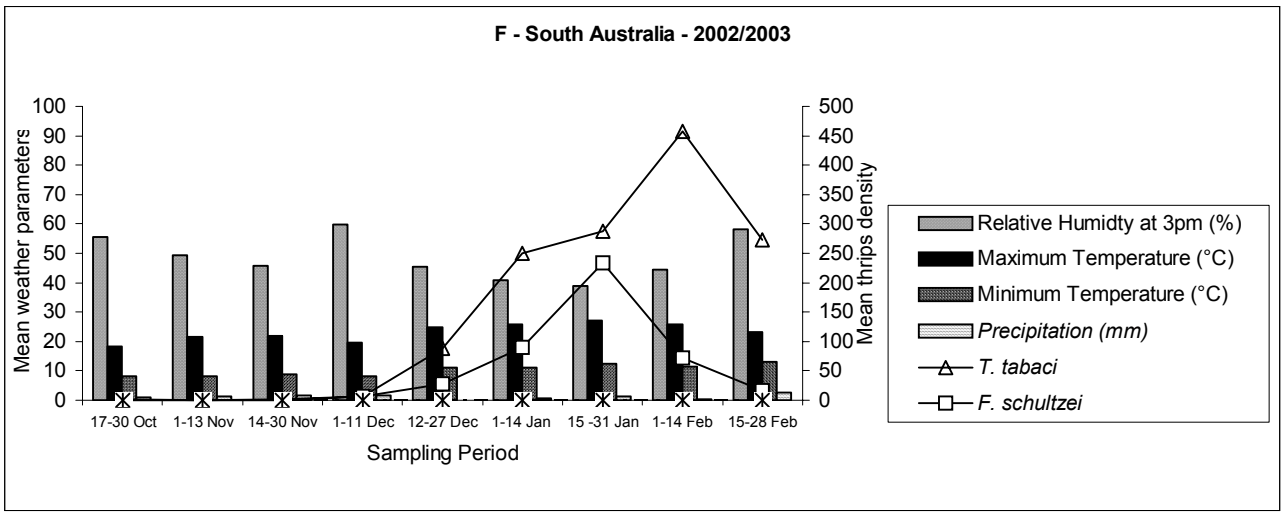

F - South Australia - 2002/2003
Mean weather parameters
Mean thrips density
100
90
80
70
60
50
40
30
20
10
0
500
450
400
350
300
250
200
150
100
50
0
17-30 Oct
1-13 Nov
14-30 Nov
1-11 Dec
12-27 Dec
1-14 Jan
15 -31 Jan
1-14 Feb
15-28 Feb
Sampling Period
Relative Humidty at 3pm (%)
Maximum Temperature (°C)
Minimum Temperature (°C)
Precipitation (mm)
T. tabaci
F. schultzei

Table 2.6. Multiple linear regression describing relationships between thrips flights and population dynamics within potato crops during 2002/2002 and 2002/2003 seasons

State	Season	Regression equation [a]	R^2	F. pr	P (0.05)
Tasmania	2001/2002†	$Y_1 = 19.2 - 0.5X_1 + 0.9X_2 + 0.2X_3 - 0.3X_4$	N/A	-	-
	2002/2003	$Y_1 = -445 - 11.6X_1 + 57.4X_2 - 57.7X_3 - 3.4X_4$	0.33	0.397	NS
Victoria	2001/2002†	$Y_1 = 2.51 - 0.08X_1 + 0.2X_2 + 0.01X_3 - 0.031X_4$	0.06	0.323	NS
		$Y_2 = 3.47 - 0.13X_1 + 0.24X_2 + 0.02X_3 - 0.04X_4$	0.67	0.001	*
	2002/2003	$Y_1 = 9.7 - 0.26X_1 + 0.48X_2 + 0.89X_3 - 0.17X_4$	0.26	0.237	NS
		$Y_2 = -0.3 + 0.12X_1 + 0.15X_2 + 0.52X_3 - 0.08X_4$	0.42	0.124	NS
South Australia	2001/2002	$Y_1 = -5522 + 366X_1 + 31.7X_2 + 4.3X_3 + 6.0X_4$	0.06	0.395	NS
		$Y_2 = -71.6 + 4.01X_1 + 0.74X_2 + 0.60X_3 - 0.18X_4$	0.29	0.184	NS
	2002/2003	$Y_1 = -1488 + 39.6X_1 + 33.4X_2 - 34.0X_3 + 8.7X_4$	0.46	0.180	NS
		$Y_2 = 173 + 1.4X_1 + 12.7X_2 + 14.7X_3 - 6.27X_4$	0.25	0.319	NS

[a] Y_1 = Expected *T. tabaci* population, Y_2 = Expected *F. Schutzei* population, X_1 = Mean daily maximum air temperature (°C), X_2 = mean daily minimum air temperature (°C), X_3 = mean daily precipitation (mm), X_4 = mean daily relative humidity at 3pm (%),

R^2 = Adjusted coefficient of multiple determination

† = Data transformed (Log_{10})

N/A = Residual variance exceeds variance of response variate

NS = Not significant ($P \leq 0.05$)

* = Significant ($P \leq 0.05$)

Validation of models

Full and reduced models fitted to the 2003/2004 data sets were statistically compared at each step of the development process. Comparisons of the percentage variance (r^2), *F* test, variance ratios and *t* probabilities of the parameter estimates, indicated a good correlation between predicted values and results observed (P=0.05). For the Freese's X^2 test, the calculated X^2 were significantly similar (P=0.05), to the critical X^2 statistics from the tables for both the Gompertz and Exponential models, again indicating the accuracy of the models. Hence, the parameters generated in the models were not statistically different from those estimated from the 2001/2002 and 2002/2003 data, thereby validating the models.

Given that the meteorological conditions were different in the sampling sites (Figure 1), and the two seasons, 2001/2002 and 2002/2003 were also different, data from the different sampling sites and the two seasons could not be pooled to improve precision of the models. Therefore, the non-linear relationships between *T. tabaci* and *F. schutzei,* daily maximum air temperature (°C), daily minimum air temperature (°C), daily precipitation (mm) and daily relative humidity at 3pm (%) in Tasmania, Victoria and South Australia are best described statistically by the Gompertz and Exponential curves (Table 2.7, Figure 2.8)

Table 2.7 Non-Linear Regressions with significant parameter estimates describing relationships between thrips flights and population dynamics and weather variables within potato crops during 2002/2002 and 2002/2003 seasons

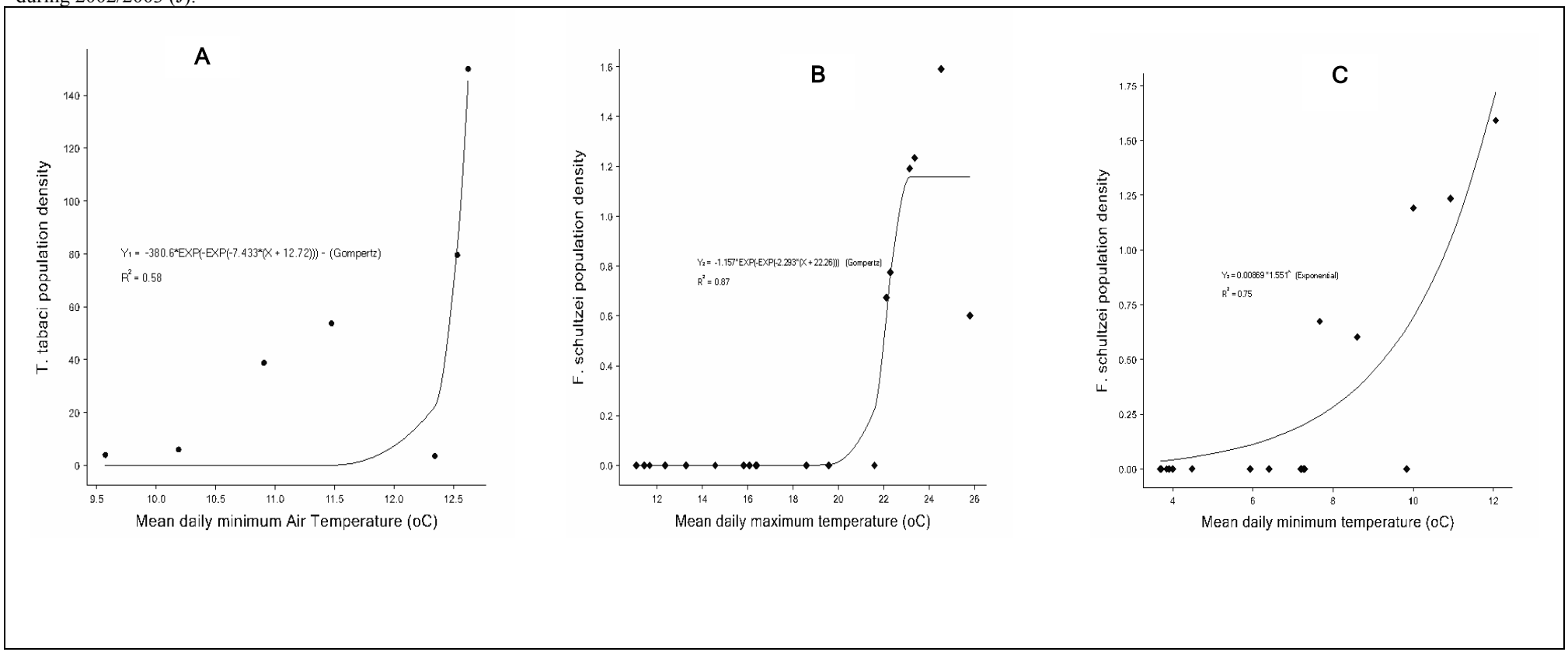
State	Season	Parameter [Ŧ]	Best fitting curve	Regression equation [a]	r^2	F. pr	V.R.	P (≤0.05)
Tasmania	2001/2002 [†]	-		-	-	-	-	-
	2002/2003	Mean Daily Minimum Air Temperature (°C)	Gompertz	$Y_1 = -380.6*EXP(-EXP(-7.433*(X + 12.72)))$	0.58	0.037	7.89	S
Victoria	2001/2002 [†]	Mean Daily Maximum Air Temperature (°C)	Exponential	$Y_1 = 0.0210 * 1.178^X$	0.22	0.001	9.99	S
		Mean Daily Minimum Air Temperature (°C)	Exponential	$Y_1 = 0.0173 * 1.486^X$	0.35	<.001	13.81	HS
		Mean Daily Precipitation (mm)	Gompertz	$Y_1 = -0.527*EXP(-EXP(-0.3916*(X + 31.42)))$	-	0.051	3.22	S
		Mean Daily Relative Humidity at 3pm (%)	Gompertz	$Y_1 = -1.171*EXP(-EXP(+1039*(X + 50.5)))$	0.12	0.008	5.61	S
	2001/2002	Mean Daily Maximum Air Temperature (°C)	Gompertz	$Y_2 = -1.157*EXP(-EXP(-2.293*(X + 22.26)))$	0.87	<.001	60.26	HS
		Mean Daily Minimum Air Temperature (°C)	Exponential	$Y_2 = 0.00869 * 1.551^X$	0.75	<.001	41.04	HS
		Mean Daily Precipitation (mm)	Gompertz	$Y_2 = -0.5418*EXP(-EXP(+10.98*(X + 1.368)))$	0.04	0.047	3.32	S
		Mean Daily Relative Humidity at 3pm (%)	Gompertz	$Y_2 = -1.059*EXP(-EXP(+1.262*(X + 50.25)))$	0.84	<.001	47.37	HS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	Gompertz	$Y_1 = -3.141*EXP(-EXP(-2.672*(X + 23.30)))$	0.53	<.001	20.77	HS
		Mean Daily Minimum Air Temperature (°C)	Exponential	$Y_1 = 0.418 * 1.188^X$	0.36	<.001	21.35	HS
		Mean Daily Precipitation (mm)	Gompertz	$Y_1 = -2.21*EXP(-EXP(+4.22E-03*(X + 1.37E+03)))$	-	0.018	6.20	S
		Mean Daily Relative Humidity at 3pm (%)	Exponential	$Y_1 = -2.208 * 0.2816^X$	-	0.004	10.46	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	Exponential	$Y_2 = 0.0147 * 1.2056^X$	0.49	<.001	21.08	HS
		Mean Daily Minimum Air Temperature (°C)	Exponential	$Y_2 = 0.210 * 1.219^X$	0.30	0.002	14.30	HS
		Mean Daily Precipitation (mm)	Gompertz	$Y_2 = -17.4*EXP(-EXP(+1.923*(X –0.9078)))$	-	0.041	4.43	S
		Mean Daily Relative Humidity at 3pm (%)	Gompertz	$Y_2 = -2.69*EXP(-EXP(+0.132*(X +39.80)))$	0.26	0.006	9.10	S
South Australia	2001/2002	Mean Daily Maximum Air Temperature (°C)	Gompertz	$Y_1 = -1088*EXP(-EXP(-12.58*(X + 14.79)))$	0.86	<.001	30.16	HS
	2001/2002	Mean Daily Minimum Air Temperature (°C)	Exponential	$Y_2 = 2.41E-13 * 27^X$	0.47	0.007	8.53	S
		Mean Daily Precipitation (mm)	Exponential	$Y_2 = 0.000 * 4.5^X$	0.47	0.007	8.54	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	Exponential	$Y_1 = 0.211 * 1.316^X$	0.51	0.005	12.05	S
		Mean Daily Minimum Air Temperature (°C)	Gompertz	$Y_1 = -341.3*EXP(-EXP(-9.955*(X + 11.07)))$	0.87	<.001	36.56	HS
		Mean Daily Relative Humidity at 3pm (%)	Gompertz	$Y_1 = -330*EXP(-EXP(+2.664*(X + 44.92)))$	0.42	0.024	6.71	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	Exponential	$Y_2 = 0.000 * 2.136^X$	0.99	<.001	450.74	HS
		Mean Daily Relative Humidity at 3pm (%)	Gompertz	$Y_2 = -129011*EXP(-EXP(+0.3673*(X +21.66)))$	0.92	<.001	44.86	HS

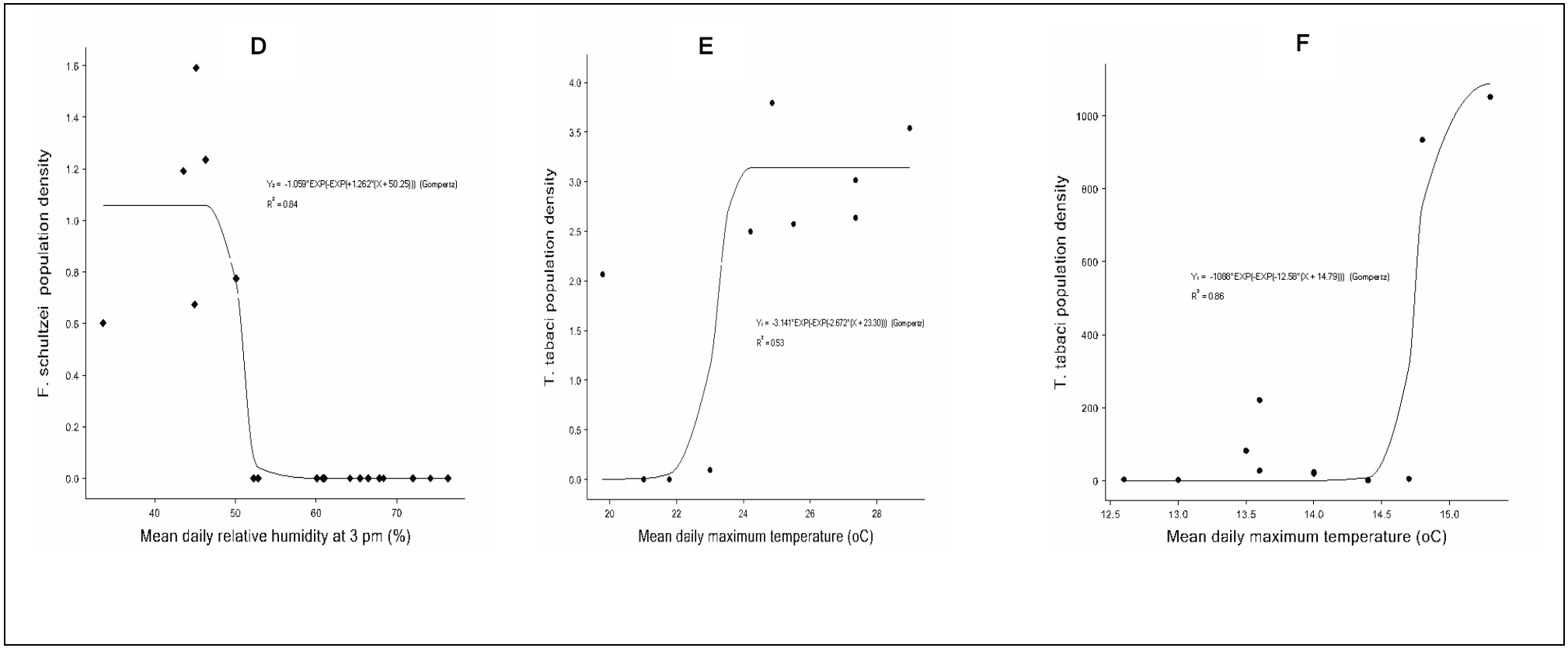
[Ŧ] Only those variables significant at P≤0.05 were included

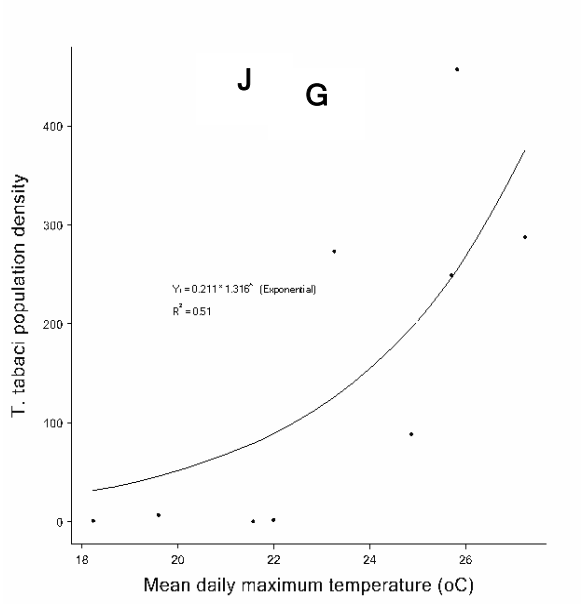
[a] Y_1 = Expected *T. tabaci* population, Y_2 = Expected *F. Schutzei* population

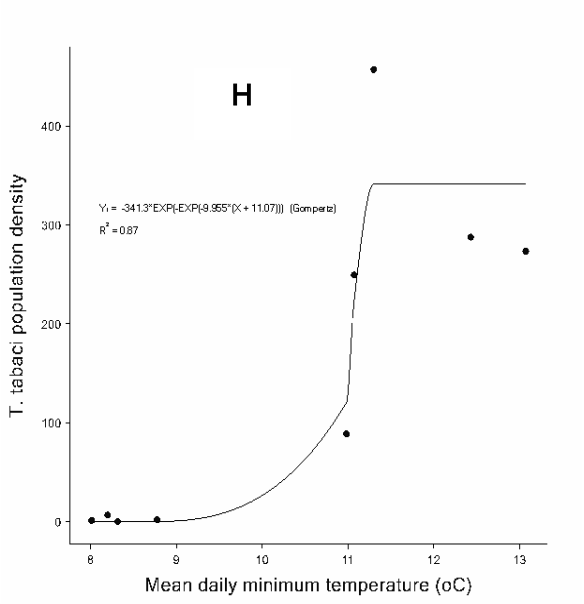
S = significant (P<0.05); HS = highly significant (P<0.001)

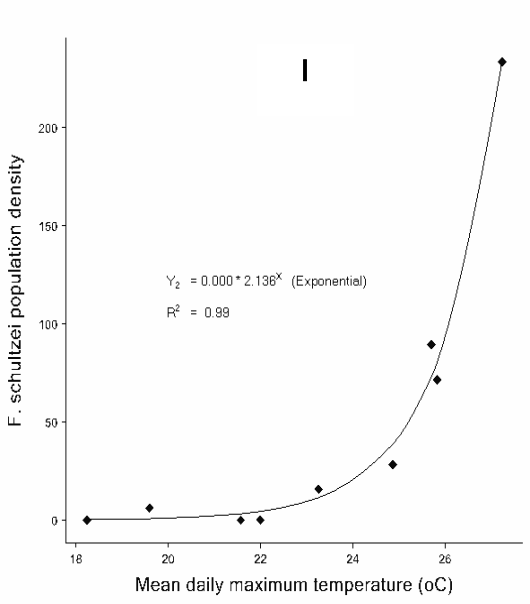
Figure 2.8 Fitted and expected population density of *T.tabaci* in response to mean daily minimum temperature (°C) in Tasmania during 2002/2003 (A), *F.schutzei* in response to mean daily maximum temperature (°C) in Victoria during 2001/2002 (B), *F.schutzei* in response to mean daily minimum temperature (°C) in Victoria during 2001/2002 (C), *F.schutzei* in response to mean daily relative humidity at 3pm (%) in Victoria during 2001/2002 (D), *T.tabaci* in response to mean daily maximum temperature (°C) in Victoria during 2002/2003 (E), *T.tabaci* in response to mean daily maximum temperature (°C) in South Australia during 2001/2002 (F), *T.tabaci* in response to mean daily maximum temperature (°C) in South Australia during 2002/2003 (G), *T.tabaci* in response to mean daily minimum temperature (°C) in South Australia during 2002/2003 (H), *F.schutzei* in response to mean daily maximum temperature (°C) in South Australia during 2002/2003 (I), *F.schutzei* in response to mean daily relative humidity at 3pm (%) in South Australia during 2002/2003 (J).

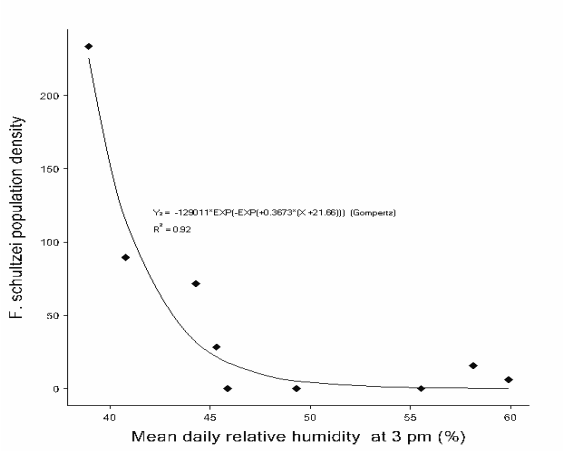



D
F. schultzei population density
Y₂ = -1.059*EXP(-EXP(+1.262*(X+50.25))) (Gompertz)
R² = 0.84
Mean daily relative humidity at 3 pm (%)
E
T. tabaci population density
Y₁ = -3.141*EXP(-EXP(-2.672*(X+23.30))) (Gompertz)
R² = 0.53
Mean daily maximum temperature (oC)
F
T. tabaci population density
Y₁ = -1088*EXP(-EXP(-12.58*(X+14.79))) (Gompertz)
R² = 0.86
Mean daily maximum temperature (oC)


J
G
T. tabaci population density
Y1 = 0.211 * 1.316^X (Exponential)
R² = 0.51
Mean daily maximum temperature (oC)


H
T. tabaci population density
Y1 = -341.3*EXP(-EXP(-9.955*(X + 11.07))) (Gompertz)
R² = 0.87
Mean daily minimum temperature (oC)


I
F. schultzei population density
Y2 = 0.000 * 2.136^X (Exponential)
R² = 0.99
Mean daily maximum temperature (oC)


F. schultzei population density
Y2 = -129011*EXP(-EXP(+0.3673*(X +21.66))) (Gompertz)
R² = 0.92
Mean daily relative humidity at 3 pm (%)

Discussion

The response of *T. tabaci* and *F. schutzei* populations was quantified across a range of weather parameters, seasons and sampling sites. Optimum daily minimum air temperature (°C) in Tasmania for *T. tabaci* populations to start rising, as estimated by fitting the Gompertz model to data from the 2002/2003 season, occurred between 11.5°C and 12.0°C (Figure 2.8, A). Optimum daily maximum and minimum air temperature (°C) for *F. schutzei* populations to start rising, as estimated by the Gompertz and Exponential models in Victoria was between 20°C and 22°C, and 4 °C and 6 °C, respectively (Figure 2.8, B – C). Daily relative humidity at 3pm of more than 45% depresses *F. schutzei* populations in Victoria (Figure 2.8, D). At about 22°C, daily maximum air temperature (°C), *T. tabaci* populations start to rise in Victoria (Figure 2.8, E). In South Australia, *T. tabaci* populations start to rise when the daily maximum air temperature (°C) is between 14.5°C and 18°C, as estimated by the Gompertz and Exponential models, respectively (Figure 2.8, F-G). The optimum minimum air temperature (°C) for *T. tabaci* populations to start rising is 10°C (Figure 2.8, H). For *F. schutzei,* in South Australia, the optimum daily maximum air temperature (°C) for the population to start rising is between 22°C and 24°C (Figure 2.8, I). Daily relative humidity at 3pm of more than 40% depresses *F. schutzei* populations in South Australia (Figure 2.8, J).

Although the response of the two thrips species differed in their weather requirement optima across the three states, population rises and depressions were observed over a fairy similar range. An exception to this was the fairly low minimum air temperature (°C) requirement for *F. schutzei* populations to start rising, as estimated by the Exponential models with a certainty of 75% in Victoria (Figure 2.8, C). Estimation by the Gompertz model produced similar range requirements although the variance ratio for the Exponential curve provided a better fit (Appendice 2 & 4). Similar range estimates were also obtained for *F. schutzei* in South Australia by both the Gompertz and Exponential models (Appendice 2 & 4). In general, *T. tabaci* population rise within potato crops require an optimum daily minimum air temperature (°C) range between 10°C and 12.0°C and daily maximum air temperature (°C) range of 14.5°C and 22°C. The observed versus predicted estimates for the daily relative humidity at 3pm in Tasmania had had low r^2 values *T. tabaci*. The general optimum daily minimum air temperature (°C) range for *F. schutzei* populations to start rising is between 4°C and 6°C and daily maximum air temperature (°C) range of 20°C and 22°C. Less than favourable daily relative humidity at 3pm of more than 40% depresses *F. schutzei* populations in both Victoria and South Australia. Both *T. tabaci* and *F. schutzei* populations are depressed by precipitation as indicated by three models across the sampling sites (Appendices 2, 3 & 4).

The ability of both *T. tabaci* and *F. schutzei* to start dispersing and build-up populations over a broad range of temperatures, and in particular at cooler temperatures between 4°C and 6°C for *F. schutzei*, plays a significant role in the epidemiology of TSWV in potato crops, as it determines if and when external inoculum will become available for infection, and consequently the initiation, sustenance and spread of an epidemic. The association between both *T. tabaci* and *F. schutzei* and the weather variables was found to be strong, as indicated by r^2 values (Table 2.7) to uncover some ecological trends and to encourage future research in this direction to better understand the epidemiology of TSWV in potato crops.

The relationship between predicted and observed values was consistent in the regression models and their validation. The unexplained variability (r^2 values, Appendices 2 –4) between the predicted and observed values takes the form of both under- and over estimation of the thrips populations during identifications and counting on the thrips from the yellow sticky traps. Both the under- and over-prediction observed in the models (Figure 2.8) has to be taken into account when interpreting the geographical thrips populations. However, beyond absolute thrips population numbers, the general TSWV incidence distribution pattern observed in the study matches the prediction trend of the models indicating the disease to be more common in both Victoria and South Australia than in Tasmania (Appendice 1). This pattern resembles variables, such as maximum and minimum air temperature, precipitation and relative humidity, selected during the exploratory analyses as relevant by the non-linear regression models and confirmed by the Gompertz and Exponential models. All these variables have a marked south-north gradient from temperate Tasmania to subtropical New South Wales. Consistently, maximum and minimum air temperature were among the variables all the models used (Gompertz, Logistic and Exponential) picked to separate thrips populations dynamics in potato crops. What both the Gompertz and Exponential models seem to suggest is that an increase in moisture (as determined by both precipitation and relative humidity) seem to correlate with depressed thrips populations and incidence of TSWV in potato crops and weeds (Appendice 1). In areas of higher precipitation and relative humidity prevalence, such as northwestern Tasmania, the negative effects of high moisture on thrips populations may account for the absence of TSWV in that part of Australia. In contrast, western Victoria and South Australia, which receive relatively higher temperatures for most part of the year, seem to experience more frequent and severe TSWV outbreaks as can be deduced from literature (Magee 1936; Bald, 1937; Norris and Bald, 1943; Conroy *et al*., 1949; Norris, 1951a, 1951b; Latham & Jones, 1996, 1997; Wilson, 2001). This may also explain the observation by Conroy *et al*., (1949), that the prevalence and severity of TSWV coincided with summer seasons with less precipitation (Conroy *et al*., 1949). However, weather variables alone do not fully explain the differences in thrips populations, as other effects evidently exist that were not measured in this study which may act in concert with these variables to affect thrips dispersion.

The fact that summer weather conditions seem very important when modeling thrips population dynamics is not surprising, given that (i), the potato growing areas in Australia has a strong seasonality, especially in temperature, precipitation, and relative humidity (Lindesay, 2003) (ii) commercial potato field growing in Australia occurs in summer (iii) the phenology of some weeds such as cape weed (*Arctotheca calendula*), an Autumn-Winter germinating annual, and Blackberry nightshade (*Solanum nigrum*) an Autumn-Spring germinating annual (Hyde-Wyatt *et al*., 1975; Wilding *et al*., 1998; Auld and Medd, 1987) synchronise well to influence the functioning and development of both thrips populations and hosts.

Is it possible to develop a model to more accurately predict TSWV outbreaks in potato crops? To answer this question, it is necessary to consider the various sources of TSWV, including those in non-cultivated ecologies (Funderburk, 2002), influence of temperature on the ability of both *T. tabaci* and *F. schultzei* to acquire and transmit the virus

(Chatzivassiliou *et al.,* 2002, Wijkamp *et al.,* 1995; Inoue *et al.,* 2002; Nagata *et al.,* 2002), flight dispersal patterns (Lewis 1997; Teulon & Penman, 1996; Sites *et al.,*1992) prevalence and virulence of TSWV strains in Australia (Latham & Jones 1998; Talty & Dietzgen, 2001; Dietzgen, 2003), host preference and subsequent performance of vector thrips species (Chapter 5; Futuyma & Moreno 1988; Thompson, 1988, 1994,1996; Courtney & Kibota 1990; Jaenike 1990; Andow, 1991; Terry 1997; Ullman *et al.*, 1997; Hobbs *et al.,* 1993; Bautista & Mau 1994; Mound, 2002), inter- and intra-specific interactions among thrips and other arthropod species and plants (Agrawal & Colfer, 2000; Agrawal, 2000; De Kogel, 2002; De Kogel & Koschier, 2002; Koschier & Sedy, 2002); trade-offs in fitness on different host plants (Jaenike, 1990; Chatzivassiliou *et al*., 1999; Agrawal 2000; Jones 1959, Paliwal 1974, 1976; Wijkamp *et al.,* 1995; Chatzivassiliou *et al*., 1998, 1999, 2001, 2002; Brodbeck *et al*., 2002; Sedy & Koschier 2003; Teulon *et al.*, 1993), differences between vector thrips species and sexes to acquire and transmit TSWV (Wijkamp *et al*., 1995; Nagata *et al.,* 2002), and cultural management tactics (Jenser *et al*., 2003; Stavisky *et al*., 2002). Most of these issues require local data to be useful in a model. Such issues were not fully considered in this study due to lack of adequate information.

The models developed in this study point at the influence of weather variables on both *T. tabaci* and *F. schutzei* dispersal and population dynamics. However, the study also indicates a more complex scenario than simply the effect of one or two variables. It is important to note that using weather variables over a limited period of two seasons may not be sufficient to understand the complexity of thrips dispersal and population dynamics on one hand, and TSWV epidemics in potato crops on the other. None of the TSWV incidences observed in both potato and weeds came up as positive correlations with thrips dispersal and population build-up, which would have shown some definite links in epidemic development. Such links are evidently in existence given the sporadic outbreaks that have occurred in potato crops in Australia.

The results of the models developed in this study can be used as a hazard prediction to orient integrated TSWV disease management (Jones 2004). To be effective and adopted successfully by growers and farm advisers, the models would need to be part of a decision support system and incorporated in such a way as to allow improvements and adjustments over time prior to each decision (Magarey *et al.,* 2002). The models are also useful in generating hypotheses and emphasizing the need for information and further research. Guided by the main findings of this study at the regional level, future research should focus on better understanding of vectoring efficiencies of the two thrips species in potato, links between external sources of inoculum from non-cultivated ecologies, and farming systems within regions, which in turn might be closely related to the observed TSWV epidemics in various crops in Australia. In this way, relationships and pattern can be integrated with plausible mechanisms.

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Chapter 3

COMPARATIVE RESISTANCE OF SELECTED POTATO CULTIVARS TO MECHANICAL AND THRIPS MEDIATED TRANSMISSION OF TOMATO SPOTTED WILT VIRUS

Among the most significant factors affecting the epidemiology of virus diseases is the inherent susceptibility of the cultivars being grown (Daughtrey *et al.*, 1997; Moury *et al*., 1997; Llamas-Llamas *et al*., 1998; Soler *et al*., 1998, 1999; Garg & Khurana, 1999; Kikkert *et al*., 1999; Wilson, 2001; Maris *et al*., 2003a; Aramburu & Martí, 2003), which in turn are influenced by the environment, type of virus, mode of transmission, cultural practices (Norris 1951a, 1951b; Roca *et al.*, 1997; Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998; Córdoba *et al*., 1991; Maris *et al*., 2003a), and age of the plant and inoculum pressure (Thresh 1974; Plumb & Thresh 1983; McLean *et al*., 1986; Moriones *et al.,* 1998; Soler *et al*., 1998; Thomas-Carroll & Jones 2003). Cultivar assessments for viral diseases can yield information that facilitates an understanding of virus epidemiology. More often than not, the main agricultural practice of monoculture, i.e., the planting of large acreages with a single monogenic crop that is susceptible to viruses, favours the rapid development of disease (Thresh 1974; Gray and Banerjee 1999), particularly if vector intensity and propensity, and sufficient inoculum exist within the environment (Duffus 1971; Irwin & Ruesink 1986). By contrast, tolerant or resistant cultivars reduce disease progress and sometimes delay disease onset (Culbreath *et al.* 1992; Camann *et al.* 1995; Maris *et al*., 2003a, 2003b; Yang *et al*., 2004). The use of resistant cultivars is a well-known means of virus disease control and applicability (Plumb & Thresh, 1983; Culbreath *et al.* 1992; Camann *et al.* 1995; Hadidi *et al.* 1998; Maris *et al*., 2003a, 2003b; Yang *et al*., 2004). This approach has been recommended repeatedly as a means of controlling TSWV in many other crops (Cho *et al*., 1998; Plasencia & Sánchez, 1999), particularly due to public concern over human health and the environmental limitations to the use of insecticides. The repeated upsurge of TSWV epidemics in potato has increased the need for virus-resistant potato cultivars. And there are obvious long-term advantages in using such a strategy, which include enhancement of productivity through reduction in damage to the crop through feeding, consequential transmission of TSWV and the opportunity for further spread by vectors.

The use of potato varieties with high levels of resistance or tolerance to TSWV presents the best option in view of the role played by vector thrips carrying the virus from external sources thereby rendering chemical control less effective. Sources of resistance to TSWV in some potato cultivars have been identified (Hooker 1981; Wilson 2001). These sources of resistance to TSWV could be exploited by incorporating them into other potato cultivars. Screening for host-plant resistance and subsequent breeding programs to introduce genetic resistance to vector thrips, should also aim at maintaining other desirable qualities for yield, processing quality and appearance in order to be of any benefit to potato growers, processors and consumers. Resistance to TSWV is not immunity and seems to be overcome under certain conditions (Thompson & van Zijl 1996; Qiu *et al.,* 1998; Qiu & Moyer 1999; Roggero *et al.,* 2002; Aramburu & Martí 2003; Thomas-Carroll & Jones 2003), thus underlining the importance of selecting and breeding for both glasshouse and field resistance.

Commercial production of potato in Australia utilizes a number of varieties (Isenegger *et al*., 2001), which are almost always grown in large patches of genetically homogeneous geneotypes. These potato varieties have many desirable attributes, but more traditional cultivars such as Shepody and Atlantic are susceptible to both foliar and tuber infections of tomato spotted wilt virus (TSWV), which pose a serious yield and quality constraint (Wilson 2001). Although potato breeding for aphid-transmitted viruses (Rodoni, 2003), yield and other qualities (Kirkham *et al.,* 2001; Isenegger *et al.,* 2001; Dawson *et al.,* 2002; Williams *et al.,* 2003), have been successfully carried in Australia, selecting and breeding for resistance to TSWV is yet to be done. Despite significant efforts and time spent to produce seed potato crops with low levels of the virus, sporadic epidemics of TSWV occur because of the many hosts of both the thrips vectors and virus in uncultivated environments, making disease management by physical, chemical or biological methods difficult. Thus, host plant resistance seems to offer the best strategy in managing the disease in potato. Indeed, in many other crops, numerous efforts have been invested in identification of sources of resistance to TSWV (Roselló *et al.,* 1998; van de Wetering *et al.,* 1999; Mandal *et al.,* 2002; Cebolla-Cornejo *et al.,* 2003; Maris *et al.,* 2003a, 2003b; Yang *et al.,* 2004) although this resistance has been overcome in many instances (Aramburu & Martí 2003; Roggero *et al.,* 2002) due to virus reasortments of the genomes (Qiu *et al.,* 1998; Qiu & Moyer 1999) and consequently leading to epidemics (Moury *et al*., 1997; Roggero *et al*., 2002; Bucher *et al*., 2003). And because outbreaks of TSWV are infrequent, but severe when they occur, potato growers in Australia apply protectant insecticides every year regardless of actual disease threat. The use of host resistance would reduce the costs of using insecticides to protect susceptible cultivars, as these are likely to be much greater than the cost of the breeding programme in the long term.

The rate of spread of TSWV within a susceptible plant population is affected by the age at which plants are infected with the virus (Thresh 1974). In potato, plants tend to become decreasingly vulnerable to infection with age (Norris 1951a, 1951b; Wilson 2001), even though they come into more contact with neighbouring plants and present an increasing catchment area to thrips. Less virus is also translocated downwards to tubers (Norris 1951a, 1951b; Wilson 2001); a process Beemster (1987) termed 'mature plant resistance'. This phenomenon has also been observed in tomato (Moriones *et al.,* 1998). Mature plant resistance to TSWV is cultivar specific and virus translocation has been observed to be higher in cultivars such as Atlantic and Shepody, but less evident in the cvs Russet Burbank and Coliban (Wilson 2001).

Wilson (2001) also observed that some cultivars such as Russet Burbank and Coliban display moderate resistance to foliar and tuber infections of TSWV. It can, therefore, be speculated that the relative effect of these tolerant cultivars on disease distribution and progress over time and space would be significant as observed in other crops (Maris *et al.,* 2003a, 2003b), and therefore, it would be beneficial to evaluate their potential as one of the tools for management of TSWV. The observation in potato (Norris 1951a, 1951b; Wilson 2001) that plants arising from second generation tubers exposed to

TSWV have lower incidence of the disease is potentially enormous, since benefits of host gene-mediated resistance (gene-silencing mediated protection) can be combined with other desirable agronomic characteristics in the breeding programme. However, there is still a need to study the role of reversion and proteolysis in virus elimination in potato. Once resistance is identified in potato, further research would also be needed on the most appropriate means of deploying TSWV-resistant cultivars to avoid rapid degradations of resistance due to selection pressure on both the virus and its vectors (Hollings 1965; Latham & Jones 1998; Aramburu & Martí 2003; Roggero *et al.,* 2002). Spatial diversification of host resistance appears to be a major technique to achieve successful and durable management of crop pathogens by genetic means (Hollings 1965; Thresh 1982, 1983a).

The components of TSWV resistance in potato, particularly restriction of virus translocation from shoots to tubers is not well understood. Remarkably, little is known about the reaction of most of the commercially grown potato varieties to TSWV infections. In this study, the objective was to assess the reaction of a broader range of potato cultivars with diverse genetic backgrounds (Isenegger *et al*., 2001) to TSWV in controlled (glassouse) and naturally field conditions consistent with normal commercial growing practice for the region, and to evaluate the effects of infections at different stages of plant growth.

Materials and Methods

Cultivar evaluation through mechanical inoculations under Glasshouse conditions

Trial planting and maintenance

Two sets of twenty seven (27) potato cultivars that had tested negative by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) protocol (Clark & Adams, 1977) using antisera to TSWV lettuce strain (Agdia, IN, USA), were planted in 24 x 30cm black polyethylene bags containing potting mix and arranged in four replicates of a randomized complete block (RCD) design. Atlantic and Russet Burbank, previously demonstrated to be susceptible and resistant to TSWV, respectively (Wilson 2001) were included as positive and negative controls. The plants were maintained in an insect-proof glasshouse (Temp.= 20-30^{o}C, RH = 55 ± 5%) at the facilities of the NewTown Research Laboratories, NewTown, Tasmania. Only single shoots were allowed to grow after emergence by roguing of additional shoots.

Inoculum source and mechanical inoculation

Although natural populations of TSWV isolates are highly heterogeneous with a great capacity for genetic variation (Moyer *et al.*, 2003), there are no significant molecular differences observed among TSWV strains in Australia (Talty & Dietzgen, 2001; Dietzgen, 2003). However, resistance-breaking strains have been reported (Latham & Jones 1998). TSWV isolate An_{WA}*-1* maintained in tomato (*cv.* Grosse Lisse, Arthur Yates & Co. Ltd, Homebush, NSW, Australia) was used for all inoculations. On one set of twenty-seven (27) potato cultivars, mechanical inoculation was done 28 days after planting (DAP), approximately 3 weeks after emergence when most plants had three or more fully expanded leaves. Inoculation was by abrading three youngest fully expanded potato leaves with infected tomato leaf sap diluted 1:20 (w/v) in 0.2M phosphate buffer (pH 7.4) containing celite and maintained on ice. The second set of twenty-seven (27) potato cultivars was also mechanically inoculated at 60 days after planting using the same virus source and inoculation protocol as in the first set. After inoculation, plants were maintained in the glasshouse for further observation. Inoculations at 28 DAP were defined as 'early' and those at 60 DAP as 'late'.

Virus incidence scoring and testing.

TSWV incidence, based on symptom expression, was scored on a binary system, 1 (present) or 0 (absent) on all plants in the trial starting at two weeks after inoculation. Symptom severity, as a measure of localised or systemic infection in shoots, was scored on an increasing rating of 1 to 5 (1 = no disease symptoms, 2 = few concentric necrotic spots, lines or specks covering less than 25% of canopy; 3 = concentric necrotic spots, lines or specks covering 25-50% of canopy; 4= concentric necrotic spots, lines or specks covering 50-75% of canopy; 5= numerous concentric spots (>70%) with significant shrivelling of leaves. TSWV infection was verified by serological assessments starting three weeks after inoculation using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) protocol with a monoclonal antibody mixture used in 1: 400 dilution for both capture and detection (Agdia Inc.). Leaf sample extracts (w/v) were prepared by crushing ~0.1g in phosphate buffered saline (pH 7.4) containing 2ml litre^{-1} Tween 20 and 20g litre^{-1} polyvinyl pyrrolidone using a press. Long-distance movement of the virus to tubers was tested by ELISA soon after harvest. Protocols for preparations of tuber extracts were previously described by Wilson (2001) and involved the use of a sliver of tuber tissue from the entire longitudinal inner half section of the tuber being tested. Expressed sap (100μl) was tested for TSWV in duplicate wells of a flat bottom microtitre plate (NUNC, maxisorb) in a crisscross plate layout. Positive and negative controls were included on each plate. Absorbances (A_{405} nm) were measured with a photometer (Titertek) 30 and 45 minutes after addition of 0.5 mg ml^{-1} p-nitrophenyl phosphate in 10 ml litre^{-1} diethanolamine, pH 9.6 as substrate. The absorbance values were corrected for blank values consisting of only extraction buffer in the sample incubation step. Samples were considered positive if they had absorbances greater than three standard deviations of the mean of the negative controls (Sutula *et al*., 1986). Percentage infection of composite samples was calculated using binomial theorem (Gibbs & Gower, 1960). The assessments of cultivars were repeated twice to confirm results.

Cultivar evaluation by thrips transmission under natural field conditions

Trial site, planting and maintenance

In comparative field evaluations, twenty (20) potato varieties from the twenty-seven (27) tested in glassouse trials were selected for testing during 2001/2002, based on analysed data used for assigning TSWV disease reaction. Twenty-one (21) varieties were evaluated during 2002/2003. These were planted at the University of Tasmania Farm, Cambridge, Tasmania, in January during 2001/2002 season as was generally done in the southern part of Tasmania due to heavier than normal rainfalls at the start of the season in November 2001. This extended the growing season to May 2002. The second trial during 2002/2003 was planted in December at the same site as the previous one. The experiment was conducted in a randomised block design with plots of 5 x 1m in four replications on a patch of land previously sprayed with a herbicide as done under commercial practice. Seed tubers of each potato variety previously certified negative by DAS-ELISA were planted at a spacing of 50cm within each ridge (10 plants in each row). The experiments were kept weed free by hand weeding and supplementary irrigation provided by overhead sprinkler irrigation. All plants in each plot were examined individually for incidence and severity of TSWV infections during each measurement date. No fungicide or insecticide was used in the trials and plants were grown until complete senescence of foliage. The evaluations were repeated over two consecutive seasons (2001/2002 and 2002/2003) to confirm results.

Inoculum source and maintainance

The same TSWV isolate *An_{WA}-1*, used in glasshouse assessments and maintained in tomato plants (*cv.* Grosse Lisse, Arthur Yates & Co. Ltd, Homebush, NSW, Australia) was mechanically inoculated onto young tomato plants of the same cultivar. Infected tomato plants were then planted on each side of the four replications in the field a week prior to potato planting to serve as sources of inoculum. This virus-infected spreader row pattern was employed to ensure a high and uniform inoculum pressure within the trial area. The advantage of using tomato plants is that they have indeterminate growth and remained viable source of TSWV throughout the duration of the trials. Thrips vectors, *T. tabaci* occurred naturally at this location. A patch of gum trees and natural vegetation flanked the trial site on its northern side. This patch of vegetation fundamentally influenced wind direction making it to blow mainly eastward or westward across all the replications and thus spreading thrips vectors.

Thrips monitoring

Thrips populations were monitored using five 20 X 25cm yellow sticky traps (Seabright Laboratories, Emeryville, CA 94608, USA) suspended just above the plant canopy and continuously adjusted with plant growth. Five traps were place around the trial site in a "Z: pattern, with four traps placed evenly around and close to the edges of the trial and one in the centre to allow for directional monitoring of incoming thrips. The traps were changed weekly, and the caught thrips were counted and identified under the microscope to species.

Virus incidence scoring and testing.

Individual plants in the trial were visually scored for TSWV symptoms and tested by ELISA at 28 and 60 days after planting (DAP). For this study, 28 DAP was defined as early vector transmission and 60 DAP as late vector transmission. TSWV incidence, based on symptom expression on the shoot, was scored on a binary system, 1 (present) or 0 (absent). Symptom severity, as a measure of localised or systemic shoot infection, was scored on an increasing rating of 1 to 5 (1 = no disease symptoms, 2 = few concentric necrotic spots, lines or specks covering less than 25% of canopy; 3 = concentric necrotic spots, lines or specks covering 25-50% of canopy; 4= concentric necrotic spots, lines or specks covering 50-75% of canopy; 5= numerous concentric spots (>70%) with significant shrivelling of leaves.

Serological assessment (DAS-ELISA) of virus incidence in leaves and tubers was also carried out as described above for glasshouse samples using antiserum to TSWV lettuce strain (Agdia, IN, USA). In shoots virus testing was done at the time of visual scoring. Virus translocation to tubers was tested soon after harvest. For leaf samples, the individual leaves from all the plants within each 5m row were tested as compound samples in each well. Positive compound samples were retested to obtain actual incidence values within each row. Shoots that tested positive for TSWV were tagged and tubers harvested separately after natural senescence of shoots. TSWV infection in tubers was tested by ELISA as described above.

Data analysis

All data were analysed using Genstat software ver 6.1.0.200 (Lawes Agricultural Trust, 2002). Initial regression analysis of data for individual assays using log-linear modeling with a count variate, poisson distribution and a log link function indicated no significant differences in response for the combination of cultivar and inoculation (i.e. Cultivar.Inoculation), Inoculation (early/late), or cultivar (Atlantic, etc.) as indicated by the absence of any significant interaction between response and inoculation, or response and cultivar. A comparison between this model and the chi-square goodness of fit allowed an assessment of lack of fit (how well the analysis described the variability in the treatment means in each season and method of inoculation) taking into account the binomial nature of the data (Little and Hills, 1978; McCullagh and Nelder 1989). Data from all experiments were therefore, subjected to analysis using a poisson generalised mixed model with a logit link which included the main effects of cultivar, time of inoculation and their interactions as fixed models, with replication over two seasons as a random model. Wald statistics was used to evaluate the fixed effects in the model (i.e cultivar, time of inoculation and a cultivar x inoculation interation). The predicted output of means for the logit transformed and back-transformed data (i.e. means in the original scale of measument) were used in the pairwise comparisons using the least significance difference. The between-assessment dispersion was used to calculate all standard errors presented and as the baseline in *F* tests done for all other comparisons and the analysis of parallelism as part of an analysis of deviance (McCullagh and Nelder 1989). Contigency table pearson and maximum likelihood chi-square tests with single variate and

grouping factors were done to assess any differences relating to the time (early vs. late) and method of inoculation (mechanical vs. thrips) (Little and Hills, 1978) and residuals generated where significant differences were identified. A probability level (under null hypothesis) of 0.05 was used for all tests to determine significance. In field experiments the mean weekly thrip population was calculated from the mean counts of the five traps used during each week.

Results

Cultivar symptom expression

Tables 3.1 and 3.2 reveals that all potato cultivars assessed by either mechanical or thrips transmission, in early and/or late growth stages, expressed TSWV symptoms and the presence of the virus was confirmed by ELISA, indicating that the cultivars possessed no immunity to the virus. The regression model indicated that the number of plants with symptoms varied significantly ($P<0.001$) between times of inoculations (plant age at inoculation), with more cultivars exhibiting symptoms when infected late than early, both under glasshouse ($\chi^2 = 114.66$, means: Early =43.5%, Late =81.0%); and field ($\chi^2 = 119.74$, means: Early = 2.9%, Late =19.1%) conditions (Table 3.1, Figure 3.2 and Table 3.2). Notable exceptions to this pattern were the cvs Kipfler and Up-To-date which had lower numbers of plants showing symptoms when mechanically inoculated late (Table 3.1). The main effects of cultivar alone or its interaction with time of inoculation were not significant (P (cultivar) $= 0.305$; P (cultivar x time of inoculation) $= 0.787$).

All cultivars exhibited varying incidence and severity levels of symptoms when mechanically inoculated. Except in the cvs Atlantic, Shepody, Bismark, Kipfler and Victoria in which systemic infections were observed, symptoms in other cultivars mainly consisted of local necrotic lesions in late mechanically inoculated plants. In cvs Atlantic, Shepody, Bismark, Kipfler and Victoria, systemic infections progressed much more rapidly after first symptoms appeared; becoming generalized chlorotic and necrotic spotting in axillary and terminal shoots. The time taken for the first TSWV symptoms to appear in cultivars varied and ranged from 15 –20 days after inoculation. Early mechanical inoculations in cvs Royal Blue, Bismark, Kipfler and Up-To-Date resulted in >75% of the plants showing TSWV symptoms. In late mechanical inoculations, all plants in cvs Bismark, Brownell, Coliban, Nicola, Ranger Russet, Victoria and McCains 1 exhibited TSWV symptoms (Table 3.1). The type and intensity of symptoms expressed also varied among cultivars (Figure 3.1).

Generally, the onset of symptoms in most cultivars under field conditions was slower than in mechanical inoculations in the glasshouse, with first symptoms appearing 20 days after sprouting. Progress of symptom expression was slow in the cvs Bintje, Brownell, Kipfler, Sebago, Tasman, Royal Blue, Goldstar and McCains 1 although virus infections had already got established in the plants as evident from ELISA results (Table 3.2). Under field conditions where plants were allowed to grow multiple stems, partial infections involving single stems or part of the stem were observed in cvs Atlantic, Shepody and Ranger Russet. The cv. McCains 1 was of strikingly particular interest as plants grew vigorously and appeared healthy until mid to late season when 21% of the plants eventually expressed necrotic symptoms reaching 100% under late mechanical inoculations. In cvs Atlantic, Bismark and Kennebec more plants expressed symptoms in early infections (Table 3.2). However, the cv. Bismark exhibited high levels of resistance to thrips feeding across two seasons (Figure 3.2), with only one plant expressing symptoms during the first season of testing suggesting the involvement of vector-mediated resistance components since the same cultivar became systemically infected and exhibited symptoms when mechanically inoculated (Table 3.1). Plants showing symptoms in Bismark had a clustered pattern close to an infector tomato plant and evident in only one replication during the second year of assessment. The cvs Atlantic, Brownell, Desiree, Pontiac, Ranger Russet, Yellow King, Russet Burbank, Royal Blue, Gold Star, Victoria and McCains 1 had 21-44% of the plants showing symptoms when infected late in the season.

Strikingly, in plants that were infected early, through both mechanical and thrips virus transmissions, most of the symptoms were confined to initially inoculated leaves in the lower parts of the plant canopy and axillary shoots. Non-inoculated leaves and newly emerging apical leaves did not exhibit symptoms until later in their growth stages.

Infected tubers of the cvs Atlantic, Russet Burbank, McCains1 had generally little or no internal tuber symptoms. Where symptoms were present, particularly in early inoculations, they tended to take the form of occasional necrotic spotting in these cultivars although patchy blotching was also observed in tubers of Atlantic (Figure 3.1). Infected tubers from late inoculated plants were in most cases symptomless. In contrast, the cvs Shepody and Bismark had conspicuous internal necrotic symptoms. In Bismark, internal tuber necrosis followed vascular tissues. In shepody irregular necrotic tissues appeared more scattered in infected tubers (Figure 3.1). Except in the cv. Bismark, infected tubers did not exhibit external symptoms. In Bismark, infected tubers were in most cases, misshapen and had a strikingly distorted external appearance.

Virus detection by ELISA in shoots

Generally, plants that exhibited TSWV symptoms also tested positive for the virus in ELISA (Table 3.1 and 3.2) although symptomless plants of the cvs Bintje (2%), Kipfler (2%), Sebago (1%), Royal Blue (1%) and McCains 1 (3%) tested positive for the virus in early infections under field conditions. ELISA results followed a similar trend to that observed for symptom expression with a statistically significant difference in the number of shoots testing positive for TSWV between mechanical ($\chi^2 = 186.54$, means: Early = 29.2%, Late =63.9%, $P < 0.001$) and thrips inoculations ($\chi^2 = 88.71$, means: Early = 2.3%, Late =12.0%, $P = 0.001$) (Table 3.1 and Table 3.2). Overall, late inoculations resulted in higher number of shoots testing positive for the virus than early inoculations with the exception of Bismark and Kennebec (mechanical inoculation) and Bismark and Kennebec, King Edward, Tasman, Up-To-Date and Shepody (thrips transmission) (Table 3.1and Table 3.2). A high level of resistance to both thrips feeding damage and TSWV was observed in the cv. Tasman in which ELISA did not detect the virus in early mechanical inoculations and both early and late field infections. This cultivar, however, succumbed to the virus in late mechanical inoculations (Tables 3.1 and Table 3.2).

Table 3.1. Mean TSWV Incidence and translocation to tubers in potato varieties mechanically inoculated during 2001/2002 and 2002/2003

Cultivar	Plants with TSWV symptoms (%) [i]		TSWV positive Shoots (%)(ELISA) [ii]		Tubers (%)(ELISA) (Positive tubers/total tested) [iii]	
	Early Inoculation	Late Inoculation	Early Inoculation	Late Inoculation	Early Inoculation	Late Inoculation
Atlantic (Susceptible Control)	25	63	63	75	31.3 (5/16)	25.0 (3/12)
Bintje	38	63	50	63	4.8 (1/21)	0.0 (0/26)
Bismark	75	100	63	63	29.4 (5/17)	7.1 (1/14)
Brownell	38	100	25	75	16.7 (4/24)	0.0 (0/19)
Coliban	50	100	25	88	6.7 (1/15)	0.0 (0/19)
Desiree	25	88	38	63	0.0 (0/17)	0.0 (0/22)
Fontenot	25	88	50	88	17.6 (3/17)	5.3 (1/19)
Granola	25	63	25	50	0.0 (0/20)	0.0 (0/26)
Kennebec	25	50	63	38	44.4 (8/18)	6.7 (1/15)
King Edward	25	63	25	38	4.3 (1/23)	0.0 (0/30)
Kipfler	75	63	25	63	26.1 (6/23)	0.0 (0/28)
Pink Eye	50	88	38	63	4.3 (1/23)	0.0 (0/20)
Pontiac	38	75	25	63	11.1 (2/18)	0.0 (0/20)
Nicola	25	100	25	75	0.0 (0/26)	0.0 (0/24)
Nooksack	25	75	13	50	0.0 (0/7)	0.0 (0/11)
Ranger Russet	50	100	25	63	14.3 (2/14)	0.0 (0/16)
Sebago	50	88	25	50	0.0 (0/15)	0.0 (0/13)
Tasman	38	88	0	50	0.0 (0/11)	0.0 (0/19)
Up-To-Date	75	63	13	63	0.0 (0/18)	0.0 (0/20)
Yellow King	38	75	0	50	0.0 (0/25)	0.0 (0/28)
Viking	63	75	38	75	0.0 (0/20)	0.0 (0/14)
Russet Burbank (Tolerant Control)	38	75	13	75	9.5 (2/21)	6.3 (1/16)
Shepody	25	75	13	63	23.5 (4/17)	0.0 (0/13)
Royal Blue	88	88	13	63	5.3 (1/19)	0.0 (0/25)
Gold Star	38	88	13	88	0.0 (0/19)	0.0 (0/24)
Victoria	50	100	50	63	18.8 (3/16)	0.0 (0/16)
McCains 1	63	100	38	75	15.0 (3/20)	5.9 (1/17)
Mean	**43.7**	**81.3**	**29.4**	**64.2**	**10.5**	**2.1**

[i] = % plants with TSWV symptoms :

P (cultivar) = 0.305
P (time of inoculation) < 0.001 (1 d.f., l.s.d. = 9.53)
P (cultivar x time of inoculation) = 0.787
Pearson Chi-square value (Time of inoculation) = 111.66 with 26 d.f
Likelihood Chi-square value (Time of inoculation) = 114.66 with 26 d.f.

Time of Inoculation [i]	Mean	
Late	81.0	a
Early	43.7	b

[ii] = ELISA : % TSWV positive shoots :

P (cultivar) = 0.351
P (time of inoculation) < 0.001 (1 d.f., l.s.d. = 8.60)
P (cultivar x time of inoculation) = 0.570
Pearson Chi-square value (Time of inoculation) = 168.07 with 26 d.f
Likelihood Chi-square value (Time of inoculation) = 186.54 with 26 d.f

Time of Inoculation [ii]	Mean	
Late	64.2	a
Early	29.4	b

[iii] = ELISA : TSWV translocation to tubers (positive tubers/total tested) :

P (cultivar) = 0.197
P (time of inoculation) = 0.005 (1 d.f., l.s.d. = 4.71)
P (cultivar x time of inoculation) = 0.985
Pearson Chi-square value (Time of inoculation) = 185.45 with 26 d.f
Likelihood Chi-square value (Time of inoculation) = 226.57 with 26 d.f

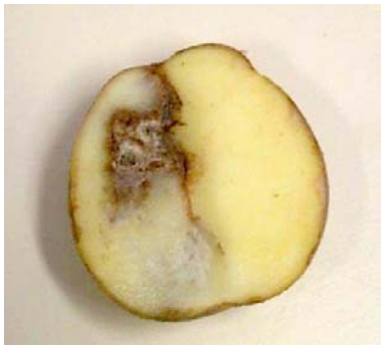
Time of Inoculation [iii]	Mean	
Late	2.1	a
Early	10.5	b

Leaf Symptoms **Tuber Symptoms**

Kipfler

Atlantic

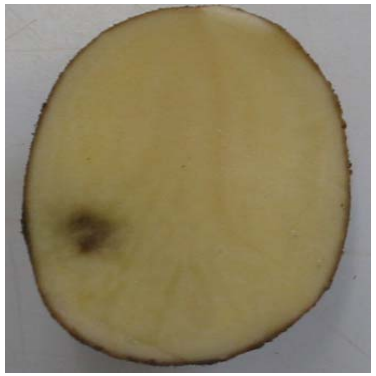
Bismark



Atlantic

Pink Eye

Shepody



McCains 1

Shepody

Figure 3.1. Variation of Leaf and tuber symptoms of TSWV in selected potato varieties

Assessment of long-distance movement of virus from shoots to tubers by ELISA

In testing the effects of cultivar and time of inoculation on the translocation of TSWV to tubers, the regression analysis indicated that in many cultivars (n=17, Table 3.1, Figure 3.4), virus translocation was significantly higher (χ^2 =226.57, *P =0.005*) in early compared to late mechanically inoculated plants (Table 3.1). Inversely, the significantly higher shoot infections resulting from late mechanical inoculations did not generally translate into detactable virus infections in tubers except in cvs Atlantic (25%), Bismark (7.1%), Fontenot (5.3%), Kennebec (6.7%), Russet Burbank (6.3%) and McCains 1 (5.9%). Exceptionally high proportions of infected tubers were detected from early mechanically inoculated plants of cvs Atlantic (31.1%), Bismark (29.4%), Fontenot (17.6%), Kennebec (44.4%), Kipfler (26.1%), Shepody (23.5%), Victoria (18.8%) and McCains 1 (15%) (Table 3.1).

Under field conditions, the virus translocation was not significantly influenced by time of infection (*p= 0.198*) but, nevertheless, cultivars differed widely in virus translocation efficiency (χ^2 = 68.41, Table 3.2). Early infections resulted in high percentages of infected tubers in the cvs Atlantic (17.1%), Bismark (15.0%) and Yellow King (13.8%). Infected tubers of the cvs Atlantic, Bismark, Russet Burbank and McCains 1 were detected across all times (early and late) and methods of inoculation (mechanical and thrips transmissions). In contrast, no virus was detectable in tubers of the cvs Sebago, Tasman, Up-To-Date and Gold Star in both early and late mechanical and thrips transmissions despite having shoot infections (Table 3.1 and Table 3.2).

Thrips population dynamics

Weekly trap counts and identification of adult thrips from November 2001 to June 2003 indicated that *Thrips tabaci* was the predominant species during both seasons at the University of Tasmania farm, Cambridge, Tasmania, accounting for 63% of the total count. Routine sex identication of *T. tabaci* population collected at the trial site found them to be 100% female, particularly toward the end of the season, although the existence of males during the season could not be discounted (L. Mound, personal communication). Most of the remaining adult thrips were *Tenothrips* (12%), *Limothrips cerealium* (11%), *Thrips imaginis* (8%), *Thrips australis* (2%), *Tubuliferan sp.* (1%), and Others (*C. manicatus, Limothrips angulicornis, Pseudanaphothrips achaetu*s, *Apterothrips apteris*)(3%). *Thrips imagines* has not been recorded previously in the region, which would suggest that this species was a relatively new introduction to Southern Tasmania. Except for *T. tabaci* the other species trapped are not known to vector TSWV. Therefore, *T. tabaci* was assumed to be responsible for the virus transmissions observed in the field trials. The initial rise in *T. tabaci* population densities from November, which peaked toward the end of January, also coincided with early infections observed in the field trial (Figure 3.3). The second thrips population increase was at the start of March, which peaked during the third week of the same month and declined in mid April. Although the presence of TSWV in thrips was not determined in these populations, it is likely that the increase in the incidence of infections (Table 3.1 and 3.2) is related to the increase in thrips abundance.

The spacial spread of infections in the trials was influenced by wind direction, which were from east- westerly and west-easterly across all the replications. Turbulence caused by a shelter-belt of mixed vegetation (mainly eucalyptus and native grasses) running along a creek on the northern side of the trial site modified these winds. Consequently, higher frequencies of infected plants within the experiments were observed in the outer replications (1 and 4), with infections originating from infector tomato plants on the sides. The trial site had no history of TSWV epidemics and no evidence of external TSWV inoculum sources were observed. The other perimeters of the trial site were surrounded by a cereal crop (Triticale), a non-host of TSWV, hence the high population of *L. cerealium* (11%), a non-vector of TSWV.

Table 3.2. Mean TSWV Incidence and translocation to tubers in potato varieties naturally infected by thrips in the field during 2001/2002 and 2002/2003

	Plants with TSWV symptoms (%) [i]		TSWV positive Shoots (%)(ELISA) [ii]		Tubers (%)(ELISA) (Positive tubers/total tested) [iii]	
Cultivar	**Early Inoculation**	**Late Inoculation**	**Early Inoculation**	**Late Inoculation**	**Early Inoculation**	**Late Inoculation**
Atlantic	8	35	6	20	17.1 (13/76)	6.5 (5/77)
Bintje	0	9	2	12	8.8 (7/80)	1.3 (1/80)
Bismark	8	11	5	4	15.0 (12/80)	5.3 (4/76)
Brownell	0	25	0	19	0.0 (0/80)	1.3 (1/79)
Desiree	5	25	1	25	3.8 (3/80)	0.0 (0/80)
Kennebec	7	7	1	0	0.0 (0/75)	0.0 (0/80)
King Edward	2	5	1	0	0.0 (0/80)	0.0 (0/80)
Kipfler	0	10	2	7	2.5 (2/80)	0.0 (0/80)
Pink Eye	2	7	4	15	1.3 (1/78)	0.0 (0/70)
Pontiac	3	26	1	15	0.0 (0/78)	0.0 (0/80)
Ranger Russet	4	44	1	29	0.0 (0/74)	8.5 (6/71)
Sebago	0	7	1	3	0.0 (0/79)	0.0 (0/80)
Tasman	0	17	0	0	0.0 (0/80)	0.0 (0/80)
Up-To-Date	4	4	4	0	0.0 (0/80)	0.0 (0/80)
Yellow King	4	21	3	3	13.8 (11/80)	0.0 (0/80)
Russet Burbank (Tolerant Control)	5	41	1	19	2.7 (2/75)	6.5 (4/62)
Royal Blue	0	26	1	12	0.0 (0/79)	1.3 (1/80)
Gold Star	0	25	0	16	0.0 (0/80)	0.0 (0/80)
Victoria	3	32	3	32	0.0 (0/76)	0.0 (0/75)
McCains 1	0	21	3	16	1.3 (1/80)	1.3 (1/80)
Shepody (Susceptible Control)	2	2	1	0	0.0 (0/37)	0.0 (0/40)
Mean	**2.7**	**19.0**	**2.0**	**11.8**	**3.2**	**1.5**

[i] = % plants with TSWV symptoms :

P (cultivar) = 0.832
P (time of infection) < 0.001 (1 d.f., l. s.d. = 6.92)
P (cultivar x time of infection) = 0.880)
Pearson Chi-square value (Time of inoculation) = 98.76 with 20 d.f
Likelihood Chi-square value (Time of inoculation) = 119.74 with 20 d.f

Time of Infection [i]	Mean	
Late	19.0	a
Early	2.7	b

[ii] = ELISA : % TSWV positive shoots :

P (cultivar) = 0.893
P (time of infection) = 0.001 (1 d.f., l. s.d. = 5.63)
P (cultivar x time of infection) = 0.843
Pearson Chi-square value (Time of inoculation) = 72.16 with 20 d.f
Likelihood Chi-square value (Time of inoculation) = 88.71 with 20 d.f

Time of Infection [ii]	Mean	
Late	11.8	a
Early	2.0	b

[iii] = ELISA : TSWV translocation to tubers (positive tubers/total tested) :

P (cultivar) = 0.346
P (time of infection) = 0.198 (1 d.f., l. s.d. = 2.74)
P (cultivar x time of infection) = 0.684
Pearson Chi-square value (Time of inoculation) = 54.70 with 20 d.f
Likelihood Chi-square value (Time of inoculation) = 68.41 with 20 d.f

Time of Inoculation [iii]	Mean	
Late	1.5	a
Early	3.2	a

Figure 3.2 The variety Bismark (third row from left) showing high levels of resistance to thrips feeding damage and TSWV infection in a variety trial (2001/2002), University of Tasmania Farm (Cambridge, Tasmania)

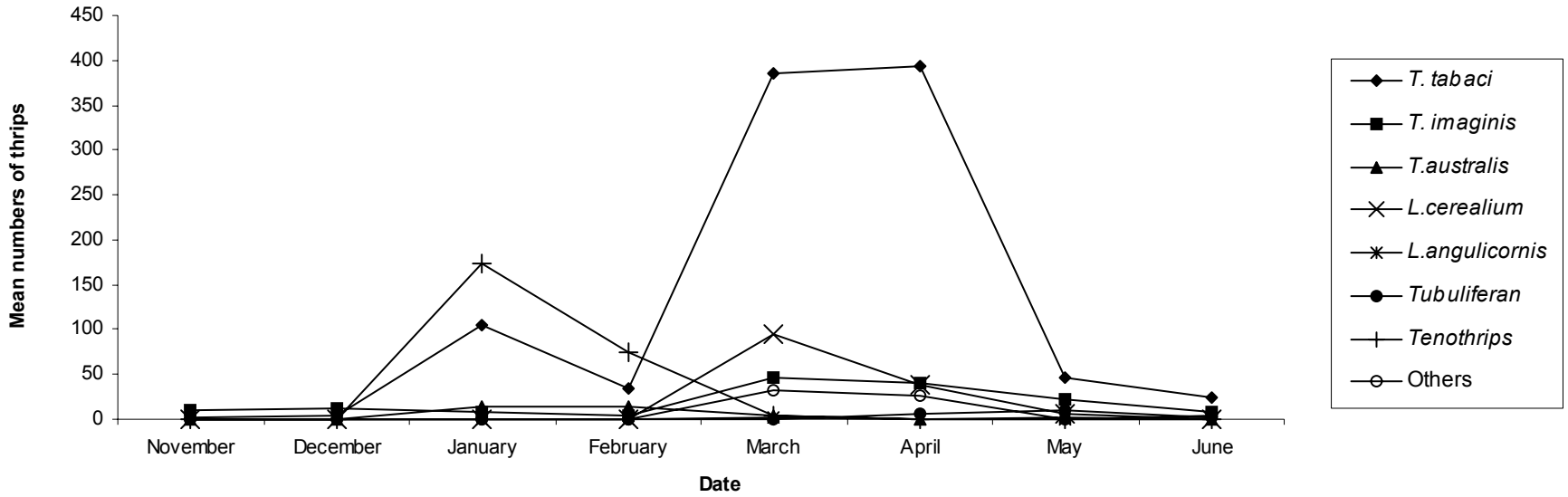


Figure 3.3. Mean seasonal variability of thrips population at the University Farm, Cambridge Tasmania, 2001-2003

Table 3.3. Weekly mean thrips numbers in the field trials, University Farm, Cambridge, Tasmania

	Average weekly thrips numbers																					
Thrips species	25-Nov	9-Dec	17-Jan	24-Jan	31-Jan	7-Feb	14-Feb	21-Feb	28-Feb	7-Mar	15-Mar	22-Mar	29-Mar	5-Apr	12-Apr	19-Apr	26-Apr	10-May	17-May	24-May	7-Jun	**Weekly mean**
Thrips tabaci	3	4	64	182	68	96	4	33	2	717	66	846	661	467	315	738	53	123	18	0	25	214
Thrips imaginis	9	12	12	8	2	4	5	5	3	89	14	90	46	52	25	61	23	47	20	2	9	26
Thrips australis	0	0	3	21	19	11	1	39	2	4	2	0	0	0	0	0	0	2	2	0	0	5
Chirothrips manicatus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limothrips cerealium	0	0	0	0	0	1	0	0	0	119	21	191	285	90	35	17	12	14	5	0	0	38
Limothrips angulicornis	0	0	0	0	0	0	0	0	0	8	0	0	3	0	0	0	0	0	0	0	0	1
Tubuliferan sp.	0	0	0	0	0	0	0	0	0	2	0	0	4	4	3	8	12	23	5	0	3	3
Tenothrips	0	0	264	198	56	200	14	68	13	4	3	0	0	0	0	0	0	0	0	0	0	39
Others	0	0	1	0	0	2	0	1	0	95	10	51	0	45	31	30	0	0	0	0	4	13

Mechanical versus Thrips inoculations

For this comparison, performance values (incidences of symptom expression, shoot and tuber infections) were calculated as means of early and late infections for mechanical and thrips inoculations and then subjected to nonparametric chisquare analysis (Table 3.4). In all these comparative analyses, significant differences ($p<0.001$) were observed between mechanical and thrips inoculations in the incidences (%) of infected plants, as measured by occurrence of foliar symptoms and presence of virus detactable by foliage and tuber ELISA (Table 3.4). However, individual cultivars differed widely based on foliar symptom development, foliage and tuber ELISA when inoculated mechanically or through thrips. Many cultivars were as susceptible as the control (Shepody), but some exhibited robust resistance, which translated into restrictions of virus translocation to tubers. The cv. Atlantic consistently expressed high levels of susceptibility to TSWV infections both in mechanical inoculations and thrips transmissions. The cvs Bismark and Atlantic also had very efficient virus translocation from shoots to tubers under both mechanical inoculations and thrips transmissions. However, the number of plants testing positive for the virus in thrips transmissions was relatively low in the cv. Bismark (5%)(Table 3.4). These results suggest that vector-mediated components may be responsible for the low number of infected plants in the field in this cultivar. In contrast no virus translocation to tubers was detected in cvs Up-To-Date, Sebago, Tasman and Gold Star in both mechanical inoculations and thrips transmissions (Table 3.4). Exceptionally high levels of resistance to TSWV infections were exhibited by the cv. Tasman in both mechanical inoculations and thrips transmissions. With the exception of cvs Royal Blue, Desiree and Bintje, all the others had more plants infected by mechanical than thrips inoculations.

Mechanical inoculations in the cv. Royal Blue resulted in large numbers of plants expressing symptoms (88%) but only 38% of these tested positive in ELISA. Under field conditions, this cultivar had the highest numbers of plants with thrips feeding marks, which resulted in 13% of plants expressing symptoms and 7% testing positive in ELISA. But only 1% of tubers were detected with TSWV in them.

Table 3.4 Comparison of potato varietal response to mechanical and thrips inoculation of tomato spotted wilt virus (2001-2003)

	Plants with symptoms (%)		Positive Shoots (ELISA)(%)		Positive Tubers (ELISA)(%)	
Cultivar	**Glasshouse (Mechanical)[a]**	**Field (Thrips)[a]**	**Glasshouse (Mechanical)[b]**	**Field (Thrips)[b]**	**Glasshouse (Mechanical)[c]**	**Field (Thrips)[c]**
Royal Blue	88	13	38 $^{d+}$	7 $^{d-}$	1	1
Bismark	88	9	63 $^{d+}$	5 $^{d-}$	18 $^{d+}$	10 $^{d+}$
McCains1	81	10	56 $^{d+}$	9 $^{d-}$	8	1
Victoria	75	17	56 $^{d+}$	17	8	0 $^{d-}$
Ranger Russet	75 $^{d-}$	24 $^{d+}$	44 $^{d+}$	15 $^{d-}$	6	5
Up-To-Date	69 $^{d+}$	4 $^{d-}$	38	2 $^{d-}$	0 $^{d-}$	0 $^{d-}$
Sebago	69 $^{d+}$	3 $^{d-}$	38	2 $^{d-}$	0 $^{d-}$	0 $^{d-}$
Pink Eye	69 $^{d+}$	4 $^{d-}$	50 $^{d+}$	10 $^{d-}$	2	1
Kipfler	69 $^{d+}$	0 $^{d-}$	44 $^{d+}$	4 $^{d-}$	11 $^{d+}$	1
Brownell	69	12	50 $^{d+}$	9 $^{d-}$	6	1
Tasman	63	8	25	0 $^{d-}$	0 $^{d-}$	0 $^{d-}$
Gold Star	63	13	50 $^{d+}$	8 $^{d-}$	0 $^{d-}$	0 $^{d-}$
Yellow King	56	12	25	3 $^{d-}$	0 $^{d-}$	7
Russet Burbank	56 $^{d-}$	23 $^{d+}$	44 $^{d+}$	10 $^{d-}$	7	5
Pontiac	56	14	44 $^{d+}$	8 $^{d-}$	4	0 $^{d-}$
Desiree	56	15	50 $^{d+}$	13 $^{d-}$	0 $^{d-}$	2
Shepody	50 $^{d+}$	2 $^{d-}$	38	1 $^{d-}$	9	0 $^{d-}$
Bintje	50	5	56 $^{d+}$	7 $^{d-}$	3	5
King Edward	44	3	31	1 $^{d-}$	2	0 $^{d-}$
Atlantic	44 $^{d-}$	21 $^{d+}$	69	13 $^{d-}$	29 $^{d+}$	10 $^{d+}$
Kennebec	38	7	50 $^{d+}$	1 $^{d-}$	18 $^{d+}$	0 $^{d-}$

Likelihood Chi-square value (mechanical vs thrips inoculations)	= 102.99 with 20 d.f[a]	= 62.41 with 20 d.f[b]	= 128.50 with 20 d.f[c]
Probability level (under null hypothesis)	$P < 0.001$ [a]	$P < 0.001$ [b]	$P < 0.001$ [c]
Residual deviance statistic	= 92.07 with 41 d.f[a]	= 816.0 with 41 d.f[b]	= 298.7 with 41 d.f[c]

$^{d+/d-}$ values have significant ($p < 0.05$) negative or positive residuals

Discussion

In this study the aim was to evaluate resistance of selected potato (s*olanum tuberosum*) cultivars to mechanical and thrips mediated transmission of tomato spotted wilt virus (TSWV). Comprehension of the components and mechanisms of resistance is critical as a means of gaining insights into the significance of different components of epidemics in potatoes. As symptom expression, foliar and tuber infections are the key criteria to measure resistance to TSWV, this variability implies that these parameters should be assessed in several experiments in both glasshouse and field conditions.

The study of symptom expression, foliar and tuber infections were consistent in all experiments, despite the variability inherent between mechanical and thrips transmissions and has demonstrated that all the potato cultivars evaluated are not immune to TSWV. Using mechanical inoculations under glasshouse conditions and natural thrips transmissions in the field, the study has indicated that more plants expressed TSWV symptoms when inoculated late (60 days after planting) than early (28 days after planting)(Table 3.1 and Table 3.2, Figure 3.6). However, most late infections were localised and attributed to the restricted distribution and movement of TSWV in some cultivars, except in cvs Atlantic, Shepody, Bismark, Kipfler and Victoria in which systemic infections were observed. TSWV translocation to tubers was comparatively more efficient in early than late mechanically inoculated plants of many cultivars (n=17). Variability was also observed between cultivars in their ability to restrict TSWV translocation to tubers in thrips transmissons, although these differences were not significantly affected by time of inoculation ($P = 0.198$; Table 3.2). This indicated the genetic basis of the components of resistance and the conditioning by the age of the plant at the time of infection as measured by symptom expression, shoot and tuber infections.

The expression of symptoms varied in both foliar and tuber infections (Figure 3.1), and the number of plants expressing these symptoms was also influenced by the time of inoculation (plant age at inoculation)(Table 3.1 and Table 3.2). Some infected plants of the cvs Bintje (2%), Kipfler (2%), Sebago (1%), Royal Blue (1%) and McCains 1 (3%), while yielding positive serological assays for TSWV, never exhibited symptoms in early field infections (Table 3.2). In cultivars that expressed symptoms, such symptoms were generally restricted to the initially inoculated leaves in the lower parts of the plant canopy and auxiliary shoots for both mechanical and thrips virus transmissions. Non-inoculated leaves and newly emerging apical leaves did not exhibit symptoms and had negative ELISA results until later in their growth stages. Such symptom expressions, although not serologically confirmed, were observed in prior research (Norris, 1951a, 1951b). And they are not unique to potato. Asymptomatic characteristics of some TSWV infected plants have also been observed in other crop systems (Matteoni & Allen 1989; Stobbs *et al.*, 1992; Latham & Jones, 1997; Moriones *et al.*, 1998; Chatzivassiliou *et al.*, 2001), including geminivirus pathosystems (Fargette & Thresh 1994; Fargette *et al.*, 1994), and has often led to underestimation of virus incidence because symptoms may be masked or infection latent. From this and other similar observations in surveys of commercial potato fields (Chapter 2), it is apparent that the health status of some cultivars cannot be assessed satisfactorily from the presence of symptoms, even if such observations are supplemented by virus detection tests. Virus-host interactions differ widely in the mechanisms involved in the display of symptoms. Although detection of TSWV based on symptom expression is fundamental to epidemic analysis, development of leaf symptoms is not always reliable as observed in the cvs Bintje, Kipfler, Sebago, Royal Blue and McCains 1 in this study. This raises a fundamental question of risk in the exclusive reliance on cultivar symptom expression or shoot infections, particularly in certification schemes. Indeed, the problems that can arise from such exclusive reliance on cultivar symptom expression or shoot infections are apparent from data in the present study, which lends itself to a hypothesis of one possible cause of epidemics in potato crops. The hypothesis proposes that the inconspicuous TSWV symptoms in the lower parts of the plants canopy are missed during routine field foliar inspections for certification of seed crops in early to mid-season. These seed crops are ultimately certified free of TSWV based on foliar inspections. Consequently, the infected tubers arising from such early-infected crops serve as sources of carry-over infections which eventuary build-up in seed stocks over time. And because certification is not routinely based on tuber serological testing, infected seed stocks get into the production streams, and therefore, lead to epidemics. Weed species that are hosts to TSWV such as *Arctotheca calendula* (Capeweed) and *Solanum nigrum* (Blackberry nightshade) that grow within and adjacent to the infected crops have previously tested positive for the virus at the end of the season during surveys of commercial potato fields (Chapter 2 this thesis). Such weeds become TSWV reservoirs from which further spread can occur to other host species in the vicinity of the fields. Hibernating thrips have been observed around the root systems of these weeds during surveys of commercial potato fields in winter. And studies that help explain the seasonal survival of thrips vectors, *Thrips tabaci* and *Frankliniella fusca* adults, have also shown that hibernating viruliferous thrips can serve as reserviour hosts for TSWV (Johnson *et al.,* 1995; Groves *et al.,* 2001, 2002; Jenser, 2003). Observations in commercial field crops in the cvs Riverina Russet in Victoria and Atlantic in New South Wales during TSWV epidemics in 2002/2003 season render credence to such a

hypothesis. It may also explain the source of epidemics that have occurred in the past. In the epidemics of 1945-46 and 1946-47 seasons, the crops that were affected were grown from certified seed (Norris, 1951a, 1951b). A similar hypothesis has also been advanced for the re-emergence of potato virus Y (PVY) in seed production areas of the northeastern United States, where asymptomatic potato cultivars were introduced (Hane & Hamm 1999; Groves & Gray 2003; Nolte *et al.,* 2004). It may also be true, in Australia, for other potato viruses such as *Potato carlavirus S* (PVS), in which infections produce inconspicuous symptoms. However, the hypothesis does not discount the contribution of primary sources of inoculum, which can be significant depending on the distances from the source (Coutts & Jones 2003).

Furthermore, data from this study indicates that late infections are not generally systemic in potato and do not translate into virus translocation to tubers except in a few cultivars (Table 3.1 and 3.2). This is consistent with the earlier report by Wilson (2001) and confirms the observations by Conroy *et al*., (1949) and Norris (1951b) that late infections generally pose less risk than those that occur early in the season. This observation suggests that field inspections carried out in late season would, therefore, unfairly overestimate the risk of TSWV infection except in cvs Atlantic and Bismark, which consistently had efficient virus translocation to tubers in both early and late infections. Late season field inspections of crops based on foliar symptoms present other problems as well. In the current study, more plants expressed symptoms when infected late (Table 3.1 and Table 3.2). A practical consequence of these results for field inspections is the problem of distinguishing TSWV symptoms from strikingly resembling fungal leafspots or early blight (*Alternaria solani*) in late season. Overall, the observations on symptom expression in this study have supported the conclusion that the date of TSWV field assessements in potato shoots can be a crucial factor in obtaining reliable and representative estimates. And because routine field inspections usually combine observations for other potato virus pathogens, cost considerations for such field visits inevitably requires that compromises be made for such inspections, taking into account the epidemiological cycles of the viruses being considered.

As with symptom expression, data from this study also indicates that most plants with detectable virus by ELISA were those infected late, both mechanically and through thrips transmissions. But such infections were localised and not generally systemic, except in cvs Atlantic and Bismark (Table 3.1 and 3.2). In contrast, tuber infection did not occur in a number of cultivars, despite the presence of foliar symptoms in early and late mechanical inoculations and late thrips transmissions, and virus detection by ELISA (Table 3.1 and 3.2). The cv. Tasman was, strikingly conspicuous in this regard. Prior research (Norris 1951a, 1951b, Wilson 2001) has indicated that TSWV infections in potato are inversely proportional to plant age in both foliar and tuber infections. Such observations, which have been made in many other viral infections and termed 'mature plant resistance' (Beemster 1976, 1987; Sigvald 1985), would suggest that the phenomenon only applies to cell-to-cell or long-distance movement of viruses, is host specific and influenced by the time of infection (plant age at infection). Cell-to-cell or long-distance movement of viruses is a key factor in determining systemic virus infections and host range, and the inhibition of either movement is a common resistance mechanism to viral infections (Lindbo *et al*., 1993; Carrington *et al*., 1996; Voinnet *et al*., 1999; Vance & Vaucheret 2001). While data in the current study is not sufficient to arrive at a definite conclusion, a closer examination of the data (Table 3.1 and 3.2) together with inference from and interpretation of the observations by Norris (1951b) reveals a pattern. In addition to reporting on mature plant resistance in the cultivar Up-To-date, Norris (1951b) also noted that "in three experiments using 61 potato plants aged approximatelly 90-100 days, …..all inoculated leaves developed numerous necrotic local lesions and most of them ultimately abscissed or shriveled (virus movement restriction), but no systemic invasion occurred in any plant". These observations lead to a hypothesis that the infection processes involving virus replication in initially infected cells and subsequent movement to adjacent cells through plasmosdesmata leading to localized systemic cell invasion were not impeded, particularly in late infections, hence the high infection rate observed. However, there was restriction of long-distance movement in resistant or late infected potato plants (Table 3.1 and Table 3.2). The rate of long-distance movement was significantly higher in early mechanical inoculations, tempting to suggest that the rapid movement of photosynthates within the plant during the early growth phase aided it. However, such a suggestion does not explain the restrictions of virus movement in resistant/tolerant cultivars during early infections or long-distance virus movements observed in susceptible cultivars like Atlantic and Bismark in both early and late infections (Table 3.1 and 3.2). And this calls for an alternative plausible hypothesis to explain the observed virus movement or lack of it. Such a hypothesis would be that long-distance movement restriction of TSWV in resistant or mature plants might have been caused by the inhibition of virus loading into the phloem, transportation through the phloem, or unloading from the phloem to nonvascular tissues. Such inhibition may be associated with an inherent lack of host factors (plant receptors) required for compartible TSWV-host interactions in long distance viral transport, presence/absence of plant proteins reacting with movement proteins of the virus and/or inability of TSWV to efficiently suppress the host gene silencing mechanisms in resistant and/or mature plants, all which may be

influenced by factors such as temperature and virus strain. Such a hypothesis of movement-restricted interactions is supported by evidence from a number of studies (Carrington *et al*., 1996; Llamas-Llamas *et al.,* 1998; Soler *et al.,* 1998; Moury *et al*., 1998; Soellick *et al*., 1999; Voinnet *et al*., 2000; Voinnnet, 2001). While temperature fluctuations in the glasshouse were generally low and fairy constant within the range 20 – 25 ^{o}C, themic fluactuations in the field were higher. Indeed, the TSWV strain, An_{WA}*-1*, used in this study has been observed to induce different resistance responses in *Capsicum chinense* accessions (Latham & Jones 1998; Thomas-Carroll & Jones 2003). In many other TSWV-crop infections, the rates of virus movement have been observed to be greater in sensitive compared with tolerant varieties (Moury *et al*., 1997; Soler *et al*., 1998, 1999; Maris *et al*., 2003a). Temperature (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998) and water stress (Córdoba *et al*., 1991) have been shown to restrict movement of TSWV in infected plants. In pepper, population segregation for the *Tsw* gene has shown that heterozygosity at the *TSw* locus increased the chance of inoculated seedlings to develop systemic necrotic symptoms and under continuous high temperature resistance in young plants was destabilised and older plants rarely developed systemic symptoms (Moury *et al*., 1998). In cucumber, cell-to-cell movement of *Cucumber mosaic virus* (CMV, pepo strain) was enhanced at 36^{o}C compared with that observed at 24^{o}C (Kobori *et al*., 2003). The study on potato leafroll virus (PLRV) translocation from aphid-inoculated shoots to uninoculated shoots sprouted from the same tubers suggests that no specific mechanisms impair PLRV movement through the tubers of the resistant genotypes (Syller 1991, 2003). From these examples, it is clear that such movement varies across hosts and viruses. Determination of such viral movements requires knowledge of regulatory sites necessary to establish systemic infections. These have not yet been determined for TSWV. It would, therefore, be helpful, for example, to identify and locate the plant receptors that recognise TSWV in an initially infected cell, movement to adjacent cells through plasmodesmata (cell-to-cell movement), mesophyll cells, phloem parenchyma cells, and/or companion cells and then transported to other organs and tissues through the phloem (long-distance movement). Such systemic movements of viruses within plants have broadly been reviewed and are thought to depend on several processes (Lucas & Gilbertson 1994; Carrington *et al*., 1996; Gilbertson & Lucas 1996; Séron & Haenni 1996; Nelson & van Bel 1998; Gray & Banerjee 1999; Bucher *et al*., 2003). However, most of the current understanding of viral-host interactions has been limited to the viral contribution of these interactions (Lucas & Gilbertson 1994; Carrington *et al*., 1996; Gilbertson & Lucas 1996; Séron & Haenni 1996; Nelson & van Bel 1998; Gray & Banerjee 1999; Bucher *et al*., 2003). Despite an enormous literature decribing various TSWV-host associations and symptom expression, little is known about the molecular and cellular mechanisms that regulate the TSWV multiplication within plant cells and determine the efficiency of cell-to-cell movement. Some progress is being made in this area. Using NSm viral movement protein (Kormelink *et al*., 1994; Gunasinghe & Buck, 2003), as a bait in a yeast two-hybrid screen, two homologous NSm-binding plant proteins of the DnaJ family from *Nicotiana tabacum* and *Arabidopsis thaliana* have been identified and hypothesed to provide a molecular basis for specific recognition of nucleocapsid structures which link the viral structures to elements of a plant machinery directing intercellular transport through plasmodesmata (Soellick *et al*., 1999). However, while it is important to understand the role of viral genes in causing plant disease (Lindbo *et al*., 1993; Carrington *et al*., 1996; Gray & Banerjee 1999; Voinnet *et al*., 1999; Vance & Vaucheret 2001; Bucher *et al*., 2003), it is equally important to understand how plants respond to TSWV infections, particularly at different growth stages as observed in this study. Systemic infections of TSWV, particularly at different growth stages, were observed in this study. But how such systemic infections may have been enhanced or impeded in young compared to mature plant cells due to the production or absence of plant proteins reacting with movement proteins of the virus is currently unknown. Identification of such proteins in plants and factor(s) affecting their function(s) would help to explain the concept of "mature plant resistance" and the lack of complete systemic infections in some cultivars as observed in this study. Searches for such proteins in plants are ongoing (Chen & Klessig, 1991; Durner & Klessig 1995; Felton *et al*., 1999; Soellick *et al*., 1999; Pierpoint 1994, 2000, 2002) and is a matter of some curiosity and interest that could be tested directly. Indeed, antiviral RNA silencing has been shown to occur in nature and proposed as a natural defence mechanism protecting plants against viruses, resulting in resistance (Lindbo *et al*., 1993; Ratcliff *et al*., 1997; Al-Kaff *et al*., 1998; Vance & Vaucheret 2001; Voinnet 2001; Li & Ding 2001; Hannon 2002; Ye *et al*., 2003a; Novina & Sharp 2004). But without access to the specific plant contributions to these interactions, it will be difficult to understand how TSWV causes disease in potato and other crops or to develop new approaches to controlling the disease. For example, it would be enlightening to determine at what level plant cell homeostasis is affected by TSWV infections at different plant growth stages (early or late) and what genes are involved. Equally beneficial would be to understand how the genes associated with these receptors evolve, and how the recognition event is able to turn on a cascade of defence genes, which limit systemic infections and translocation of the virus to tubers, particularly in late infections as observed in this study. It would also be enlightening to investigate the antiviral RNA silencing mechanism(s) operating in TSWV infected plants by targeting viral RNA sequences that are highly conserved and normally invariant between

different TSWV strains and the messenger RNA molecules they encode, and therefore preventing their expression. Broad mechanisms involved in such viral-host interactions have been identified (Voinnnet *et al*., 1999, 2000; Voinnnet, 2001). Molecular techniques are available which can aid to define how TSWV causes disease in many plants, and how plants resist the disease (Lindbo *et al*., 1993; Voinnet *et al*., 1999; Vance & Vaucheret 2001; Bucher *et al*., 2003; Novina & Sharp 2004). And rapid progress is being made in this area. For instance, negative-strand TSWV have been shown to carry a gene for a suppressor of gene silencing at analogous genomic positions which suppresses posttranscriptional silencing of a green fluorescent protein transgene in infected *Nicotiana benthamiana* (Bucher *et al*., 2003). Transforming tobacco plants with a construct comprising the nucleocapsid-protein (N) gene of TSWV and the 5' non-translated leader sequence of *Plum pox virus* (PPV) as a translation enhanser has been shown to confer unusually broad resistance against TSWV and groundnut ringspot virus (GRSV) by blocking systemic spread (Schwach *et al*., 2004)

Thrips catches from traps in the field exposure trial revealed that *T. tabaci* was the only known vector and the most abundant species for the most part of the growing season (Figure 3.3, Table 3.3), and, therefore, assumed to be responsible for the virus transmissions observed. Several other thrips species were identified from the traps and these included *Tenothrips* (12%), *Limothrips cerealium* (11%), *Thrips imaginis* (8%), *Thrips australis* (2%), *Tubuliferan sp.* (1%), and Others (*C. manicatus, Limothrips angulicornis, Pseudanaphothrips achaetu*s, *Apterothrips apteris*)(3%). The species *T. imagines* has not been recorded previously in the region, which would suggest that this species was a relatively new introduction to Southern Tasmania. *T. imagines* has not been implicated in the transmission of TSWV, but a study by Milne & Walter (2003), demonstrated that *Prunus necrotic ringspot virus* (PNRSV) (family *Bromoviridae*) can be readily transmitted when *T. tabaci, Thrips imaginis* and *Thrips Australis* and virus-bearing pollen are placed together onto test plants. Ironically, the competencies of these thrips species to transmit TSWV, with the exception of *T. tabaci*, have not been experimentally tested in Australia. It would have been enlightening to investigate the contribution of these species in the TSWV infections observed in the current study or any other epidemics in potato and other crops in the country.

Strikingly, the *T. tabaci* population at the field trial site in the current study only comprised of females. *T. tabaci,* a haplodiploid species, is known to exist in two population forms, one unisexual and parthenogenetic and the other bisexual (Mound 1997; Terry 1997; Mound 2004). Zawirska (1976, reference in Nagata & Peters 2001) argues that *T. tabaci* comprises two taxonomically identical "types" from among which the populations on tobacco (*Nicotiana tabacum*) are considered as *T. tabaci* subsp. *tabaci* and those living on *Galinsoga parviflora*, potato and other hosts as *T. tabaci* subsp. *communis*. Populations of *T. tabaci* subsp. *communis* on different plant populations, mainly on onion, propagate parthenogenetically and not considered virus vectors. Different biotypes of *T. tabaci* have not been reported in Australia. Transmission studies have indicated that parthenogenetic and arrhenotokous populations of *T. tabaci* reared on beans (Wijkamp *et al.,* 1995) and leek (Chatzivassiliou *et al*., 1999) were unable to transmit TSWV isolates. These observations have also been made by Jenser *et al*., (2002), and appears to be a common phenomena on many host plants. However, in Australia, bisexual populations have not been observed although they may exist (L. Mound, personal communication). *T. tabaci* females are reported to be inefficient vectors of TSWV, and populations in which males are absent have been incapable of transmitting the virus (Wijkamp *et al.,* 1995; Chatzivassiliou *et al*., 1998a). These factors may help to explain the low infection rates observed in the field trial despite abundant TSWV inoculum and a high thrips population (Figure 3.3, Table 3.3). Another plausible explanation of the low infection rates observed may have been due to effects of host-diversity resulting in restrictions of polyphagy in *T. tabaci* by trade-offs in fitness on different potato cultivars. The diversity resistance hypothesis, argues that diverse communities of plants are highly competitive and usually less prone to rapid and severe epidemics because host genotypes are more diverse and distributed in small patches. This hypothesis is supported by both theory (Thresh 1974, 1982; Fox & Morrow 1981; Futuyma and Moreno 1988; Jaenike 1990; Thompson 1994; Terry 1997; Holmgren & Getz 2000; Kennedy *et al.,* 2002; Ye *et al.,* 2003b) and experimental studies on preferences, associations and performance (Kirk 1985; Yudin *et al.,* 1988; Teulon 1993; Bautista & Mau 1994; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 1999, 2001; Agrawal & Colfer 2000; Terry 1997; Ochoa *et al.*, 1999; Herrin & Warnok, 2002), providing insight into the potential constraints and selection pressures on the evolution of host range.

Field observations also indicated that thrips feeding damage varied among potato cultivars assessed. The cultivar Royal Blue had the most thrips feeding damage (data not shown), which was initially observed 14 days after planting. Ironically, despite heavy thrips feeding damage and 88% of plants expressing symptoms, with up to 63% ELISA detectable foliar infections, only 1.3% of tubers were infected in this cultivar (Table 3.2). These field observations and data suggest differences in potato cultivar preferences by vector thrips, particularly in early season. Such thrips host preferences of plants that are susceptible to the virus have been shown to influence thrips transmission efficiency (Allen & Broadbent, 1986; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 2002) as virus acquisition must be by the immobile larvae (Lindorf, 1931, 1932; Sakimura, 1963a; Van de Wetering *et al.,* 1996),

that is able to complete its development on the host selected by the adult thrips (Hobbs *et al.,* 1993; Bautista & Mau 1994; Terry 1997). Published evidence has shown that differences exist in cultivar preferences by vector thrips (Herrin & Warnock, 2002; Maris *et al*., 2003b), and incidence of virus is less in TSWV-resistant cultivars than in susceptible ones (Maris *et al*., 2003a; Culbreath *et al.* 1992; Camann *et al.* 1995; Yang *et al*., 2004). Therefore, host preference by vector adult thrips among plants susceptible to the virus becomes a critical aspect of epidemiology of virus diseases (Bautista & Mau 1994; Gray and Banerjee 1999). Vector thrips performance studies on the cv. Royal Blue would, therefore, assist in determining the contribution of such cultivars to secondary viral spread in potato crops. In contrast, the cv. Bismark, which was highly susceptible to virus infections and had efficient translocation to tubers when mechanically inoculated (Table 3.1), exhibited strikingly robust resistance to thrips feeding (Figure 3.5), and consequently TSWV infection in the field, despite a cluster of infected plants located near a tomato infector plant which resulted in early (15%) and late (5.3%) tubers infections, suggesting that resistance to the virus and to the vector are determined by two distinct genetic mechanisms, and that resistance to the virus does not imply and is not caused by resistance to the vector. This level of field resistance to vector could be useful in breeding programs and warrants further investigations. Large-scale deployment of vector resistant cultivars could, however, decrease the overall thrips population density in potato fields and reduce the subsequent risk of virus spread.

The nonparametric comparative analyses of methods of assessments (mechanical versus thrips transmissions) of potato cultivars revealed significant differences ($P<0.001$) between mechanical and thrips inoculations for all parameters tested (Table 3.4). The analyses show that TSWV resistance as measured by cultivar symptom expression, foliar and tuber infections, was most effeciently evaluated by mechanical inoculation. However, exclusive use of mechanical inoculation may result in the loss of valuable germplasm because cultivars with natural vector resistance may not be identified. This was highlighted by the responses of the cv. Bismark, which despite being highly susceptible to foliar and tuber viral infections through mechanical inoculations (Table 3.1 and 3.2), exhibited high field resistance to vector thrips (Figure 3.2). Such observations suggest that the two methods of inoculations are suited for evaluating different parameters of resistance in potato cultivars. Mechanical inoculation was useful for direct TSWV resistance such as cultivar symptom expression, systemic foliar and tuber infections. Thrips transmissions in field trials were useful for identifying vector-mediated components of TSWV resistance. Similar observations have been made in the evaluation of *Lycopersicon* germplasm for TSWV resistance (Kumar *et al.,* 1993). Resistance to vector thrips and/or TSWV is not immunity and seems to be overcome under certain conditions (Thompson & van Zijl 1996; Qiu *et al.,* 1998; Qiu & Moyer 1999; Roggero *et al.,* 2002; Aramburu & Martí 2003; Thomas-Carroll & Jones 2003), thus underlining the importance of selecting and breeding for resistance under both glasshouse and field conditions. Such dual assessments also avoids the possible reliance on defective TSWV generated during chains of mechanical inoculations and serial passages through maintenance hosts plants (Qiu *et al.,* 1998; Latham & Jones 1998; Qiu & Moyer 1999; Thomas-Carroll & Jones 2003; Moyer *et al.,* 2003a; Aramburu & Martí 2003; Bucher *et al*., 2003).

Overall, some of the practical consequences of the results from this study suggest that the risk of TSWV epidemics depends on when and to what extent the plants develop mature plant resistance in relation to the age of the crop. If the migration of viruliferous vector thrips during the growing season is early, when the plants are young and still vulnerable, the risks of TSWV epidemics increase. The implication of this phenomenon in selection and breeding for TSWV resistance is that the stage of plant development at which plants are inoculated for this purpose needs to be defined when using these cultivars.

Despite the acquisition of crucial data on potato cultivar responses to TSWV infections from the current study, there are limitations on the use of such information. In particular, many authors have argued that the development of a predictive model requires data from representative sites over sufficient multiple seasons (Thresh, 1974; Barnett 1986) and a careful evaluation and determination of individual risk factors playing a role in the epidemic (Duffus, 1971; Kranz, 1974, 1990; Thresh 1974; Plumb & Thresh, 1983; Gray & Banerjee, 1999). The level of resistance in the potato cultivars assessed is variable, underlining the need for additional evaluation under different conditions from those in Tasmania before deployment in attempts to control the disease. Genetic relationships exist among some of the potato cultivars evaluated in this study (Isenegger *et al.*, 2001), with its consequential restrictions on heterozygosity, and which could have resulted in inbreeding depression in some parental clones. This limits genotypic variability and may have been reflected in a possible TSWV isolate and/or site x genotype interactions, which should be further explored. Furthermore, the use of only one isolate of TSWV, An_{WA}-*1*, imposed a limit on the resistance responses of cultivars in the current evaluation. Although only minor (4.3%) molecular differences in TSWV strains have been observed in Australia (Talty & Dietzgen, 2001; Dietzgen, 2003), biological differences in such features as symptom expression have been reported in tomato (Norris 1946), potato (Norris 1951a, 1951b) and *Capsicum chinense* accessions (Latham & Jones 1998; Thomas-Carroll & Jones 2003). To what extent potato cultivar host-diversity in field assessments may have affected the amount of inoculum or

genetically screened inoculum at an early stage in the evaluations through genome reasortment (Qiu *et al.,* 1998; Qiu & Moyer 1999) and selective transmission by vector thrips (Nagata *et al.,* 2000; Naidu *et al.,* 2003; Sin *et al.,* 2003) is unclear. Genome reasortments and selective acquisition and spread of different TSWV isolates by different species of vector thrips may also impose selective advantages that may be further enhanced, albeit inadvertently, by cropping practices and quality of recipient and donor plants in different areas. Diversity resistance is known to restrict polyphagy in arthropods and consequently virus transmissions (Thresh 1974, 1982; Fox & Morrow 1981; Kirk 1985; Yudin *et al.,* 1988; Futuyma and Moreno 1988; Jaenike 1990; Teulon 1993; Bautista & Mau 1994; Thompson 1994; Wijkamp *et al*., 1995; Terry 1997; Ochoa *et al.*, 1999; Holmgren & Getz 2000; Agrawal & Colfer 2000; Chatzivassiliou *et al*., 1999, 2001; Herrin & Warnok, 2002; Kennedy *et al.,* 2002; Ye *et al.,* 2003b). Furthermore, only one known thrips vector species,*T. tabaci* caught in the traps is assumed to have transmitted the viruses. In other parts of Australia, four thrips vector species have been identified; onion thrips, *Thrips tabaci,* tomato thrips, *Frankliniella schultzei* Trybom; the western flower thrips, *F. occidentalis* (Pergande); and the melon thrips, *Thrips palmi* Karney (Malipatil *et al*., 1993; Mound, 1996). Due to host preferences, TSWV virus strains and transmission competencies among the vector species, results of subsequent assessements of the cultivars used in the present study may be different.

It is hoped that results from the present study, together with those of prior research (Norris, 1951a, 1951b; Wilson 2001) will assist in the evaluation, refinement and submission of adjusted national seed certification guidelines appropriate to and reflecting real risks of TSWV in potatoes. It is also hoped that the acquisition of knowledge from this study will help to explain the sporadic nature of the epidemics and, therefore, facilitate the development of risk assessment models (Nutter, 1997; Madden *et al*., 1990; Madden and Campbell, 1986; Kranz, 1988, 1990; Madden and Hughes, 1995), and ultimately, lead to the development of an early warning system for potato growers, processors and farm advisers, and consequently, better decisions regarding cropping patterns and sequences and disease control strategies in space and time (Thresh, 1974).

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Chapter 4

EFFECTS OF TEMPERATURE AND PLANT AGE AT THE TIME OF INOCULATION ON TSWV INFECTION IN POTATO

Cell-to-cell or long-distance movement of viruses is a key factor in determining systemic virus infections and host range (Lucas & Gilbertson 1994; Carrington *et al*., 1996; Gilbertson & Lucas 1996; Séron & Haenni 1996; Nelson & van Bel 1998; Voinnet *et al*., 1999; Li & Ding 2001; Bucher *et al*., 2003; Gunasinghe & Buck 2003; Ye 2003), and the inhibition of either movement is a common natural resistance mechanism to viral infections (Lindbo *et al*., 1993; Ratcliff *et al*., 1997; Al-Kaff *et al*., 1998; Vance & Vaucheret 2001; Voinnet 2001; Hannon 2002). The inherent sensitivity of the host (Daughtrey *et al.*, 1997; Moury *et al*., 1997; Llamas-Llamas *et al*., 1998; Soler *et al*., 1998, 1999; Garg & Khurana, 1999; Kikkert *et al*., 1999; Wilson, 2001; Maris *et al*., 2003; Aramburu & Martí, 2003), virus isolate (Norris 1951a, 1951b; Roca *et al.*, 1997; Maris *et al*., 2003), temperature (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998), water stress (Córdoba *et al*., 1991), and age of the plant and inoculum pressure (Moriones *et al.,* 1998; Soler *et al*., 1998; Thomas-Carroll & Jones 2003) have been shown to restrict movement of TSWV in infected plants. Such phenomenon has been observed in other virus pathosystems including soilborne wheat mosaic virus infections of hard red winter wheat (Myers *et al*., 1993). Studies in pepper with TSWV resistance conferred by the *Tsw* gene indicate that continuous high temperatures of 32^{o}C lead to systemic infections and necrotic symptoms in plants that are totally resistant at a lower temperature of 22^{o}C (Moury *et al*., 1998). In similar studies on pepper, Soler *et al*., (1998), also investigated two temperature regimes corresponding to early and late cultivation cycles and their interactions with the developmental stage of the plants. All plants subjected to 30/18^{o}C (day/night) temperatures developed systemic infections in comparison to those at 25/18 ^{o}C (day/night) where only those inoculated at 2-leaf stage became systematically infected. Plants inoculated at the 4-leaf stage under 25/18 ^{o}C (day/night) were resistant. In cucumber, cell-to-cell movement of *Cucumber mosaic virus* (CMV, pepo strain) was enhanced at 36^{o}C compared with that observed at 24^{o}C (Kobori *et al*., 2003).

Temperature also modulates the generation of defective interfering RNA (Inoue-Nagata *et al*., 1997) that has been consistently associated with disease symptom attenuation in some species (Resende *et al*., 1991). This phenomenon has also been reported for *Broad bean mottle virus* infection (Llamas *et al*., 2004). Consequently, TSWV infections in many plants induce highly variable symptoms (Best 1968; German *et al.*, 1992; Goldbach & Peters, 1996; Latham & Jones, 1996; Roselló *et al.*, 1996).

Tolerance to TSWV infections has been observed in some potato cultivars (Wilson, 2001; Chapter 3 this thesis). However, information regarding the factors and mechanism(s) modulating this tolerance is lacking. There is no direct evidence that TSWV infections in all crops behave in a similar manner. Effects of temperature have been studied on the susceptibility of potato plants to infections and accumulation of potato leafroll virus (Syller 1991; 2003). But anecdontal evidence from reports of TSWV epidemics in potato in Australia clearly indicate that more frequent and severe epidemics have occurred in South Australia, New South Wales and Victoria where day/night temperatures are generally higher during the growing season than in Tasmania which experiences lower temperatures. This implies the seemingly increasing difficulty of growing TSWV-susceptible potato varieties such as Shepody and Atlantic (Chapter 3) in regions with higher temperatures and low themic fluactuations without disease symptoms. The age of the plant at the time of inoculation is also an important factor affecting the stability of the resistance. Thus, Norris (1951a, 1951b), Wilson (2001) and Chapter 3 of this thesis, observed that there was a general decline in foliage systemic and tuber infection efficiency across potato cultivars tested with increasing plant age at inoculation in both glasshouse and field trials.

The objectives of the current work was to quantify (1) how temperature modulates infection and the expression of symptoms and (2) how it interacts with plant age at the time of inoculation to influence infection and symptom expression in commonly commercially grown potato cultivars Shepody (susceptible) and Russet Burbank (tolerant); two factors that should be considered in the management of TSWV in potato crops. A better understanding of resistance to TSWV would also facilitate development of assays for evaluation of germplasm with resistance to TSWV. The acquisition of such information is essential in any attempt to develop risk assessment models (Duffus, 1971; Thresh, 1974; Plumb & Thresh, 1983; McLean *et al.* 1986; Barnett 1986; Kranz, 1974, 1990; Jeger & Chan 1995; Gray & Banerjee, 1999), which would assist in making strategic decisions for managing TSWV in potatoes.

Materials and Methods

Potato test plants and growing conditions

Two potato varieties, one susceptible (Shepody) and one tolerant (Russet Burbank), widely grown commercially were selected. The potato plants were grown from tuber setts prior tested negative by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) protocol (Clark & Adams, 1977) using antisera to TSWV lettuce strain (Agdia, IN, USA. The plants were grown in 12 x 25cm black polyethelene bags containing potting mix (Figure 4.1). Tuber setts were presprouted before planting to ensure an even growth during inoculation. Only single shoots were allowed to grow after emergence by roguing of additional shoots and moisture within potting bags was maintained at non-limiting levels to plant growth. All the plants were Initially maintained in an insect-proof glasshouse (Temp.= 20–25 ^{o}C; R.H =55±5%) at the NewTown Research Laboratories in Tasmania, until three days before inoculation, which was at 3 weeks after emergence when most plants had three fully expanded leaves.

Figure 4.1. Shepody and Russet Burbank plants grown in 12 x 25cm black polyethelene bags in the phytotrons at 7 days after inoculation.

Virus inoculations

In order to avoid genome reasortment and genome segment-specific adaptation (Qiu & Moyer 1999) and the possible reliance on defective TSWV generated during chains of mechanical inoculations and serial passages through maintenance hosts plants (Kumar *et al.,* 1993; Qiu *et al.,* 1998; Latham & Jones 1998; Thomas-Carroll & Jones 2003; Moyer *et al.,* 2003; Aramburu & Martí 2003; Bucher *et al*., 2003), mechanical inoculations were done using TSWV isolate An_{WA}*-1* inoculum from infected potato plants grown from field-infected tubers. Inoculation was by abrading three youngest fully expanded potato leaves with infected potato leaf sap diluted 1:20 (w/v) in 0.2M phosphate buffer (pH 7.4) containing celite.

Treatments arrangements and test conditions

Two sets of trials, each arranged in a randomised complete block split-split plot design with four replications, were carried out in controlled environments. Temperature was the main plot (treatment) over time of inoculation (sub-plot),

which in turn, were over potato varieties. Two growth chambers were used with temperatures set at 22^{o}C and 16^{o}C after determining the optimum growth conditions for potato varieties used. The first set of replicated trials involved mechanically inoculating plants early (21 days after planting) and late (35 days after planting) and then immediately exposing them to different treatment temperature regimes (**T1** = 16 ^{o}C continuously, **T2** = 16 ^{o}C for 48 hours, inoculate and then transferred to 22 ^{o}C; **T3** = 22 ^{o}C continuously; **T4** = 22 ^{o}C for 48 hours, inoculate and then transferred to 16 ^{o}C). The second set of experiments also involved mechanically inoculating plants early (21 days after planting) and late (35 days after planting) and then maintaining them under glasshouse conditions for 3 days (72 hours) to allow the infection process to occur before exposing them to different treatment temperature regimes as in experment 1. For both experiment #1 and experiment # 2, all plants were maintained at 55% relative humidity and light intensity of 450 μmol-m^{-2}.s^{-1} (Photosynthetically Active Radiation – PAR) under a light/dark cycle of 16 and 8 hours, within each growth chamber. Each of these experiments was repeated 3 times to confirm results.

Virus incidence scoring and testing.
Both TSWV incidence and severity scoring, using symptom expression, were done on each plant starting one week after inoculation. TSWV incidence scoring was based on the whole plant and recorded on a binary system, 1 (present) or 0 (absent). TSWV severity scoring was on three sections of each plant: top, middle and lower canopy. Symptom severity, as a measure of localised or systemic infection in shoots, was scored for the number of leaves with symptoms and total number of lesions on leaves in the top, middle and lower canopy of each plant. Plants were observed for TSWV symptoms for one and half months (45 days) after inoculation and tested by enzyme-linked immunosorbent assay (ELISA)(Clark &Adams, 1977; Gonsalves & Trujillo, 1986) at each rating date to determine virus incidence. After harvest, ELISA was also used to test long-distance movement of the virus from shoots to tubers for each treatment. The experiment was repeated three times.

Data Analysis
All data were analysed using Genstat software ver 6.1.0.200 (Lawes Agricultural Trust, 2002). Data were subjected to analysis of variance for a split-split-plot design using a cultivar x days after inoculation x temperature x time of inoculation treatment structure which included the main effects of temperature as whole plots, time of inoculation as subplots and potato cultivars as sub-sub-plots over four replications. A probability level (under null hypothesis) of 0.05 was used for all tests to determine significance.

Results

Experiment 1: Mechanical inoculation and immediate exposure to different temperature regimes.

Determination of TSWV incidence by ELISA
Cultivar, days after inoculation, leaf position, and time of inoculation and interactively, cultivar x leaf position, days after inoculation x leaf position, cultivar x time of inoculation, leaf position x time of inoculation and temperature regime x time of inoculation, all had significant (P =0.05) influences on detactable TSWV in cv. Shepody and Russet Burbank immediately subjected to different temperature regimes after mechanical inoculation (Table 4.1)

Plants immediately and continuously exposed to 22^{o}C (T3) did not produce tubers. In tubers produced from plants subjected to other temperature conditions, ELISA after harvest returned negative results for TSWV.

Table 4.1 Influence of cultivar, time of inoculation, temperature regime, leaf position and days after inoculation on TSWV incidence determined by ELISA in cvs Shepody and Russet Burbank plants immediately exposed to different temperature regimes after mechanical inoculation.

			TSWV incidence (%)															
			Leaf Position on Plant / Days after Inoculation															
			Top				Middle				Lower				Mean			
Cultivar	Time of Inoculation [a]	Temperature Treatment [b]	7	12	21	28	7	12	21	28	7	12	21	28	7	12	21	28
Shepody	Early	**T1**	0	0	0	0	0	75	75	25	0	50	100	75	0	42	58	33
Shepody	Early	**T2**	25	0	0	*	75	50	50	100	50	75	75	3	50	42	42	*
Shepody	Early	**T3**	50	0	*	*	100	75	100	75	100	100	99	100	83	58	*	*
Shepody	Early	**T4**	0	0	0	0	75	100	75	100	50	100	75	75	42	67	50	58
Shepody	Late	**T1**	0	0	0	0	0	100	0	0	0	50	0	0	0	50	0	0
Shepody	Late	**T2**	0	0	0	0	0	75	0	0	0	0	0	0	0	25	0	0
Shepody	Late	**T3**	0	*	*	*	0	50	0	0	0	*	*	*	0	*	*	*
Shepody	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**9**	**0**	**0**	**0**	**31**	**66**	**38**	**37**	**25**	**47**	**44**	**32**	**22**	**35**	**19**	**11**
Russet Burbank	Early	**T1**	0	0	0	0	100	100	0	0	0	50	75	100	33	50	25	33
Russet Burbank	Early	**T2**	0	0	0	*	0	0	0	4	25	0	2	16	8	0	1	*
Russet Burbank	Early	**T3**	0	0	*	*	75	0	5	*	25	100	54	68	33	33	*	*
Russet Burbank	Early	**T4**	0	0	0	0	0	50	25	25	50	100	75	65	17	50	33	30
Russet Burbank	Late	**T1**	0	0	0	0	0	25	0	0	0	100	0	25	0	42	0	8
Russet Burbank	Late	**T2**	0	0	0	0	0	25	0	0	0	0	0	50	0	8	0	17
Russet Burbank	Late	**T3**	0	2	*	*	0	*	*	*	0	*	*	*	0	*	*	*
Russet Burbank	Late	**T4**	0	0	0	0	0	0	0	0	0	99	0	0	0	33	0	0
Mean			**0**	**0**	**0**	**0**	**22**	**25**	**4**	**4**	**13**	**56**	**26**	**40**	**11**	**27**	**7**	**11**

[a] Early = 21 days after planting; Late = 35 days after planting

[b] **T1** = 16 °C continuously, **T2** = 16 °C for 48 hours, inoculate and then transferred to 22 °C, **T3** = 22 °C continuously, **T4** = 22 °C for 48 hours, inoculate and then transferred to 16 °C.

Cultivar (Cv)	P < 0.001	(1 d.f., l.s.d. = 5.83)	DAI x TI	P = 0.054
Days After Inoculation (DAI)	P < 0.001	(3 d.f., l.s.d. = 8.24)	LP x TI	P < 0.001 (2 d.f., l.s.d. = 10.09)
Leaf Position (LP)	P < 0.001	(2 d.f., l.s.d. = 7.14)	TR x TI	P < 0.001 (3 d.f., l.s.d. = 11.65)
Temperature regime (TR)	P = 0.029	(3 d.f., l.s.d. = 8.24)	Cv x DAI x LP	P = 0.011 (6 d.f., l.s.d. = 20.18)
Time of inoculation (TI)	P < 0.001	(1 d.f., l.s.d. = 5.83)	Cv x DAI x TR	P = 0.301
Cv x DAI	P = 0.688		Cv x LP x TR	P = 0.904
Cv x LP	P < 0.001	(2 d.f., l.s.d. = 10.09)	DAI x LP x TR	P = 0.006 (18 d.f., l.s.d. = 28.54)
DAI x LP	P < 0.001	(6 d.f., l.s.d. = 14.27)	Cv x DAI x TI	P = 0.220
Cv x TR	P = 0.060		Cv x LP x TI	P = 0.146
DAI x TR	P = 0.003	(9 d.f., l.s.d. = 16.48)	DAI x LP x TI	P = 0.056
LP x TR	P = 0.076		Cv TR x TI	P = 0.003 (3 d.f., l.s.d. = 16.48)
Cv x TI	P < 0.001	(1 d.f., l.s.d. = 8.24)	DAI x TR x TI	P = 0.061
			LP x TR x TI	P = 0.039 (6 d.f., l.s.d. = 20.18)
			Cv x DAI x LP x TR	P = 0.311
			Cv x DAI x LP x TI	P = 0.005 (6 d.f., l.s.d. = 28.54)
			Cv x DAI x TR x TI	P = 0.131
			Cv x LP x TR x TI	P = 0.024 (6 d.f., l.s.d. = 28.54)
			DAI x LP x TR x TI	P = 0.041 (16(2) d.f., l.s.d. = 40.36)
			Cv x DAI x LP x TR x TI	P = 0.078

Mean separation within parameters contributing significantly to detactable TSWV indicated that overall, the cv. Shepody had a significantly higher foliar incidence of TSWV, which was observed 12 days after inoculation in middle and lower canopies (Table 4.2)

The number of early-inoculated plants was significantly higher than in late inoculation. And the interactive effects of cultivar x leaf position indicate that both cultivars had significantly similar numbers of plants with detectable TSWV in both top and lower canopies, except in the middle where cv. Shepody was significantly higher. The number of plants with detactable TSWV was significantly similar across all rating days after inoculation in top and middle canopies. However, in lower canopies, significantly more plants had detectable TSWV at 12 days after inoculation (Table 4.3)

The interation between cultivar and time of inoculation had significantly different influence in early and none in late inoculated plants, with cv. Shepody having more plants with detectable TSWV than Russet Burbank. In early-inoculated plants, significantly more plants were tested positive for TSWV in lower canopies. In late inoculated plants, detectable TSWV was found significantly more in middle and lower canopies (Table 4.4)

Except for plants exposed to 16^{o}C for 48 hours, inoculated and then transferred to 22^{o}C (T2), all early-inoculated plants had significantly higher and similar numbers of plants with detectable TSWV. In early-inoculated plants, exposure to 16 ^{o}C continuously resulted in significantly higher TSWV incidence (Table 4.5)

Table 4.2 Independent influence of cultivar, days after Inoculation and leaf position on foliar TSWV incidence determined by ELISA in potato cultivars Shepody and Russet Burbank with instant exposure after inoculation.

Cultivar	Mean (%)[§]	Days After Inoculation	Mean (%)[§]	Leaf Position	Mean (%)[§]
Shepody	28.9a	7	16.7b	Top	0.4b
Russet Burbank	15.0b	12	35.4a	Middle	30.2a
d.f	1	21	17.9b	Lower	35.2a
l.s.d	5.83	28	17.9b	d.f	2
		d.f	3	l.s.d	7.14
		l.s.d	8.24		

[§] Figures followed by different letters within each column indicate a significant difference at the 0.05% probability level

Table 4.3 Independent influence of time of inoculation and interactive effects of cultivar x leaf position and days after inoculation x leaf position on foliar TSWV incidence determined by ELISA in potato cultivars Shepody and Russet Burbank with instant exposure after inoculation.

Time of inoculation[Ŧ]	Mean (%)[§]	Cultivar	Leaf Position[§]			Days After Inoculation	Leaf Position[§]		
			Top	Middle	Lower		Top	Middle	Lower
Early	36.1a	Shepody	2.1a	47.7a	36.8a	7	4.7a	26.6a	18.7c
Late	7.8b	Russet Burbank	0.0a	12.8b	33.7a	12	0.1a	54.6a	51.5a
d.f	1	d.f		2		21	0.0a	20.6a	34.7b
l.s.d	5.83	l.s.d		10.09		28	0.0a	19.1a	36.1b
						d.f		6	
						l.s.d		14.27	

[Ŧ] Early = 21 days after planting; Late = 35 days after planting
[§] Figures followed by different letters within each column indicate a significant difference at the 0.05% probability level

Table 4.4 Interactive influence of cultivar x time of inoculation and leaf position x time of inoculation on foliar TSWV incidence determined by ELISA in potato cultivars Shepody and Russet Burbank with instant exposure after inoculation.

	Time of inoculation [Ŧ]	
Cultivar	Early	Late
Shepody	48.9a	6.8a
Russet Burbank	23.3b	8.9a
d.f	1	
l.s.d	8.24	

	Time of inoculation [Ŧ]	
Leaf Position	Early	Late
Top	0.7c	0.1b
Middle	47.2b	13.3a
Lower	60.4a	10.1ab
d.f	2	
l.s.d	10.09	

[Ŧ] Early = 21 days after planting; Late = 35 days after planting
[Ŧ] Figures followed by different letters within each column indicate a significant difference at the 0.05% probability level

Table 4.5 Interactive influence of temperature regime x time of inoculation on foliar TSWV incidence determined by ELISA in potato cultivars Shepody and Russet Burbank with instant exposure after inoculation.

	Time of inoculation [Ŧ]	
Temperature regime	Early	Late
T1 = 16 ^{o}C continuously	34.4a	18.8a
T2 = 16 ^{o}C for 48 hours, inoculate and then transferred to 22 ^{o}C	22.9b	6.2b
T3 = 22 ^{o}C continuously	43.8a	2.1b
T4 = 22 ^{o}C for 48 hours, inoculate and then transferred to 16 ^{o}C	43.4a	4.1b
d.f	3	
l.s.d	11.65	

[Ŧ] Early = 21 days after planting; Late = 35 days after planting
[Ŧ] Figures followed by different letters within each column indicate a significant difference at the 0.05% probability level

Incidence of foliar TSWV symptoms

Days after inoculation and time of inoculation (age of the plant at the time of inoculation) and two-way interactions at different levels of cultivar x temperature regime, cultivar x time of inoculation (age of the plant at the time of inoculation), days after inoculation x time of inoculation (age of the plant at the time of inoculation), and temperature regime x time of inoculation (age of the plant at the time of inoculation) all significantly ($P < 0.05$) influenced the incidence of TSWV symptoms in both Shepody and Russet Burbank plants that were inoculated and then immediately exposed to different temperature regimes.

Each of these parameters showed a wide range of values. The number of plants with TSWV symptoms was significantly higher ($P < 0.001$) at 12 days postinoculations in both cultivars and after which senescence and absission of infected leaves started to occur. Therefore, the decrease in the incidence during the last two sampling times (21 & 28 days after inoculation) is due to combined effects of a decrease in infected leaf area resulting from leaf necrosis and virus degradation in senescent leaf tissues.

Most of the plants expressing symptoms were those inoculated early and the differences on incidence of TSWV infection were significant ($P < 0.001$). Late inoculated plants of the cultivar Shepody immediately exposed to 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) did not exhibit any symptoms.

The interactive effect of cultivar x temperature was significantly different ($P < 0.05$) in all but plants immediately exposed to 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4). When immediately exposed to 16^{o}C continuously, significantly more plants of the cv. Russet Burbank expressed TSWV symptoms. However, when exposed to 16^{o}C for 48 hours, inoculated and then transferred to 22^{o}C (T2) or continuously to 22^{o}C (T3), significantly more plants of cv. Shepody expressed TSWV symptoms. Exposure to 22^{o}C for 48 hours, inoculating and then transferring plants of both cultivars to 16^{o}C (T4) did not predispose plants to significantly different TSWV expression.

There was a significant effect of time of inoculation on TSWV foliar symptom expression in both cultivars with early inoculations inducing more plants to exhibit symptoms than late inoculations. Significantly higher numbers of plants in cv. Shepody expressed TSWV symptoms than in cv. Russet Burbank in early-inoculated plants. No significant difference was observed in late inoculated plants.

The interaction of days after inoculation and time of inoculation significantly increased the number of early-inoculated plants that expressed foliar TSWV symptoms at 12, 21 and 28 days after mechanical inoculation. In late inoculated plants, significantly more plants had TSWV symptoms at 12 days after inoculation.

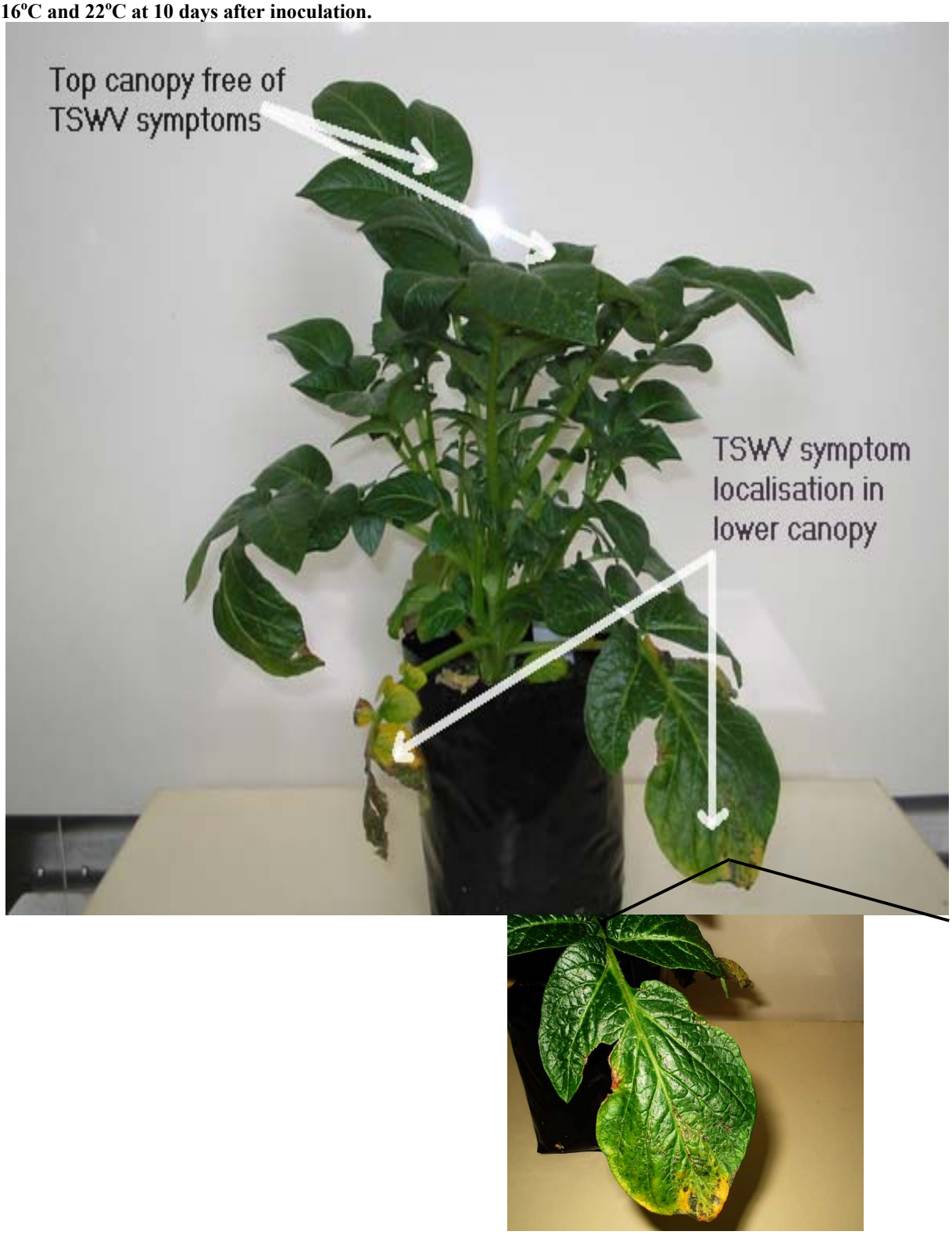
Instant and continuous exposure of early inoculated plants to 16^{o}C (T1) or 22^{o}C (T3) or 22^{o}C for 48 hours, inoculating and then transferring them to 16^{o}C (T4) resulted in a significantly higher number of plants expressing TSWV symptoms.

All the symptoms expressed in both cultivars were restricted to the lower and middle parts of the plant canopy and auxiliary shoots (Figure 4.2). In cv. Shepody, continuous exposure to 16^{o}C (T1) and 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) induced numerous necrotic spots (Figure 4.3). These necrotic spots also delayed in appearing and lesion expansion was restricted (Figure 4.3). In cv. Russet Burbank, fewer necrotic symptoms appeared when plants were exposed to 16^{o}C continuously (T1). On leaves where necrotic lesions appeared, chlorotic hues rapidly developed around them, followed by leaf necrosis and senescence. In contrast, plants of both cultivars continuously exposed to 22^{o}C developed TSWV symptoms two days earlier (5 days after inoculation). Continuous exposure to 22 ^{o}C also caused rapid lesion expansion in both cultivars and was followed by leaf and plant death in cv. Russet Burbank at 21 days after inoculation. In cv. Shepody, necrotic leaves remained attached to the stems and lodged within the canopy while those in cv. Russet Burbank senescenced as a mechanism to restrict long distance movement of the virus (Figure 4.3)

Number of leaves with TSWV lesions

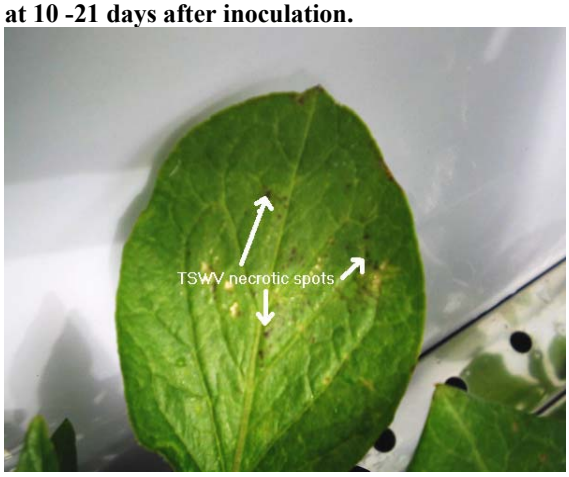
The number of leaves with TSWV spots and lesions was significantly dependent (P = 0.05) on cultivar, days after inoculation, position of leaf on the plant, temperature regime, time of inoculation (plant age at inoculation), two-way interactive effects between cultivar and leaf position, leaf position and temperature regime, cultivar and time of inoculation (plant age at inoculation), days after inoculation and time of inoculation (plant age at inoculation), leaf position and time of inoculation (plant age at inoculation), temperature regime and time of inoculation (plant age at inoculation), three-way interactions between leaf position, temperature regime and time of inoculation (plant age at inoculation), four-way interactions between cultivar, leaf position, temperature regime and time of inoculation (plant age at inoculation), and days after inoculation, leaf position, temperature regime and time of inoculation (plant age at inoculation). A three-way interaction of cultivar, leaf position and time of inoculation (plant age at inoculation) also had some influence (P = 0.001). The number of leaves with TSWV spots and lesions was independent of the highiest level interaction between cultivar x days after inoculation x leaf position x temperature regime x time of inoculation (plant age at inoculation)(P = 0.359)(Table 4.6). Residue distribution indicating the quantitative singular and interactive contribution of each of the assessed parameters to the number of leaves with TSWV spots and lesions is provided in Table 4.3.

Figure 4.2 TSWV symptoms localization in lower leaf canopy in cvs Shepody with immediate exposure to 16°C and 22°C at 10 days after inoculation.

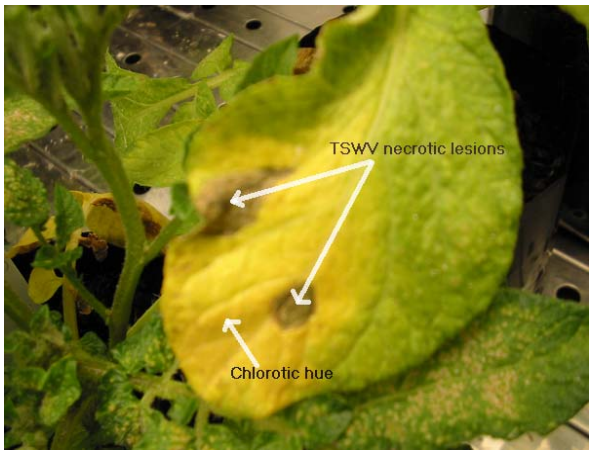


TSWV symptom localization to lower leaves in cv. Shepody

Figure 4.3 TSWV symptoms in cvs Shepody and Russet Burbank with immediate exposure to 16°C and 22°C at 10 -21 days after inoculation.

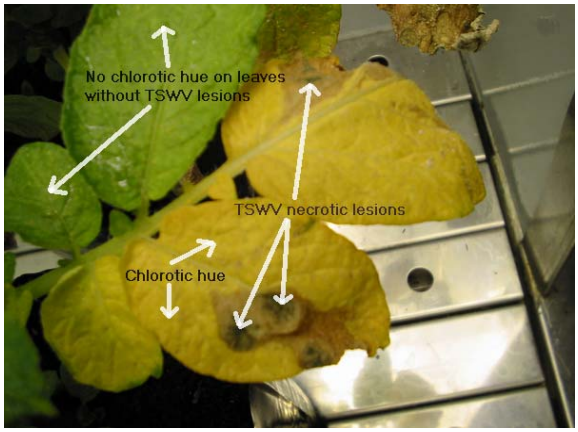


Systemic TSWV necrotic spots at 7 days after inoculation in cv. Shepody at 16°C



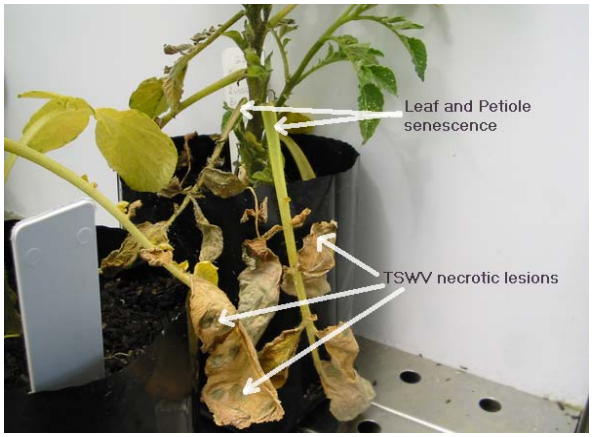
Chlorotic hue surrounding TSWV necrotic spots to restrict virus movement in cv. Russet Burbank at 16°C

Systemic TSWV necrotic spots in cv. Shepody at 16°C



Chlorotic hue surrounding TSWV necrotic spots to restrict virus movement in cv. Russet Burbank at 16°C

Rapidly coalescing TSWV lesions and start of leaf necrosis in cv. Shepody at 22°C



Rapidy leaf senescence in cv. Russet Burbank to restrict virus movement at 22°C

Table 4.6 Influence of cultivar, time of inoculation, temperature regime, leaf position and days after inoculation on the number of leaves with TSWV spots and lesions in cvs Shepody and Russet Burbank plants immediately exposed to different temperature regimes after mechanical inoculation.

			Number of leaves with lesions															
			Leaf Position on Plant / Days after Inoculation															
			Top				Middle				Lower				Mean			
Cultivar	Time of Inoculation [a]	Temperature Treatment [b]	7	12	21	28	7	12	21	28	7	12	21	28	7	12	21	28
Shepody	Early	**T1**	0	0	0	0	0	1	1	1	0	1	5	4	0	1	2	2
Shepody	Early	**T2**	0	0	0	0	1	3	1	1	1	1	1	0	1	1	1	0
Shepody	Early	**T3**	1	0	0	0	4	3	6-	4-	2	2	2	2	2	2	3	2
Shepody	Early	**T4**	0	0	0	0	1	3	6-	4	1	2	4-	5-	1	2	3	3
Shepody	Late	**T1**	0	0	0	0	0	3	0	0	0	3	0	0	0	2	0	0
Shepody	Late	**T2**	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T3**	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**0**	**0**	**0**	**0**	**1**	**2**	**2**	**1**	**1**	**1**	**2**	**1**	**0**	**1**	**1**	**1**
Russet Burbank	Early	**T1**	0	0	0	0	1	1	0	0	0	1	1	2	0	1	0	1
Russet Burbank	Early	**T2**	0	0	0	*	0	0	0	*	1	0	0	*	0	0	0	*
Russet Burbank	Early	**T3**	0	0	*	*	2	0	*	*	0	6	*	*	1	2	*	*
Russet Burbank	Early	**T4**	0	0	0	0	0	1	0	0	1	2	2	1	0	1	1	0
Russet Burbank	Late	**T1**	0	0	0	0	0	0	0	0	0	7	0	1	0	2	0	0
Russet Burbank	Late	**T2**	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Russet Burbank	Late	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T4**	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0
Mean			**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**2**	**0**	**1**	**0**	**1**	**0**	**0**

[a] Early = 21 days after planting; Late = 35 days after planting

[b] **T1 =** 16 ºC continuously,
T2 = 16 ºC for 48 hours, inoculate and then transferred to 22 ºC,
T3 = 22 ºC continuously
T4 = 22 ºC for 48 hours, inoculate and then transferred to 16 ºC.

* = Plant died

Cultivar (Cv):	P < 0.001	(1 d.f., l.s.d. = 0.1889)
Days after inoculation (DAI):	P < 0.001	(3 d.f., l.s.d. = 0.2671)
Leaf Position (LP):	P < 0.001	(2 d.f., l.s.d. = 0.2313)
Temperature Regime (TR):	P < 0.001	(3 d.f., l.s.d. = 0.2671)
Time of inoculation (TI):	P < 0.001	(1 d.f., l.s.d. = 0.1889)
Cv x DAI:	P = 0.022	(3 d.f., l.s.d. = 0.3778)
Cv x LP:	P < 0.001	(2 d.f., l.s.d. = 0.3272)
DAI x LP:	P = 0.002	(6 d.f., l.s.d. = 0.4627)
Cv x TR:	P = 0.182	
DAI x TR:	P = 0.026	(9 d.f., l.s.d. = 0.5342)
LP x TR:	P < 0.001	(6 d.f., l.s.d. = 0.4627)
Cv x TI:	P < 0.001	(1 d.f., l.s.d. = 0.2671)
DAI x TI:	P < 0.001	(3 d.f., l.s.d. = 0.3778)
LP x TI:	P < 0.001	(2 d.f., l.s.d. = 0.3272)
TR xTI:	P < 0.001	(3 d.f., l.s.d. = 0. 3778)
Cv x DAI x LP:	P = 0.011	(6 d.f., l.s.d. = 0. 6543)
Cv x DAI xTR:	P = 0.617	
Cv x LP xTR:	P = 0.309	
DAI x LP x TR:	P = 0.144	
Cv x DAI x TI:	P = 0.074	
Cv x LP xTI:	P = 0.001	(2 d.f., l.s.d. = 0.4627)
DAI x LP x TI:	P = 0.008	(6 d.f., l.s.d. = 0.6543)
Cv x TR x TI:	P = 0.002	(3 d.f., l.s.d. = 0.5342)
DAI x TR x TI:	P = 0.002	(9 d.f., l.s.d. = 0.7555)
LP x TR x TI:	P < 0.001	(6 d.f., l.s.d. = 0. 6543)
Cv x DAI x LP x TR:	P = 0.898	
Cv x DAI x LP x TI:	P = 0.482	
Cv x DAI xTR xTI:	P = 0.367	
Cv x LP xTR xTI:	P < 0.001	(6 d.f., l.s.d. = 0. 9253)
DAI x LP x TR xTI:	P < 0.001	(16 (2) d.f., l.s.d. = 1.3086)
Cv x DAI x LP x TR xTI:	P = 0.359	

Mean separation within parameters of the two highiest and parallel four-way interactions that were significant (P = 5%)(Table 4.6) reveals the contribution of each to the number of leaves with TSWV symptoms. Cv. Shepody had more leaves with TSWV symptoms than cv. Russet Burbank. Early-inoculated plants had significantly more leaves with TSWV compared to those inoculated late. Late inoculation did not generally predispose plants of both cultivars to significantly different number of leaves with TSWV symptoms except in plants of cv. Russet Burbank continuously exposed to 16^{o}C (T1) in lower parts of the canopies. There were generally no TSWV symptoms observed in top parts of the canopies of both cultivars, confirming the significant differences observed in the two-way interactions of leaf position and time of inoculation (plant age at inoculation). Significantly more leaves with TSWV symptoms were observed in middle followed by lower canopies. Analysis of interactions between the different parameters confirmed these general observations and further revealed that in cv. Shepody, more leaves with TSWV symptoms were observed in the middle part of the canopy. In cv. Russet Burbank, the number of leaves with TSWV symptoms was significantly higher in the lower than any other part of the canopy. Continuous exposure of early-inoculated plants of cv. Shepody to 22^{o}C (T3) produced the highiest number of leaves with symptoms although not significantly different from those subjected to 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4). The lowest number of leaves with symptoms in both cultivars was obtained by exposing early-inoculated plants to 16 ^{o}C for 48 hours, inoculating and then transferring them to 22^{o}C (T2). In late inoculated plants, the cv. Russet Burbank had significantly more leaves with TSWV symptoms in lower parts of the canopies in plants that were continuously exposed to 16^{o}C (T1).

Generally early-inoculated plants had significantly more leaves with TSWV compared to those inoculated late. At 7 days after inoculation, significantly more leaves with TSWV symptoms were observed in early-inoculated plants continuously exposed to 22^{o}C (T3) in middle canopies. The same phenomenon was observed in plants exposed to similar temperature conditions at 12 days after inoculation in lower canopies of plants. At 21 days after inoculation, significant numbers of leaves were observed in middle canopies in early inoculated plants exposed continuously to 22^{o}C (T3) and 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) and lower canopies in plants continuously exposed to 16^{o}C and 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4). When plants were continuously exposed to 16^{o}C and 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4), significantly more leaves with TSWV symptoms were observed in lower canopies at 28 days after inoculation. Late inoculations did not generally induce plants of both cultivars to exbit TSWV sysmptoms. However, more leaves with symptoms, including the significantly greatest number of leaves with TSWV (mean= 5.250) were observed at 12 days after inoculation in the lower and middle parts of the plant canopies and auxiliary shoots of late inoculated plants.

Area covered by TSWV spots and lesions

The total area covered by TSWV spots and lesions on leaves in different parts of the plant canopy was scored on an increasing scale of 1 = no symptoms, 2 = very few spots covering less than 25% of leaves, 3 = spots covering 25-50% of leaves, 4 = spots covering 50-75% of leaves with some defoliation, 5 = numerous spots with significant defoliation and death of the plant. The total area covered by TSWV spots and lesions on leaves was significantly (*P* = 0.05) influenced independently by all the parameters tested (cultivar, days after inoculation, leaf position, temperature regime, time of inoculation (plant age at inoculation) and the interactive influence of cultivar and leaf position (Cv x LP), days after inoculation and leaf position (DAI x LP), days after inoculation and temperature regime (DAI x TR), leaf position and temperature regime (LP x TR), cultivar and time of inoculation (plant age at inoculation)(Cv x TI), leaf position and time of inoculation (plant age at inoculation)(LP x TI), temperature regime and time of inoculation (plant age at inoculation)(TR x TI), cultivar, leaf position and time of inoculation (plant age at inoculation)(Cv x LP x TI), leaf position, temperature regime and time of inoculation (plant age at inoculation)(LP x TR x TI) and days after inoculation, leaf position, temperature regime and time of inoculation (plant age at inoculation)(DAI x LP x TR x TI). The highiest level of interations between cultivar x days after inoculation x leaf position x temperature regime x time of inoculation (plant age at inoculation) was not significant (P = 0.102).

The total leaf area covered by TSWV spots and lesions was independent of the two-way interactions between cultivar and days after inoculation (P = 0.005), which consequently produced two significant parallel interactions involving both parameters. With two-way interactions between cultivar and temperature regime not being significant for the area covered by TSWV spots and symptoms, the highiest interaction, which included cultivar, was between cultivar x leaf position x time of inoculation (plant age at inoculation). Parallel to that was a four-way interaction involving days after inoculation x leaf position x temperature regime x time of inoculation (plant age at inoculation)(DAI x LP x TR x TI).

Mean separation within the three-way interactions between cultivar x leaf position x time of inoculation (plant age at inoculation) revealed the interactive influences of these parameters on the leaf areas affected by TSWV. Significantly more early-inoculated plants of cv. Shepody had larger leaf areas (25-50% of leaves) covered by TSWV spots and lesions in middle and lower parts of the canopy than other plants and cv. Russet Burbank. In late inoculated plants, the cv. Russet Burbank had more leaf area with TSWV necrotic spots and lesions covering up to 25% of leaves in lower parts of the canopy and cv. Shepody in middle canopies. In the top parts of the canopy, no TSWV spots or lesions symptoms were observed in both cultivars.

The interactions of days after inoculation x leaf position x temperature regime x time of inoculation (plant age at inoculation) resulted in significantly larger leaf areas covering 25-75% of leaves with TSWV spots and lesions and some defoliation in early inoculated plants continuously exposed to 22^{o}C (T3), in middle, lower, and middle and lower canopies at 7, 12 and 21 days after inoculation respectively. In late inoculated plants, lower canopies of plants exposed continuously to 16^{o}C (T1) at 12 days after inoculation had significantly more leaf area (25 - 50% of leaves), with TSWV symptoms. The decline in leaf area with TSWV spots and lesions, particularly in the cv. Russet Burbank, 21 days after inoculation was largely due to leaf senescence. Comparative observations of plant growth, symptom development and lesion size characteristics are given in Table 4.7

Table 4.7 Comparative symptom characteristics in mechanically inoculated plants of cvs Shepody and Russet Burbank immediately exposed to different temperature regimes.

		Parameter		
Cultivar	**Temperature Regime [Ŧ]**	**Plant growth**	**Symptom development**	**Lesion size**
Shepody	**T1**	Good	Very slow and systemic	Appearing as numerous necrotic spots
Russet Burbank	**T1**	Good	Very slow & localized in inoculated lower leaves	Appearing as few necrotic spots
Shepody	**T2**	Average & better than in T3	Slow and localized in inoculated lower leaves	Larger than in T1 but less than in T3 and appearing as medium sized necrotic spots
Russet Burbank	**T2**	Poor but better than at T3	Slow and localized in inoculated lower leaves	Larger than in T1 but less than in T3 and appearing as medium sized necrotic spots
Shepody	**T3**	Poor growth	Rapid developing & conspicuous on inoculated leaves	Fewer and large. Rapidly coalescing leading to leaf necrosis
Russet Burbank	**T3**	Poor, leaf and petiole senescence, early plant death	Rapid developing & conspicuous on inoculated leaves. Leaf absission rapidly follows.	Fewer and large Rapidly coelescing
Shepody	**T4**	Average & better than in T2 & T3	Slow and localized in inoculated lower leaves. Appears earlier than in T1 but later than in T3	Appearing as necrotic spots fewer but larger than in T1
Russet Burbank	**T4**	Average & better than in T2 & T3	Slow and localized in inoculated lower leaves. Appears later than in T3	Appearing as necrotic spots but larger than in T1

[Ŧ] **T1** = 16 ^{o}C continuously,
[Ŧ] **T2** = 16 ^{o}C for 48 hours, inoculate and then transferred to 22 ^{o}C,
[Ŧ] **T3** = 22 ^{o}C continuously,
[Ŧ] **T4** = 22 ^{o}C for 48 hours, Inoculate and then transferred to 16 ^{o}C.

Experiment 2: Mechanical inoculation followed by a 72 hours delayed exposure to different temperature regimes.

Determination of TSWV incidence by ELISA

All parameters tested significantly influenced (P =0.05) detectable TSWV in cv. Shepody and Russet Burbank that had 72hours delayed exposure to different temperature regimes after mechanical inoculation. Means of TSWV incidence (%) resulting from parameter interactions and distribution of total residues are given in Table 4.8. Mean separation within the highiest significant level interaction of cultivar x days after inoculation x leaf position x temperature regime x time of inoculation indicate that the incidence of TSWV was significantly higher in cv. Russet Burbank plants exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) and all (100%) had detectable TSWV in the top, top and middle and top canopies at 28, 35 and 43 days after inoculation respectively. This response indicate that exposure to higher followed by lower temperatures, promotes more efficient virus translocation in cv. Russet Burbank than exposure to constantly lower temperatures. In cv. Shepody, the only plants detected with TSWV were also those inoculated early and exposed to T4 temperature conditions with mean TSWV incidence much lower (25%) than in cv. Russet Burbank.This result suggest that although exposure to these conditions promotes virus migration within cv. Shepody plants, the pace of such movement is much more delayed in this cultivar. The combined response of the two cultivars in this experiment indicate that virus movement into middle and top parts of the canopy was delayed and dependent on both the temperature (T1 & T4) and time of inoculation (21 days after planting)(Table 4.15). For both temperature regimes (T1 & T4), the virus did not reach the middle and top canopies until 28 days after inoculation in cv. Russet Burbank plants and 43 days after inoculation in cv. Shepody. Clearly, lowering temperatures to 16^{o}C (T1 & T4) combined with inoculation at an early growth stage promoted TSWV infections observed. The time of inoculation was particularly important when exposure to different temperature regimes was delayed by 72 hours, in which ELISA detected no TSWV in late inoculated plants of both cultivars (Table 4.8).

Table 4.8 Influence of cultivar, days after inoculation, leaf position, temperature regime and time of inoculation on TSWV incidence determined by ELISA in cvsShepody and Russet Burbank with a 72hours delayed exposure after inoculation

			TSWV incidence (%)																					
			Leaf Position on Plant / Days after Inoculation																					
			Top						Middle						Lower						Mean			
Cultivar	Time of Inoculation [a]	Temperature Treatment [b]	7	12	21	28	35	43	7	12	21	28	35	43	7	12	21	28	35	43	7	12	21	28	35	43
Shepody	Early	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T4**	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.3
Shepody	Late	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**0**	**0**	**0**	**0**	**0**	**3.1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**
Russet Burbank	Early	**T1**	0	0	0	75	75	75	0	0	0	25	50	50	0	0	0	0	0	0	0	0	0	33	42	42
Russet Burbank	Early	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Early	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Early	**T4**	0	0	0	100	100	100	0	0	0	50	100	100	0	0	0	0	0	0	0	0	0	50	67	67
Russet Burbank	Late	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**0**	**0**	**0**	**22**	**22**	**22**	**0**	**0**	**0**	**9.4**	**18.8**	**19**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**14**	**14**

[a] Early = 21 days after planting; Late = 35 days after planting

[b] **T1** = 16 ºC continuously, **T2** = 16 ºC for 48 hours, inoculate and then transferred to 22 ºC, **T3** = 22 ºC continuously, **T4** = 22 ºC for 48 hours, inoculate and then transferred to 16 ºC.

Cultivar (Cv)	P	< 0.001 (1 d.f., l.s.d. = 1.015)
Days After Inoculation (DAI)	P	< 0.001 (5 d.f., l.s.d. = 1.759)
Leaf Position (LP)	P	< 0.001 (2 d.f., l.s.d. = 1.244)
Temperature regime (TR)	P	< 0.001 (3 d.f., l.s.d. = 1.436)
Time of inoculation (TI)	P	< 0.001 (1 d.f., l.s.d. = 1.015)
Cv x DAI	P	< 0.001 (5 d.f., l.s.d. = 2.487)
Cv x LP	P	< 0.001 (2 d.f., l.s.d. = 1.759)
DAI x LP	P	< 0.001 (10d.f., l.s.d. = 3.046)
Cv x TR	P	< 0.001 (3 d.f., l.s.d. = 2.031)
DAI x TR	P	< 0.001 (15 d.f., l.s.d. = 3.517)
LP x TR	P	< 0.001 (6 d.f., l.s.d. = 2.487)
Cv x TI	P	< 0.001 (1 d.f., l.s.d. = 1.436)
DAI x TI	P	< 0.001 (5 d.f., l.s.d. = 2.487)
LP x TI	P	< 0.001 (2 d.f., l.s.d. = 1.759)
TR x TI	P	< 0.001 (3 d.f., l.s.d. = 2.031)
Cv x DAI x LP	P	< 0.001 (10 d.f., l.s.d. = 4.308)
Cv x DAI x TR	P	< 0.001 (15 d.f., l.s.d. = 4.974)
Cv x LP x TR	P	< 0.001 (6 d.f., l.s.d. = 3.517)
DAI x LP x TR	P	< 0.001 (30 d.f., l.s.d. = 6.092)
Cv x DAI x TI	P	< 0.001 (5 d.f., l.s.d. = 3.517)
Cv x LP x TI	P	< 0.001 (2 d.f., l.s.d. = 2.487)
DAI x LP x TI	P	< 0.001 (10 d.f., l.s.d. = 4.308)
Cv TR x TI	P	< 0.001 (3 d.f., l.s.d. = 2.872)
DAI x TR x TI	P	< 0.001 (15d.f., l.s.d. = 4.974)
LP x TR x TI	P	< 0.001 (6 d.f., l.s.d. = 3.517)
Cv x DAI x LP x TR	P	< 0.001 (30 d.f., l.s.d. = 8.616)
Cv x DAI x LP x TI	P	< 0.001 (10 d.f., l.s.d. = 6.092)
Cv x DAI x TR x TI	P	< 0.001 (15 d.f., l.s.d. = 7.035)
Cv x LP x TR x TI	P	< 0.001 (6 d.f., l.s.d. = 4.974)
DAI x LP x TR x TI	P	< 0.001 (30 d.f., l.s.d. = 8.616)
Cv x DAI x LP x TR x TI	P	< 0.001 (30 d.f., l.s.d. = 12.184)

Incidence of foliar TSWV symptoms

A 72 hours delayed exposure of mechanically inoculated plants of cv. Shepody and Russet Burbank to different temperature regimes had a completely different influence on TSWV incidence. In cv. Shepody, foliar TSWV symptoms were only observed at 43 days after inoculation in plants that were inoculated early and exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4). In cv. Russet Burbank, TSWV were only observed at 28, 35 and 43 days after mechanical inoculation in early-inoculated plants exposed continuously to 16^{o}C (T1) and 22^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4). All other plants of both cultivars and tubers after harvest had no detectable TSWV by ELISA, and cosenquently, analyses are based only on foliar TSWV infections.

Mean separation of the highiest level interactions of cultivar x days after inoculation x temperature regime x time of inoculation ($P < 0.001$,15 d.f., l.s.d. = 14.205) reveals that significantly more early-inoculated plants of the cv Russet Burbank exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4) had TSWV symptoms at 28, 35 and 43 days after inoculation. TSWV was not detected in late inoculated plants of both cultivars.

Number of leaves with TSWV spots and lesions

Analysis of variance for the number of leaves with TSWV spots and lesions also shows significance for all parameters assessed after back transformation of data. In contrast to observations in mechanically inoculated plants that were instantly exposed to different temperature regimes, significantly higher numbers of leaves with TSWV spots and lesions were in top and middle canopies (Table 4.9). Mean separation of the highiest level interactions of cultivar x days after inoculation x leaf position x temperature regime x time of inoculation ($P < 0.001$, 30 d.f., l.s.d. = 0.7448) indicated that significantly more leaves in the top canopies of cv. Russet Burbank plants that were exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4) had TSWV spots and lesions at 43 days after inoculation (mean = 8.50). In cv. Shepody, the only TSWV symptoms observed were in a few early-inoculated plants exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4) at 43 days after inoculation. No TSWV spots or lesions were observed in late inoculated plants in both potato cultivars (Table 4.9).

Table 4.9 Influence of cultivar, time of inoculation, temperature regime, leaf position and days after inoculation on the number of leaves exhibiting TSWV symptoms in cvs Shepody and Russet Burbank with a 72hours delayed exposure after inoculation.

			Mean number of leaves with lesions																	
			Leaf Position on Plant / Days after Inoculation																	
			Top						Middle						Lower					
Cultivar	Time of Inoculation [a]	Temperature Treatment [b]	7	12	21	28	35	43	7	12	21	28	35	43	7	12	21	28	35	43
Shepody	Early	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Early	**T4**	0	0	0	0	0	0.25	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Shepody	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**0**	**0**	**0**	**0**	**0**	**0.03**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Russet Burbank	Early	**T1**	0	0	0	3.25	5.5	7.0	0	0	0	0.25	1.25	1.75	0	0	0	0	0	0
Russet Burbank	Early	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Early	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Early	**T4**	0	0	0	3.75	7.25	8.5	0	0	0	0.5	1.5	1.75	0	0	0	0	0	0
Russet Burbank	Late	**T1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T2**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T3**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Russet Burbank	Late	**T4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean			**0**	**0**	**0**	**0.88**	**1.6**	**1.9**	**0**	**0**	**0**	**0.09**	**0.34**	**0.44**	**0**	**0**	**0**	**0**	**0**	**0**

[a] Early = 21 days after planting; Late = 35 days after planting

[b] **T1 =** 16 °C continuously,
T2 = 16 °C for 48 hours, inoculate and then transferred to 22 °C,
T3 = 22 °C continuously,
T4 = 22 °C for 48 hours, inoculate and then transferred to 16 °C.

Cultivar (Cv):	P < 0.001 (1 d.f., l.s.d. = 0.0621)
Days after inoculation (DAI):	P < 0.001 (5 d.f., l.s.d. = 0.1075)
Leaf Position (LP):	P < 0.001 (2 d.f., l.s.d. = 0.0760)
Temperature Regime (TR):	P < 0.001 (3 d.f., l.s.d. = 0.0878)
Time of inoculation (TI):	P < 0.001 (1 d.f., l.s.d. = 0.0621)
Cv x DAI:	P < 0.001 (5 d.f., l.s.d. = 0.1520)
Cv x LP:	P < 0.001 (2 d.f., l.s.d. = 0.1075)
DAI x LP:	P < 0.001 (10 d.f., l.s.d. = 0.1862)
Cv x TR:	P < 0.001 (3 d.f., l.s.d. = 0.1241)
DAI x TR:	P < 0.001 (15 d.f., l.s.d. = 0.2150)
LP x TR:	P < 0.001 (6 d.f., l.s.d. = 0.1520)
Cv x TI:	P < 0.001 (1 d.f., l.s.d. = 0.0878)
DAI x TI:	P < 0.001 (5 d.f., l.s.d. = 0.1520)
LP x TI:	P < 0.001 (2 d.f., l.s.d. = 0.1075)
TR xTI:	P < 0.001 (3 d.f., l.s.d. = 0.1241)
Cv x DAI x LP:	P < 0.001 (10 d.f., l.s.d. = 0.2633)
Cv x DAI xTR:	P < 0.001 (15 d.f., l.s.d. = 0.3040)
Cv x LP xTR:	P < 0.001 (6 d.f., l.s.d. = 0.2150)
DAI x LP x TR:	P < 0.001 (30 d.f., l.s.d. = 0.3724)
Cv x DAI x TI:	P < 0.001 (5 d.f., l.s.d. = 0.2150)
Cv x LP xTI:	P < 0.001 (2 d.f., l.s.d. = 0.1520)
DAI x LP x TI:	P < 0.001 (10 d.f., l.s.d. = 0.2633)
Cv x TR x TI:	P < 0.001 (3 d.f., l.s.d. = 0.1755)
DAI x TR x TI:	P < 0.001 (15 d.f., l.s.d. = 0.3040)
LP x TR x TI:	P < 0.001 (6 d.f., l.s.d. = 0.2150)
Cv x DAI x LP x TR:	P < 0.001 (30 d.f., l.s.d. = 0.5266)
Cv x DAI x LP x TI:	P < 0.001 (10 d.f., l.s.d. = 0.3724)
Cv x DAI xTR xTI:	P < 0.001 (15 d.f., l.s.d. = 0.4300)
Cv x LP xTR xTI:	P < 0.001 (6 d.f., l.s.d. = 0.3040)
DAI x LP x TR xTI:	P < 0.001 (30 d.f., l.s.d. = 0.5266)
Cv x DAI x LP x TR xTI:	P < 0.001 (30 d.f., l.s.d. = 0.7448)

Area covered by TSWV spots and lesions

The area covered by TSWV spots and lesions followed the same trend as other parameters tested. Mean separation for the significant highest level interaction between cultivar x days after inoculation x leaf position x temperature regime x time of inoculation indicate that the largest leaf area covering >75% of leaves with TSWV spots and/or lesions and significant defoliation and death of the plants was observed at 35 and 43 days after inoculation in the top canopies of early inoculated plants of cv. Russet Burbank exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4). This was followed by leaf area under similar conditions in middle canopies of the same cultivar. In cv. Shepody, the only leaf areas covered by TSWV spots and lesions were at 28 and 43 days after inoculation in the top canopies of plants exposed to 22 ^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4). No TSWV symptoms were observed in the lower canopies of early inoculated plants or in late inoculated plants.

Discussion

The progress of TSWV infection in plants is thought to be accomplished through specific recognition of homologous NSm-binding plant proteins of the DnaJ family nucleocapsid structures (Soellick *et al*., 1999) by the NSm viral movement protein (Kormelink *et al*., 1994; Gunasinghe & Buck, 2003) which link the viral structures to elements of a plant machinery directing intercellular traversal of several cell types from an initially infected cell, through plasmodesmata, mesophyll cells, phloem parenchyma cells, and/or companion cells and then transported to other organs and tissues through the phloem and unloading from the phloem to nonvascular tissues (Lucas & Gilbertson 1994; Storms *et al*.,1995; Carrington *et al*., 1996; Gilbertson & Lucas 1996; Séron & Haenni 1996; Nelson & van Bel 1998). Such viral systemic movement and the induction of resistance responses by plants infected with TSWV has been shown to be affected by host variety (Sela 1981; Soler *et al*., 1998; Llamas-Llamas *et al*., 1998), temperature (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998) and water stress (Córdoba *et al*., 1991). In many TSWV-crop infections, the rates of virus movement have been observed to be greater in sensitive compared with tolerant varieties (Sela 1981; Moury *et al*., 1997; Soler *et al*., 1998, 1999; Maris *et al*., 2003).

The current study aimed at quantifying the influence of temperature and its interaction with plant age at the time of inoculation on foliar and tuber infections, and symptom expression in commonly commercially grown potato cultivars Shepody (susceptible) and Russet Burbank (tolerant). The results presented in this study provide evidence that the rresistance to TSWV in the two cultivars, as measured by the key criteria of foliar and tuber infections, and symptom expression, is temperature-sensitive and plant age dependent. And the number of days taken after inoculation for the virus and symptoms to be detectable in different parts of the plants gives an indication of translocation efficiency.

In this study, it was demonstrated that in plants that were inoculated and immediately exposed to different temperature treatments, TSWV incidence, and the number of leaves and leaf areas with symptoms as a measure of severity, that was also confirmed by ELISA, was generally, restricted to lower and middle parts of the plant canopy and auxiliary shoots and more leaves of early-inoculated plants expressed symptoms than in late inoculations. In early-inoculated cv. Shepody plants continuously exposed to 22^{o}C (T3) virus migration went even further and induced TSWV symptoms in top canopies two days earlier (5 days after inoculation) than similar plants under continuous 16^{o}C (T1)(Table 4.3). In both cultivars, plants were more resistant to TSWV infections when inoculated late and instantly exposed to 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) (Table 4.7). But even under these conditions, some infections were detected in lower inoculated leaves of cv. Russet Burbank plants at 12 days after inoculation. In contrast to virus movement patterns in plants instantly exposed to different temperature conditions after inoculation, the incidence of TSWV, number of leaves and leaf areas with symptoms in plants that were inoculated and had a 72 hours delay before exposure were in the middle and top canopies. The effects of plant age on virus translocation were also evident in these plants. The virus was detected only in plants inoculated early. Except for plants of both cultivars exposed continuously to 16^{o}C (T1) and 22^{o}C for 48 hours, inoculated and then transferred to 16^{o}C (T4) in which virus movement into middle and top canopies was comparatively efficient but only detected at 28 days after inoculation in cv. Russet Burbank and 43 days after inoculation in cv. Shepody, all other plants did not exhibit TSWV symptoms and the virus was not detected in their shoots or tubers by ELISA. Delayed exposure to different temperature regimes was meant to allow the initial infection process to take place under glasshouse conditions. But from data presented and the observed pattern of infections, it would suggest the existence of an inhibition mechanism which was triggered when plants were exposed to such growing conditions which prevented either virus replication within newly infected cells and cell-to-cell movement, or deactivation of a cascade of homologous NSm-binding plant proteins of the DnaJ family nucleocapsid structures (Soellick *et al*., 1999) that facilitates infection; a process that inhibits or enhances resistance in other viral systems (Loebenstein & Gera 1981, 1988; Sela 1981; Moser *et al*., 1988; Deom *et al*., 1991; Lindbo *et al*., 1993; Myers *et al*., 1993; Lucas & Gilbertson 1994; Carrington *et al*., 1996; Al-Kaff *et al*.,1998; Garg *et al*., 1999; Voinnnet 2001; Vance & Vaucheret 2001; Bucher *et al*., 2003; Kobori *et al*. 2003; Syller 2003; Ye *et al*., 2003; Novina & Sharp

2004). The efficiency of cell-to-cell movement is often related to that of vascular movement (Séron & Haenni 1996). And this was demonstrated in tomato plants by Córdoba *et al*., (1991) in which, although TSWV inoculation was effective both on water-stressed and well-watered plants, systemic virus infection was somewhat slower in the water stressed plants. In commercial agriculture in Australia, production of cv. Russet Burbank is known to require more irrigation water than cv. Shepody, and therefore, restricted to certain areas. This was also evident in this study when trying to adjust optimum temperature and relative humidity in the phytotrons (environmental chambers). Russet Burbank plants developed oedemma more often in temperatures above 22°C and 55% relative humidity. This would suggest that the transpiration rate is higher in cv. Russet Burbank than in Shepody. Theoretically, such high transpiration rate is expected to enhance intercellular and vascular viral transport to tissues that are sinks for photoassimilates (Carrington *et al*., 1996; Séron & Haenni 1996). The fact that this was not the case in cv. Russet Burbank suggests the involvement of other genetic factors.

Figure 4.4. TSWV incidence determined by ELISA in potato cultivars Shepody and Russet Burbank plants instantly exposed to different temperature regimes after mechanical inoculation.

	Early inoculation [Ŧ]								Late inoculation [Ŧ]							
	Shepody				Russet Burbank				Shepody				Russet Burbank			
	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
Uninoculated top leaves	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-
Uninoculated middle leaves	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-
Inoculated lower leaves	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+

[Ŧ] Early inoculation = 21 days after planting; Late inoculation = 35 days after planting
[Ŧ] **T1** = 16 °C continuously
[Ŧ] **T2** = 16 °C for 48 hours, inoculate and then transferred to 22 °C
[Ŧ] **T3** = 22 °C continuously
[Ŧ] **T4** = 22 °C for 48 hours, inoculate and then transferred to 16 °C

Comparisons of virus infection patterns and spread within plants from inoculated leaves and symptom expressions in plants subjected to the two exposure methods (instant vs 72 hrs delay) suggest that in inoculated plants that were instantly exposed to different temperature treatments, foliar and tuber infections, and symptom expression were generally restricted to lower and middle leaves of the canopies (Figure 4.4). However, in three cases the virus managed to reach the top parts of the plants and was briefly detectable by ELISA in early inoculated cv. Shepody plants exposed to 16°C for 48 hours after inoculation and then transferring to 22°C (T2), 22°C continuously (T3) and late inoculated cv. Russet Burbank plants exposed continuously to 22°C (T3) (Figure 4.4).

In cv. Shepody, virus movement and symptom expression was enhanced by a combination of early inoculation (21 days after planting) and generally high temperature conditions (T2, T3, T4) and slowed down by lower temperatures (T1). Under lower temperatures (16°C continuously) the establishment of infection and virus translocation from inoculated lower leaves into middle canopies was slow in early-inoculated cv. Shepody plants. The slow movement of the virus is manifected by the detection of the virus by ELISA at 12 days after inoculation in early-inoculated Shepody plants instantly exposed to16°C continuously (T1) compared to 7 days after inoculation in early-inoculated plants of the same cultivar exposed to other temperature regimes (Table 4.7). An increase in temperature from 16°C for 48 hours after inoculation and then transferring to 22°C (T2) or 22°C continuously (T3) or 22°C for 48 hours, inoculating and then transferring to 16 °C (T4) (Table 4.1) increased virus movement into upper canopies as shown by the early detectation of the virus at 7 days after inoculation. These results suggest that while TSWV infections can occur both at 16°C and 22°C, accumulation and translocation is more efficient at 22°C in cv. Shepody. The plant age effects on infections in cv. Shepody are elegantly demonstrated by the fact that the same temperature conditions i.e 22°C for 48 hours, inoculated and

then transferred to 16°C (T4), which enhanced virus movement to reach middle canopies of early inoculated plants within 7 days after inoculation was also responsible for the induction of total virus movement restriction in late inoculated plants of the same cultivar (Tables 4.1). However, in addition to lowering resistance in early inoculated cv. Shepody plants, continuous exposure to high temperature conditions (22°C -T3) was generally detrimental to plant growth and led to general leaf necrosis and senescence in most plants which resulted in early plant death after 12 days post inoculation (Tables 4.1).

Exposure of cv. Russet Burbank plants to generally low temperature conditions (T1 & T4) was required to establish systemic infections and symptom expression. Under these conditions, virus movement was rapid in early inoculated plants and the virus incidence reached 100% within 7 days after inoculation but declined rapidly as plants developed chlorotic hues around TSWV necrotic spots, which were followed by leaf abscission at 21 days after inoculation (Figure 4.3; Table 4.7), a mechanism known to confine virus movement (Culver *et al*., 1991; 2002). Virus movement was restricted in both early and late inoculated cv. Russet Burbank plants exposed to 16°C for 48 hours, inoculated and then transferred to 22°C (T2), a point which also confirms that high temperatures enhanced TSWV resistance in this cultivar by restricting virus replication and movement. However, continuous exposure to these high temperature conditions (22°C -T3) was also not condusive to plant growth and most plants died after 12 days post inoculation.

Overall, the virus movement patterns in early-inoculated cv. Russet Burbank plants exposed to 22°C for 48 hours, inoculated and then transferred to 16°C (T4) were similar to that observed in cv. Shepody plants exposed to 16°C continuously (T1). However, a contrasting influence of temperature regimes was observed in plants of the two cultivars exposed to 16°C for 48 hours after inoculation and then transferred to 22°C (T2) and 22°C continuously (T3). In cv. Shepody plants, these conditions enabled the rapid long-distance movement into uninoculated leaves of middle canopies but were restrictive in cv. Russet Burbank plants (Table 4.7). Clearly these results suggest that lower temperatures viz 16°C continuously (T1) and 22 °C for 48 hours, inoculated and then transferred to 16°C (T4) were restrictive to either virus multiplication or long-distance movement in cv. Shepody but not in cv. Russet Burbank. Such host dependence systemic viral replication, accumulation and transversal restrictions within or from initially infected cells or primary infection foci or after entry into the phloem suggest the existence of different genetic systems controlling resistance in the two cultivars and are elegantly supported by similar observations of TSWV infections in other hosts. In *Datura stramonium, Nicotiana tabacum* cv. White Burley and *Physalis inocarpa*, TSWV accumulation in the inoculated leaves was higher at low temperature. However, long distance movement in *N. tabacum* cv. White Burley leading to virion accumulation in other plant organs was favoured by high temperature but relatively little effect in *P. inocarpa* and *D. stramonium* (Llamas-Llamas *et al*., 1998). And Susi (1999) showed that while replication and spread of *tobacco mosaic tobamovirus* (TMV) in the phloem of wild-type tobacco plants was restricted by low temperature, vascular transport of the virus from lower inoculated leaves to upper non-inoculated leaves via a stem segment kept at low temperature (4°C) was not affected, suggesting that replication is not necessary for the efficient vascular transport of TMV.

Further evidence of specific host genetic factors playing key roles in controlling viral movement was obtained from the exposure of plants to fluctuating themic conditions of 16°C for 48 hours, inoculating and then transferring to 22°C (T2), 22°C continuously (T3) and 22°C for 48 hours, inoculating and then transferring to 16°C (T4). These temperature conditions facilitated virus multiplication and transport to uninoculated leaves in upper canopies of Shepody plants but were generally restrictive in cv. Russet Burbank plants. Experimental evidence in other plants infected by TSWV (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998) and a number of different viruses (Carrington *et al*., 1996; Susi 1999; Kobori *et al*., 2003) support these observations. In TSWV-resistant accessions of *Capsicum chinense*, exposure to a day/night temperature of 30/18°C induced systemic infections in plants inoculated at both 2-leaf and 4-leaf stages. At 25/18°C day/night temperature, only plants inoculated at 2-leaf stage became systemically infected (Soler *et al*., 1998). Moury *et al*., (1998) also demonstrated that continuous exposure of *C. chinense* plants to 32°C for at least nine days led to systemic spread and necrotic symptoms in plants that were totally resistant at a lower temperature (32°C). In *pepo-cucumber mosaic virus* infections of *Tetragonia expansa,* systemic accumulation was restricted after entry into the phloem from inoculated leaves and no viral RNA signal was detected in the upper leaves from plants grown at 24°C. In contrast, *Cucumber mosaic virus* coat protein gene fragments were detected in uninoculated upper leaves of plants grown at 36°C (Kobori *et al*., 2003).

A variety of symptoms occurred when inoculated plants of both cultivars were exposed to different temperature regimes (Figure 4.3). The symptoms ranged from numerous and very slow developing systemic necrotic spots in cv. Shepody plants exposed to continuous lower temperatures of 16°C, slow forming localized necrotic spots surrounded by chlorotic hues in cv. Russet Burbank plants under continuous exposure to 16°C, medium sized and localized necrotic spots in plants of both cultivars exposed to fluctuating themic conditions (T2 & T4) to conspicuous and rapidly coalescing lesions and leaf necrosis in cv. Shepody plants at 22°C and leaf senescence in cv. Russet Burbank. In Shepody the dead leaves remained attached to the stems while in Russet Burbank, resistance to TSWV was manifested by the abscission of infected leaves (Figure 4.3) which occurred independently of temperature regime, plant age at time of infection and days after inoculation. Such resistance responses have been observed in *Capsicum chinense* accession 'PI – 152225' exposed to continous high temperature (Soler *et al*., 1998; Moury *et al*., 1998) and may be a mechanism to restrict virus movement into the stem (Carrington *et al*., 1996; Gilbertson & Lucas 1996; Séron & Haenni 1996). In many virus-host

systems, confinement of the virus by the necrotic response is a race between how fast the virus replicates and moves and how fast the hypersentivity reponse occurs. Manipulations that alter either the rate of viral replication or the kinetics of hypersentivity reponse affect the outcome (Culver *et al*., 1991; 2002). These differences in symptoms between plants exposed to continuous 16^{o}C temperature and those moved to 22^{o}C are similar to symptoms observed under field conditions. In Tasmania where the mean temperatures are generally cooler during the growing season, symptoms in Shepody appear as numerous necrotic spots (also see Wilson 2001) whereas in Victoria, South Australia and New South Wales, where temperatures are much warmer (22 ^{o}C and above) TSWV symptoms in the same cultivar are mainly characterised by larger but fewer necrotic lesions.

In the current study, a few infected plants of both cultivars, while yielding positive serological assays for TSWV, never exhibited symptoms in top canopies, particularly those exposed to16 ^{o}C for 48 hours, inoculated and then transferred to 22 ^{o}C (T2) and 22^{o}C continuously (T3). Similar observations of symptom expression attenuation in top parts of the canopies of several potato cultivars were observed in both mechanical and thrips transmissions (Chapter 3). Some virulent strains of tobamoviruses infecting *Nicotiana glutinosa* have been observed to induce similar temperature dependent responses involving development of lesions in inoculated leaves and no necrosis in upper systemically infected leaves (Culver *et al.*, 1991). The observation in the present study coupled with those on several potato cultivars observed in both mechanical and thrips transmissions (Chapter 3), suggests that the TSWV isolate *An_{WA}-1* used induces temperature dependent symptom attenuation in some potato cultivars. As argued in Chapter 3, the consequence of this kind of symptom expression would be the underestimation of virus incidence during field inspections for certification because symptoms may be masked or infection latent and thus lead to carryover infections in untested seed stocks. Exposure to high temperatures is also known to modulate the generation of defective interfering RNA in TSWV infections (Inoue-Nagata *et al*., 1997) that has been consistently associated with disease symptom attenuation in some species (Resende *et al*., 1991).

This contrasting host dependence of virus invasions and symptom expressions in both cvs Shepody and Russet Burbank provide credence to the notion that specific host genetic factors are responsible for the observed viral movement patterns. The underlying mechanism by which temperature influences these virus movements and symptom expressions in TSWV infections is still unclear. But both specific viral and host factors are thought to play key roles in this movement (Carrington *et al*., 1996). And from the available data, it cannot be ascertained to what extent temperature affected specific virus biological processes. But such mechanisms could be operating at both biochemical and structural level.

However, once the initial TSWV infection is achieved, it has been observed that the negative-strand TSWV, which carries a gene for a suppressor of gene silencing at analogous genomic positions, suppresses posttranscriptional silencing of a green fluorescent protein transgene in infected *Nicotiana benthamiana* through interference with the intrinsic RNA silencing in plants by the tospoviral NS_s protein (Bucher *et al*., 2003).

At a biochemical level it could involve the alteration to the coat protein that have multifunctional roles in virus biology including vector transmission, suppression of gene silencing involving protein-protein or protein-RNA interactions of NSm protein, which allows transcription and replication within newly infected cells and recognises and selectively traffic proteins and protein-nucleic acid complexes through plasmodesmata as part of fundamental transport and signaling processes (Sela 1981; Kormelink *et al*., 1994; Lucas & Gilbertson 1994; Storms *et al*.,1995; Carrington *et al*., 1996; Soellick *et al*., 1999; Bucher *et al*., 2003; Novina & Sharp 2004; Kainz *et al*., 2004), cross-protection and eliciting of symptom development through the hypersensitive response (Dawson *et al*. 1988; Culver 2002). Potential host proteins that may be involved in the TSWV infection process by interacting with the NSm in plants have been identified. Using NSm viral movement protein as a bait in a yeast two-hybrid screen, two homologous NSm-binding plant proteins of the DnaJ family from *Nicotiana tabacum* and *Arabidopsis thaliana* have been identified and hypothesed to provide a molecular basis for specific recognition of nucleocapsid structures which link the viral structures to elements of a plant machinery directing intercellular transport through plasmodesmata (Soellick *et al*., 1999).

The other biochemical process potentially involved could be the prevention of salicyclic acid accumulation by high temperatures (Chen & Klessig 1991; Yalpani *et al*., 1991; Chen *et al*., 1995; Durner & Klessig 1995). Salicyclic acid, a recognised plant hormone, is an essential component of processes, which either induce or inhibit defence reactions against specific, necrotising plant pathogens and herbivorous insects (Pierpoint 2002). Manipulations either through temperature as done in the present or other studies (Fraser 1986; Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998; Kobori *et al*. 2003; Syller 2003) or transformations that alter either the rate of viral replication or the kinetics of cell-to-cell movement (Fraser 1986; Carrington *et al*., 1996; Schwach *et al*., 2004) is likely to affect the infection process.

At a structural level the influence of temperature could involve the alteration in the development and growth of vascular tissues which would affect systemic accumulation of TSWV as hypothised by Kobori *et al*. (2003) for cucumber mosaic virus infections in *Tetragonia expansa* plants grown at a higher (36^{o}C) versus lower (24^{o}C). A physical barrier such as callose deposition caused by temperature changes could also potentially alter virus movements by inhibiting the unloading of the virus from the external phloem into the surrounding cells (refs in Kobori *et al*. 2003). Such temperature effects were not determined in the present study. But the lowering of resistance to TSWV by exposure to high temperatures as observed in cv. Shepody have also been reported in other plants (Soler *et al*., 1998; Llamas-Llamas *et al*., 1998; Moury *et al*., 1998) and viruses (Kobori

et al. 2003; Syller 2003). Equally, the lowering of resistance to TSWV by exposure to low temperatures as observed in cv. Russet Burbank has been observed in *Physalis ixocarpa* (Llamas-Llamas *et al*., 1998).

Results from the present study support the conclusion that the independent and interactive influence of temperature, plant age at time of infection, days after inoculation and leaf position reveals a quantitative perspective of the genetic variability in relation to predisposition of the two cultivars, Shepody and Russet Burbanks, to TSWV foliar and tuber infections, and symptom expression. Of utmost interest it can be concluded that resistance to TSWV systemic infections and symptom expression in cultivars, Shepody and Russet Burbanks was enhanced by a combination of late inoculation and instant exposure to 22^{o}C for 48 hours, inoculating and then transferring plants to 16^{o}C (T4) under 55% relative humidity and light intensity of 450 µmol-$m^{-2}.s^{-1}$ (Photosynthetically Active Radiation – PAR) and a light/dark cycle of 16 and 8 hours. Resistance was also enhanced by delaying exposure by 72hrs of early inoculated cv. Shepody plants to 16^{o}C continuously (T1), 16 ^{o}C for 48 hours, inoculated and then transferred to 22 ^{o}C (T2), and 22 ^{o}C continuously (T3) or late inoculated plants to 16^{o}C continuously (T1), 16^{o}C for 48 hours, inoculated and then transferred to 22^{o}C (T2), 22^{o}C continuously (T3) and 22^{o}C for 48 hours, inoculated and then transferred to 16 ^{o}C (T4). In cv. Russet Burbank, TSWV resistance was also enhanced by a 72hrs delayed exposure of early inoculated plants to 16^{o}C for 48 hours, inoculated and then transferred to 22^{o}C (T2), 22^{o}C continuously (T3) and late inoculated plants to 16^{o}C continuously (T1), 16^{o}C for 48 hours, inoculated and then transferred to 22^{o}C (T2), 22^{o}C continuously (T3) and 22^{o}C for 48 hours, inoculate and then transferred to 16 ^{o}C (T4). How this plays out under field conditions where day/night time temperature, light and relative humidity fluctuates is unclear. However, it is clear from the present results that the synchronisation and interactive effects of temperature conditions with those of light and relative humidity used in the experiments plays a role in the TSWV infection process and symptom expression.

Overall, the consequences of the results from this study would be twofold; provision of practical insights into the rational selection of suitable conditions when genetically screening for host-plant resistance by breeding programs, and for setting further investigations of the temperature-sensitive resistance mechanisms of potato against TSWV. Although a difficult practical proposition to extrapolate to field situations, the results from this study could also be used to select potato cultivars for production in appropriate climatic conditions

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Chapter 5

PREFERENCES AND PERFORMANCES BY *THRIPS TABACI* IN RELATION TO EXPERIENCE ON SPECIFIC HOST PLANTS.

Thrips catches from traps during surveys in commercial potato fields in southern Australia (Chapter 2) and the field exposure trials at the University Farm, Cambridge, Tasmania (Chapter 3), revealed that some weed species and potato cultivars were more prone to thrips feeding preference and damage and/or TSWV infection. A diverse fauna of thrip species identified from the thrip catches included *Thrips tabaci, Thrips imagines, Frankliniella schultzei, Thrips australis, Chirothrips manicatus, Tubuliferan sp., Limothrips cerealium, Limothrips angulicornis, Pseudanaphothrips achaetu*s, *Apterothrips apteris, Tenothrips* and a few other native species. Among these thrips species, *T. tabaci* and *F. schultzei* were the only known vectors of tomato spotted wilt virus (TSWV) and, therefore, assumed to be responsible for the virus transmissions observed.

Over many decades, some innovative research on several aspects of thrips vector transmission have been done in many parts of the world (Sakimura, 1963a, 1963b; German *et al.*, 1992; Wijkamp *et al*., 1995; Mumford *et al.,* 1996a; Goldbach & Peters, 1996; Ullman *et al.,* 1997; Chatzivassiliou *et al*., 1999, 2001, 2002; Nagata & Peters, 2001; Sakurai *et al.*, 2002; Nagata *et al.*, 2002; Inoue *et al.*, 2002; De Kogel, 2002). These studies have documented exceedingly complex thrips/host plant interactions. The two important components found to determine adaptation by phytophagous insects in host range ecology and evolution are host preference and subsequent performance on the plant (Futuyma & Moreno 1988; Thompson, 1988, 1996; Jaenike 1990; Andow, 1991; Terry 1997; Ullman *et al.*, 1997). Adaptation and performance of thrips on the diverse host range is driven by complex dispersal patterns and host utilization (Thompson, 1988; Terry 1997). For example, female insects may exhibit an oviposition preference for specific plants. Such preference can be influenced or induced by female experience as larvae or early adults (Terry, 1997). Experience on a previous host may also affect fertility and transmission fitness on subsequent hosts (Jaenike, 1990; Chatzivassiliou *et al*., 1999). Such trade-offs in fitness on different host plants has become a central hypothesis in explaining the evolutionary specialization of herbivores in many insect pest/host interactions (Agrawal 2000). Additionally, cues emanating from thrips infested plants have also been shown to mediate other inter- and intra-specific interactions among thrips and other arthropod species and plants (Agrawal & Colfer, 2000; Agrawal, 2000; De Kogel, 2002; De Kogel & Koschier, 2002; Koschier & Sedy, 2002). The preference of hosts that are susceptible to TSWV influence thrips vector transmission efficiency (Allen & Broadbent, 1986; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 2002) since acquisition of the virus is by the immobile larvae (Lindorf, 1931, 1932; Sakimura, 1963a; Van de Wetering *et al.,* 1996), which must be able to complete its development on the host selected by the adult thrips (Courtney & Kibota 1990; Thompson 1996; Hobbs *et al.,* 1993; Bautista & Mau 1994; Terry 1997). Many studies in Europe and North America (Jones 1959, Paliwal 1974, 1976; Wijkamp *et al.,* 1995; Van de Wetering *et al.,* 1998; McPherson *et al.,* 1999; Chatzivassiliou *et al*., 1998a, 1999, 2001; De Kogel, 2002; Inoue *et al.,* 2002), and South America (Nagata *et al.,* 2002) have indicated differences between vector thrips species and sexes in host preferences and subsequent performance and TSWV transmission. Vector propensity (movement and feeding behaviour)(Irwin & Ruesink 1986) of different thrips has shown different patterns and specificity in different crop-weed systems, which occur even within a particular species, and may also be evident at the insect/host plant level (Bautista & Mau 1994; Wijkamp *et al*., 1995; Agrawal & Colfer, 2000; Chatzivassiliou *et al*., 1999, 2001, 2002; Groves *et al.*, 2001; Mound, 2002; De Kogel, 2002; Funderburk, 2002; Brodbeck *et al*., 2002; Jenser *et al*., 2003; Sedy & Koschier 2003; Teulon *et al.*, 1993) and cultural management tactics (Stavisky *et al*., 2002). A given thrips species may respond differently to chemical changes in one host plant than to similar changes in another host (Brodbeck *et al.,* 2002).

Notwithstanding the plethora of detailed knowledge gained from the above studies that has helped in the understanding of thrips/host interactions, there is still a conspicuous paucity of emperical data in many aspects of thrips fitness and host resistance. For example, there is still a great dearth in the understanding of the chemical/nutritional basis driving thrips/host plant interactions and the mechanisms causing the vector preferences and trade-offs in different hosts and ecosystems (Stanton, 1983; Jenser *et al*., 2002; Brodbeck *et al*., 2002). This knowledge is essential in the understanding of host plant resistance. Interestingly, since the pioneering research of Pittman (1927) and Samuel *et al.*, (1930) on the transmission of TSWV by *T. tabaci* was done, the competencies of thrips species to transmit TSWV in Australia have not been experimentally tested. Any strategy designed to reduce TSWV inoculum requires knowledge of the inoculum source within an area and of the dispersal of viruliferous thrips from the source. Through the examination of the vector thrips preferences and subsequent performance on different plants, insights into the relative importance of different potential TSWV transmission patterns can be gained. Such knowledge can assist in any management strategy to optimize the reduction of TSWV inoculum and spread into and potentially from crops. Thrips preferences and performance on various hosts can be directly assessed by examining the number of adults and larvae on various host species. The number of adults present on each host provides a combined measure of preferences for feeding, mating and oviposition, while that of colonially overlapping generations of adults and larvae indicates the acceptability of the plant for both feeding and reproduction, since larval Thysanoptera cannot move far to locate other hosts (Terry 1997). Choice and no-choice type of experiments provide a unique tool to study these mechanisms and are the only way of establishing the preferences, performances and trade-offs in different crops and ecologies. Such

investigations would be enlightening in any effort to determine the contribution of different plant species in TSWV infections as observed in many other studies and this thesis. Many pertinent questions in the context of TSWV epidemics in potato have received little attention. Does*T. tabaci* in Australia has host-plant specificity and transmission efficiency? What is the relative importance of *T. tabaci* and its transmission capacity from different host plants in the context of the observed TSWV transmissions in potato crops? What are the primary feeding and ovipositional preferences and performances, which determine thrips distribution and consequently, transmission efficiency in the epidemics occurring in potato crops? Clear identification of exceedingly complex issues in this line of research may lead to protocols that can assist in bringing these research efforts to the centre of TSWV epidemiology in Australia.

The objectives of this study were, therefore, to (1) assess *T. tabaci* preference for different potato cultivars and weeds, (2) quantify the development and feeding damage caused to different potato cultivars and weeds, (3) assess the reproductive ability of *T. tabaci* on different potato cultivars and weeds and (4) assess the TSWV transmission efficiency to different potato cultivars and weeds.

Materials and Methods

Test Plants

Seven potato cultivars, viz Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic and Victoria and three weeds; *Arctotheca calendula* (Cape weed), *Chenopodium album* (Fat hen) and *Solanum nigrum* (Blackberry nightshade) were used in this study. Previous research and observations (Chapter 3) had indicated that Bismark was very susceptible to the virus and resistant to thrips feeding. Russet Burbank was susceptible to thrips feeding but highly tolerant to virus infection and translocation. Royal Blue was very susceptible to thrips damage but tolerant to virus infection. Tasman was highly tolerant to both thrips feeding and virus infection. Shepody, Atlantic and Victoria were susceptible to thrips damage, virus infection and translocation. Plants of these cultivars were grown from cut seed tubers previous tested negative for TSWV in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)(Clark & Adams, 1977). The selected weeds were commonly found positive for TSWV during field surveys of commercial potato crops in 2001/2002 and 2002/2003 (Chapter 2). The weeds were grown from seed collected locally in southern Tasmania. All the above plants used were grown in a separate glasshouse under the same climatic conditions.

Thrips colony

Due to regulatory quarantine considerations, *T. tabaci,* the only known vector species trapped in Tasmania was used in the current studies. The initial collection of *T. tabaci* was from onion plants growing directly opposite a site previously used for the comparative resistance potato trials (Chapter 3), where natural TSWV infections had occurred at the University of Tasmania Farm, Cambridge, Tasmania. Strikingly, this *T. tabaci* population, individually sexed, was thelytokous, with no males observed during both field collection and transmission trials (Lawrence Mound, Entomologist – CSIRO). The adult thrips collected in the field were reared on bean pods (*Phaseolus Vulgaris* L) using a modified method from Loomans and Murai (1997) under controlled summer temperature conditions of 25^{o}C ±1^{o}C and 65 ± 5% relative humidity with a 16-hour light and 8-hour dark photoperiod. The thrips were transferred weekly onto fresh pods.

Virus source, acquisition and inoculation

In order to achieve uniformity for comparisons, the TSWV isolate An_{WA}*-1* used in previous studies was also used in these experiments. The virus isolate was used in three different plants that acted as source plants (1) infected potato plants (cv. Victoria) grown from thrips-inoculated field-infected tubers, (2) tomato plants (*cv.* Grosse Lisse, Arthur Yates & Co. Ltd, Homebush, NSW, Australia) mechanically inoculated from potato plants, (3) *Datura stramonium,* mechanically inoculated from potato plants (Victoria). In all inoculations, single mechanical transfers were used from potato to avoid envelope-deficient mutant RNA with lost sequences encoding the glycoproteins generated by serial mechanical transfers through hosts (Resende *et al.,* 1991; Inoue-Nagata *et al.,* 1997), which impede virus transmission by vector thrips (Nagata *et al.,* 2000; Sin *et al.*, 2003). The source plants were maintained in an insect-proof glasshouse at the NewTown Research Laboratories, Tasmania. Inoculation was by abrading three youngest fully expanded leaves with infected potato leaf sap diluted 1:20 (w/v) in 0.2M phosphate buffer (pH 7.4) containing celite. For all transmission tests, first instar larvae of *T. tabaci* hatching on beans were placed on TSWV systemically-infected tomato or potato or *D. stramonium* plants and given unlimited access to acquire the virus. The thrips were allowed to remain on the same plants from the first instar larval stage until they were adults.

Test conditions, determination of thrips preference, performance and virus transmission.

Host plant preferences and performances (survival, TSWV transmission competency and reproduction) were evaluated under choice and no-choice conditions in two types of experiments; (1) test plant preference and performance by thrips in relation to experience on TSWV systemically-infected specific hosts (2) thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected specific weed hosts.

In both choice and no choice experiments, treatments were randomized in four replications and carried out under controlled conditions with temperature = 25°C ± 0.5 °C; relative humidity = 55 ± 5% and light intensity = 450 μmol-m^{-2}.s^{-1} (Photosynthetically Active Radiation – PAR), under a light/dark cycle of 16 and 8 hours, within the growth chamber. Using contingency tables (Conover 1980), the relationship between the performance of thrips on different test plants was examined. Thrips preferences and performance was determined by counting the number of adults and larvae, scoring feeding marks and development of TSWV symptoms on each test plant at the end of 14 days exposure. Thrips feeding damage was scored on an increasing scale of : 0 = no damage, 1 = very few spots covering less than 25% of canopy, 2 = spots covering 25-50% of canopy, 3 = spots covering 50-75% of canopy with some defoliation, 4 = numerous spots with significant defoliation and death of the plant. Systemic TSWV infections were determined in test plants by DAS-ELISA using antisera to TSWV lettuce strain (Agdia, IN, USA) with a monoclonal antibody dilution ratio of 1: 400 for both capture and detection. Extracts were prepared by crushing ~0.1g (w/v) leaf samples in phosphate buffered saline (pH 7.4) containing 2ml litre^{-1} Tween 20 and 20g litre^{-1} polyvinyl pyrrolidone using a leaf press. Expressed sap (100μl) was tested for TSWV in duplicate wells of a flat bottom microtitre plate (NUNC, maxisorb) in a crisscross plate layout. Positive and negative controls were included on each plate. Absorbances (A_{405}nm) were measured with a photometer (Titertek) 30 minutes after addition of 0.5mg ml^{-1} p-nitrophenyl phosphate in 10 ml litre^{-1} diethanolamine, pH 9.6 as substrate. The absorbance values were corrected for blank values consisting of only extraction buffer in the sample incubation step. Samples were denoted positive if they had absorbances greater than three standard deviations of the mean of the negative controls (Sutula *et al*., 1986). Percentage infection of composite samples was calculated using binomial theorem (Gibbs & Gower, 1960). All assays were conducted three times.

Test plant preference and performance by *T. tabaci* in relation to experience on TSWV systemically-infected specific host.

Choice and no-choice type assays were used in these studies to determine if experience of prior feeding by thrips on a specific host affects subsequent feeding and reproduction (oviposition and larvae development) on other hosts. This also tested the potential spread of TSWV by *T. tabaci* from potato to potato and potato to weed. In choice experiments, a single potato or tomato or *D. stramonium* plant systemically infected with TSWV was placed in the center of a thrips-proof cage (80 x 50 x 60cm) on which 20 *T. tabaci,* reared on the same plant from the first instar larval stage to adulthood, were allowed to remain and given unlimited access to acquire the virus. Two-week old plants or 3-leaf stage of each test plant were randomly placed in a circle around the plant with thrips and left for 14 days. In this way, thrips experience on TSWV systemically-infected potato or tomato or *D. stramonium* plants was tested for subsequent preference for and performance on potato cultivars Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic and Victoria and three weeds; *A. calendula*, *C. album* and *S. nigrum.* Under no-choice situations, a single potato or tomato or *D. stramonium* plant systemically infected with TSWV was placed in the center of a thrips-proof cage on which 20 *T. tabaci,* reared on the same plant from the first instar larval stage to adulthood, were allowed to remain and given unlimited access to acquire the virus. In each of the cages, three plants of either Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic, Victoria, *A. calendula*, *C. album* or *S. nigrum* were placed in a circle around the plant with thrips and left for 14 days.

Thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected specific weed hosts.

To determine if preference and performance on potato cultivars is related to experience of thrips on TSWV systemically-infected specific weed hosts, *A. calendula* and *S. nigrum* plants were used under no-choice conditions. By inference, these assays tested the potential for primary spread of TSWV from weeds to crops by *T. tabaci.* Twenty first instar larvae (0 to 4 hour old) of *T. tabaci* were placed on TSWV systemically-infected *A. calendula and S. nigrum* plants in separate insect-proof cages for 24 hours. Three plants of potato cultivars Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic and Victoria were then placed randomly around either TSWV systemically-infected *A. calendula* or *S. nigrum* plants in separate cages and left for 14 days under conditions described above.

Data Analysis

For both types of assays above, estimates of the transmission competencies (p) of the thrips was calculated using the formula of Gibbs & Gower (1960) $p = 1 - (1 - R/N)^{1/i}$

Where p = the probability of a single thrips transmitting
R = the number of plants infected
N = the number of plants exposed to infection
i = the number of thrips per test plant

All data from experiments were analysed using Genstat software ver 7.2.0.208 (Lawes Agricultural Trust, 2002). Initial exploratory data analysis involving analyses of variance on individual variates of adult thrips, larvae and feeding damage in response to single and interactive effects of TSWV inoculum source and test plants indicated no significant differences. However, correlations indicated links between adult thrips, larvae and feeding damage in both choice and no-choice experiments suggesting that the observed feeding damage was a factor of adult and larvae

thrips populations. A linear mixed model was then fitted to the thrips phenology (adult and larvae) and feeding damage parameters in a residual maximal likelihood (REML) analysis to explore the single and interactive effects of TSWV inoculum source and test plants. Wald statistics was used to evaluate the fixed effects in the model. A probability level (under null hypothesis) of 0.05 was used for all tests to determine significance.

Results

Test plant preference and performance by *T. tabaci* in relation to experience on TSWV systemically-infected specific host.

***T. tabaci* unable to transmit TSWV isolate An_{WA}-1 :** After an inoculation access period (IAP) of 14 days, no evidence of TSWV isolate An_{WA}-1 transmission was observed with *T. tabaci* on any of the test plants in both choice and no-choice experiments or when assessed for virus transmission from systemically infected weeds to potato cultivars (Table 5.1 & 5.2) as indicated by the absence of symptoms and verified by negative ELISA results. Repeated tests produced similar results. For all assays, the estimates of the transmission competencies (p) of thrips calculated using the formula of Gibbs & Gower (1960) was zero, indicating lack of virus transmission by any thrips to any test plant exposed to infection. Consequently, it was concluded that the *T. tabaci* population used in the study could not transmit the TSWV isolate An_{WA}-1 from the systemically infected potato, tomato, *D. stramonium, A. calendula* or *S. nigrum* to any of the test plants assessed. TSWV transmission parameters were, therefore, excluded from further analysis. However, the thrips survival, as measured by how many individual thrips remained on the test plants, reproduction patterns and total feeding damage at the end of the trial, provided a meaningful measure in determining host preference, adaptation and subsequent performance on the plants (Figure 5.1, 5.2).

Choice experiments. The number of adult and larval thrips on each test plant were correlated. There were positive relationships (P=0.05) between adult thrips population, larvae and feeding damage as indicated by the correlation matrix (Table 5.1).

Table 5.1. Correlation Matrix for thrips density and feeding damage in *T. tabaci* host preference and performance under choice conditions .

Adult	1.000		
Larvae	0.829	1.000	
Thrips Feeding Damage	0.713	0.604	1.000
	Adult	**Larvae**	**Thrips Feeding Damage**

A linear mixed model fitted to the thrips phenology (adult and larvae) and feeding damage parameters in a residual maximal likelihood (REML) analysis indicated that within the first generation, *T. tabaci* showed extremely variable responses (P<0.001) in both adult and larval performance (survivorship, feeding and breeding), at levels between type of plant on which they had fed before exposure to different test plants (source plant), number of larvae produced and the feeding damage caused (Table 5.2, Figure 5.1). Testing for fixed effects in the model using wald statistics revealed a highly significant interaction between source plant x test plant and test plant x thrips phenology and feeding damage (Table 5.2).

Overall, the cummulative adult thrips density declined on all the different test plants from the initially introduced population of 20 insects, even on test plants where breeding had occurred. There was a significant variability (P<0.001) in thrips host selection and performance based on the interactive effects of both the source plants and test plant genotype (Table 5.2). Both adult and larval performance was highiest when thrips were initially exposed to potato (Figure 5.1 - A) and lowest when exposed to *D. stramonium* (Figure 5.1 - C). The experiment was repeated three times and throughout the assays, both adult and larval performance showed a qualitatively and quantitatively similar trend with survivorship, feeding and breeding at 0% on Bismark, Russet Burbank, Tasman and *C. album* when previously fed on either potato, tomato or *D. stramonium* before giving them a choice of test plants. These test plants can, therefore, be considered as the least appreciated by the *T. tabaci* population used, for both feeding and reproduction. The maximum intrinsic rate of adult thrips survivorship was on *A. calendula* at 45% of the introduced population, while that of larvae was on *S. nigrum* (40%) when adult insects had initially fed on potato (Figure 5.1 – A). Larvae was also found on cvs. Shepody and Atlantic, with higher densities on the former, indicating these potato cultivars to be breeding hosts for *T. tabaci*. The thrips feeding marks were more abundant on Royal Blue, followed by *S. nigrum* and then *A. calendula* (Figure 5.1). These results indicate that while thrips feeding preference was significantly higher on Royal Blue, that of oviposition and both adult survivorship and larvae development was best on *S. nigrum* and *A. calendula.* Adult thrips were proportionately more abundant relative to larvae numbers except when the source plant was *D. stramonium*. Averaging across all test plants relative to source plants, the ratios of adult thrips to larvae was 2.6:2.2 for potato, 1.8:1.1 for tomato and 0.5:0.7 for *D. stramonium* (Figure 5.1).

Table 5.2 Residual maximal likelihood (REML) analysis using wald statistics for single and interactive effects in *T. tabaci* host preference and performance under choice conditions.

Fixed term	Wald Statistic	df	Wald/d.f.	Chi-Square Prob.	$P^{\mathcal{T}}$
Sequentially adding terms to fixed model					
Source Plant (SP)	2.7	2	1.35	0.259	NS
Test Plant (TP)	24.07	9	2.67	0.004	*
Thrips Phenology (Adult & larvae) and Feeding Damage (TPFD)	12.25	2	6.13	0.002	*
SP x TP	45.66	18	2.54	<0.001	*
SP x TPFD	2.83	4	0.71	0.587	NS
TP x TPFD	52.73	18	2.93	<0.001	*
SP x TP x TPFD	19.52	36	0.54	0.989	NS
Dropping individual terms from full fixed model					
SP x TP x TPFD	19.52	36	0.54	0.989	NS

$^{\mathcal{T}}$ NS = Non significant at P = 0.05, * = significant at P = 0.05.

No-Choice experiments. Correlation coefficients between adult and larvae population densities and feeding damage are given in Table 5.3.

The performance dynamics of both adult and larval thrips on test plants under no-choice situations are given in Table 5.4, Figure 5.2. There were marked variations in thrips preferences and performance among test plants, which reflected the general pattern of observed preferences and performances under choice conditions. However, thrips performance was generally higher under no-choice situations. By REML analysis, one fixed term parameter (thrips phenology (adult & larvae) and feeding damage) and two parallel interactions (TSWV inoculum source plant x test plant and test plant x thrips phenology (adult & larvae) and feeding damage) were significant (Table 5.4), indicating a qualitatively consistent pattern of the effects of these parameters.

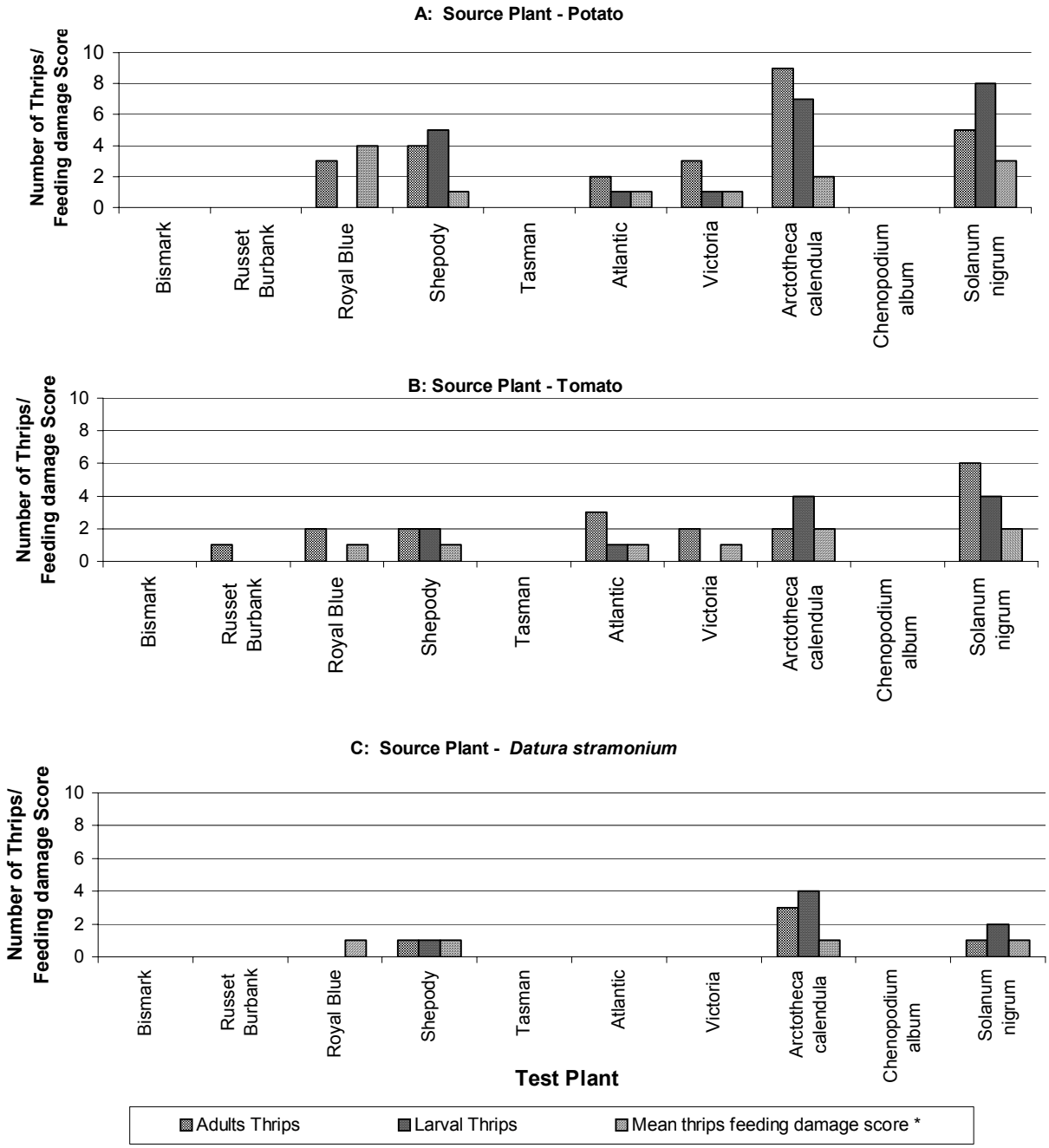
Repeated tests had the same trend as shown in Figure 5.2, with adult thrips density values significantly higher on *A.. calendula* (65%) and on *S. nigrum* (60%) for populations initially fed on potato and tomato, respectively and lowest on Bismark and Tasman. The highiest number of larvae on test plants was found on *A.. calendula* followed by *S. nigrum* and then Shepody when adult thrips had previously fed on potato. Significantly higher (P<0.05) numbers of larvae were counted on *S. nigrum* followed by Sheopdy and then *A.. calendula* when adults were initially fed on tomato. When adult thrips had initially fed on *D. stramonium*, significantly similar larvae densities were found on both Shepody and *A.. calendula.* These results suggest that an important part of the variation observed in the thrips performance on test plants could be accounted for by the plants on which the adult thrips had previously fed before exposure. Overall, thrips performance was higher on most test plants when adult thrips had initially fed on potato and tomato than on *D. stramonium* (Figure 5.2).

Table 5.3. Correlation Matrix for thrips density and feeding damage in *T. tabaci* host preference and performance under no-choice conditions .

Adult	1.000		
Larvae	0.847	1.000	
Thrips Feeding Damage	0.741	0.650	1.000
	Adult	**Larvae**	**Thrips Feeding Damage**

Figure 5.1. Final Thrips density and feeding damage in *T. tabaci* host preference and performance under choice conditions.

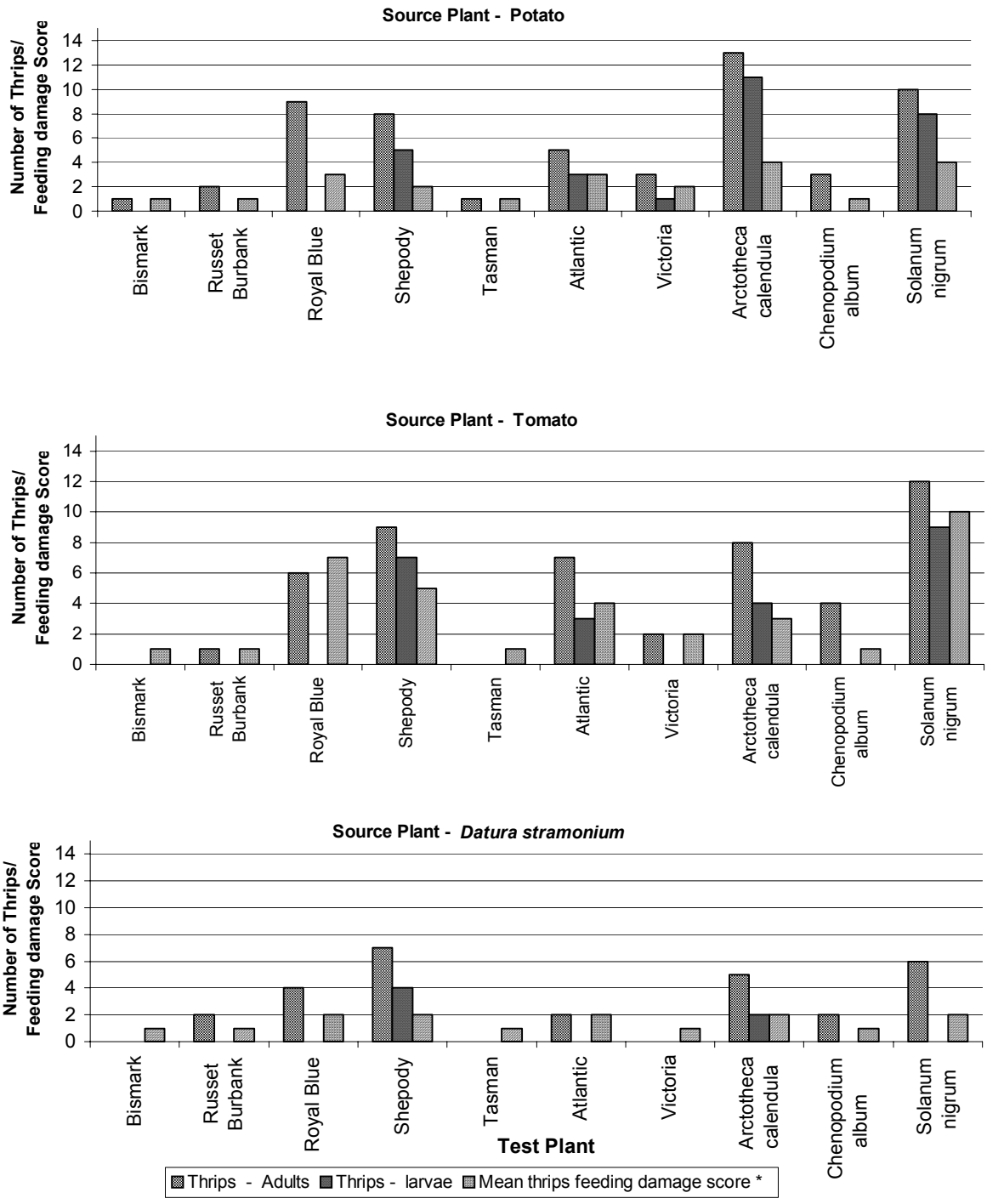
$P \leq 0.005$,
df = 18
l.s d (TSWV Inoculum Source Plant x Test Plant) = 1.3974
l.s.d (Test Plant x Thrips Phenology and Feeding Damage) = 1.3974



* Mean thrips feeding damage scoring: 0 = no damage, 1 = very few spots covering less than 25% of canopy, 2 = spots covering 25-50% of canopy, 3 = spots covering 50-75% of canopy with some defoliation, 4 = numerous spots with significant defoliation and death of the plant.

Figure 5.2 Final Thrips density and feeding damage in *T. tabaci* host preference and performance under no-choice conditions.

$P \leq 0.005$,
df = 18
l.s d (TSWV Inoculum Source Plant x Test Plant) = 2.414
l.s.d (Test Plant x Thrips Phenology and Feeding Damage) = 2.414



* Mean thrips feeding damage scoring: 0= no damage, 1 = very few spots covering less than 25% of canopy, 2 = spots covering 25-50% of canopy, 3 = spots covering 50-75% of canopy with some defoliation, 4 = numerous spots with significant defoliation and death of the plant.

Table 5.4 Residual maximal likelihood (REML) analysis using wald statistics for single and interactive effects in *T. tabaci* host preference and performance under choice conditions .

Fixed term	Wald Statistic	df	Wald/d.f.	Chi-Square Prob.	$P^{\dagger}$
Sequentially adding terms to fixed model					
TSWV Inoculum Source Plant (TISP)	2.00	2	1.00	0.367	NS
Test Plant (TP)	10.31	9	1.15	0.326	NS
Thrips Phenology (Adult & larvae) and Feeding Damage (TPFD)	15.24	2	7.62	<0.001	*
TISP x TP	37.34	18	2.07	0.005	*
TISP x TPFD	0.94	4	0.24	0.919	NS
TP x TPFD	56.27	18	3.13	<0.001	*
TISP x TP x TPFD	19.01	36	0.53	0.991	NS
Dropping individual terms from full fixed model					
TISP x TP x TPFD	19.01	36	0.53	0.991	NS

† NS = Non significant at P = 0.05, * = significant at P= 0.05.

Thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected specific weed hosts.

Tables 5.5, 5.6 and Figure 5.3 summaries the results of experiments on thrips host preference and performance on potato test plants when initially fed on either *A.. calendula* or *S. nigrum.* A weak positive correlation (r =0.311) was found between larvae and thrips damage (Table 5.3), suggesting that the feeding marks observed on test plants could be attributed to the slightly higher population density of larvae than by adult thrips, particularly on *S. nigrum*. No significant difference (P> 0.005) was observed in thrips performance on test plants between the two source plants, *A.. calendula* and *S. nigrum.* However, there were significant differences (P=0.051) in thrips phenology and feeding damage between test plants as indicated by residual maximal likelihood (REML) analysis using wald statistics (Table 5.6). The ratio for larvae to feeding damage when adults were previously fed on *A.. calendula* was 2:0 and on *S. nigrum*, 3:1 suggestive of the influence of these plants on supporting both the breeding and subsequent slight bias in thrips feeding preferences (Figure 5.3).

Table 5.5. Correlation Matrix for thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected *A. calendula* and *S. nigrum.*

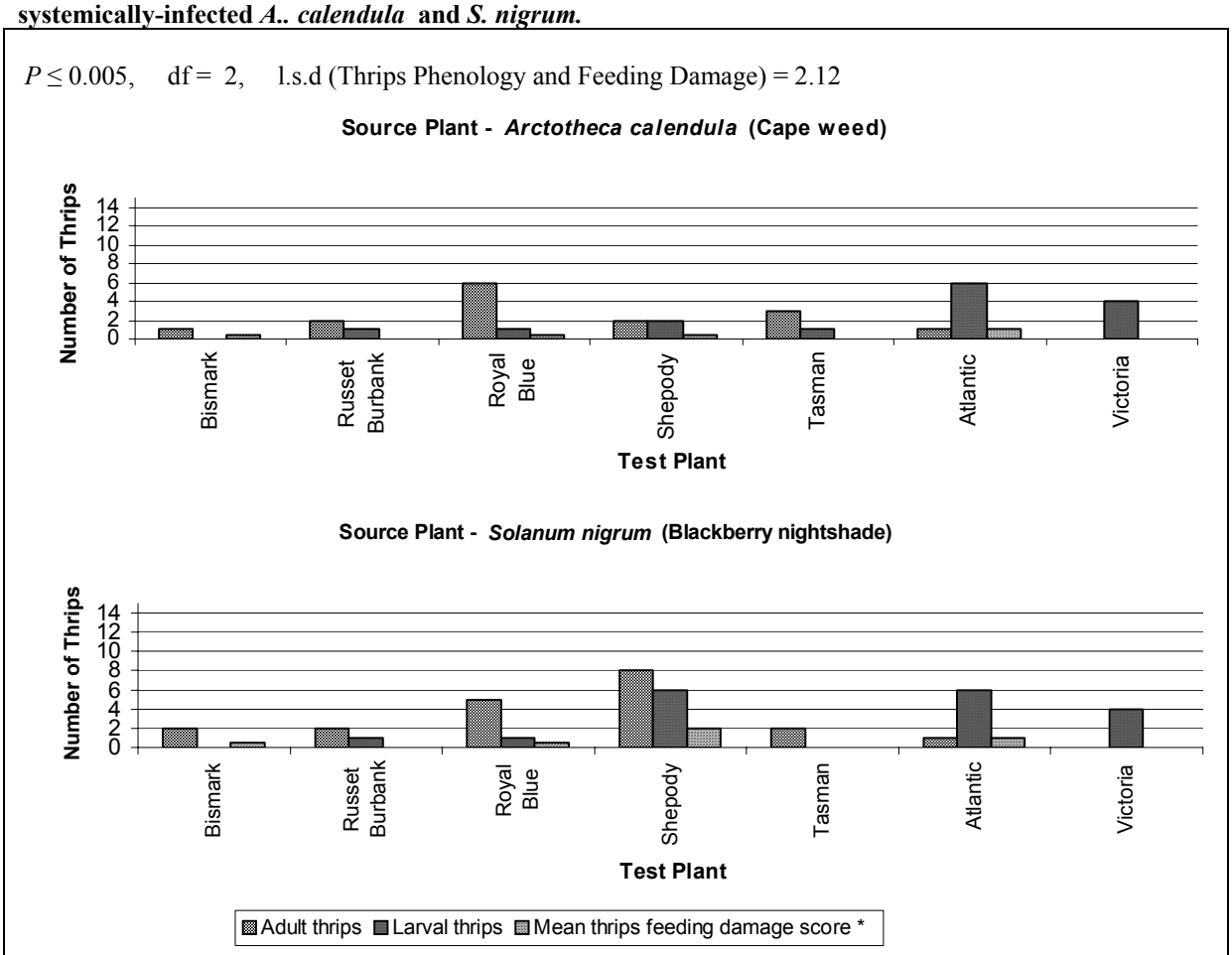
Adult	1.000		
Larvae	-0.041	1.000	
Thrips Feeding Damage	-0.113	0.311	1.000
	Adult	**Larvae**	**Thrips Feeding Damage**

Table 5.6 Residual maximal likelihood (REML) analysis using wald statistics for single and interactive effects in thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected *A. calendula* and *S. nigrum*.

Fixed term	Wald Statistic	df	Wald/d.f.	Chi-Square Prob.	$P^{\dagger}$
Sequentially adding terms to fixed model					
TSWV Inoculum Source Plant (TISP)	0.00	1	0.00	1.000	NS
Test Plant (TP)	6.13	6	1.02	0.409	NS
Thrips Phenology (Adult & larvae) and Feeding Damage (TPFD)	5.97	2	2.98	0.051	*
TISP x TP	0.00	6	0.00	1.000	NS
TISP x TPFD	0.00	2	0.00	1.000	NS
TP x TPFD	16.95	12	1.41	0.152	NS
TISP x TP x TPFD	0.00	12	0.00	1.000	NS
Dropping individual terms from full fixed model					
TISP x TP x TPFD	0.00	12	0.00	1.000	NS

† NS = Non significant at $P \leq 0.05$, * = significant at $P \leq 0.05$.

Figure 5.3 Thrips preference and performance on potato cultivars in relation to experience on TSWV systemically-infected *A.. calendula* and *S. nigrum.*



***** Mean thrips feeding damage scoring: 0= no damage, 1 = very few spots covering less than 25% of canopy, 2 = spots covering 25-50% of canopy, 3 = spots covering 50-75% of canopy with some defoliation, 4 = numerous spots with significant defoliation and death of the plant.

Discussion

This study has demonstrated that the *T. tabaci* population used could not transmit the TSWV isolate An_{WA}*-1* from the systemically infected potato, tomato, *D. stramonium, A. calendula* or *S. nigrum* to seven potato cultivars; Bismark, Russet Burbank, Royal Blue, Shepody, Tasman, Atlantic and Victoria and three weeds; *A. calendula*, *C. album* and *S. nigrum.* In repeated assays, no symptoms of TSWV infection on any test plant in both choice and non-choice assays were apparent after an IAP of 14 days, an observation verified by the use of ELISA. For all assays, the estimates of the transmission competencies (p) of thrips calculated using the formula of Gibbs & Gower (1960) was zero. However, typical feeding scarring were observed on leaves of different test plants, confirming thrips feeding activities. The *T. tabaci* population used in this study was collected from onion plants growing on a plot separated by a farm road from a comparative resistance potato trial (Chapter 3), where TSWV infections with incidences ranging from 0 – 32% had occurred naturally during the season. The population comprised of female *T. tabaci* only as determined by individual sexing of insects done at the start of the study at both NewTown Research Laboratories in Tasmania and the Commonwealth Scientific and Industrial Research Organisation (CSIRO – Entomology, Canberra). Bisexual populations of *T. tabaci* have not been observed in Australia although they may exist (L. Mound, personal communication). For many decades now there has been constrasting findings on the vector competency of *T. tabaci* to transmit TSWV in different parts of the world (Pittman, 1927; Samuel *et al.*, 1930; Sakimura, 1939; Jones 1959; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 1999, 2001; Tavella *et al*., 2002; Jenser *et al*., 2002, 2003) and great diversity and biological variations among populations have been reported (Zawirska 1976 cited in Nagata & Peters 2001; Jenser *et al*., 2002; Murai & Toda, 2002; Mound 1997, 2004). The prevalent observation in *T. tabaci* vector competency has been the lack of consistency in TSWV transmission (Jones 1959; Paliwal 1974, 1976; Sakimura, 1963b; Wijkamp *et al*., 1995; Chatzivassiliou *et al*., 1999, 2001; Tavella *et al*., 2002; Jenser *et al*., 2002, 2003). The theory advanced by Zawirska and supported by Jenser *et al*., (2002) to be the plausible explanation to the different *T. tabaci* transmission competencies, suggests that *T. tabaci* comprises two

taxonomically identical "types" from among which the populations on tobacco (*Nicotiana tabacum*) are considered as *T. tabaci* subsp. *tabaci* and those living on *Galinsoga parviflora*, potato and other hosts as *T. tabaci* subsp. *communis*. Populations of *T. tabaci* subsp. *communis* on different plant populations, mainly on onion, propagate parthenogenetically and not considered virus vectors. This theory is yet to be experimentally tested (Mound 1997; Jenser *et al*., 2002). However, assuming that the *T. tabaci* "type" found on both onion and potato in the present study was *T. tabaci* subsp. *communis,* according to the theory above, and propagating parthenogenetically and lacked fitness to transmit TSWV, then the differences in competency to transmit the same TSWV isolate An_{WA}*-1* from mechanically inoculated tomato to potatoes in the comparative resistance potato trials (Chapter 3) and the present study would have to be ascribed to different reasons other than "type". But without access to the molecular (Gillings *et al.,* 1996; Klein & Gafni 1996; Gyulai *et al*., 2002) and biological data (Brodbeck *et al*., 2002; Mound 1997, 2004) to demonstrate differences between the two populations, it is difficult from the present study, to draw conclusions based on "type". Morphologically, the *T. tabaci* trapped in comparative resistance potato trials (Chapter 3) and those from the colony used in this study were identical. Destructive epidemics of TSWV have been reported in southern Tasmania on lettuce (Wilson 1998), where *T. tabaci* is the only known vector found in open fields, and by inference, the observed transmissions to be by females.

The virus titre in both thrips and accumulation hosts was high as determined by ELISA prior to commencement of each experiment and could not possibly have been the cause of lack of transmission fitness. The amount of virus accumulated by thrips at larval and adult stages have been observed to influence transmission fitness in *T. tabaci* and other vector thrips species (Inoue 2002). Plausible explanations to the lack of fitness to transmit TSWV by the *T. tabaci* population may have been the (1) effects of temperature and (2) trade-off in fitness due to serial feeding on some hosts. The transmission tests in the current study were carried out under controlled conditions with temperature = 25^{o}C ± 0.5 ^{o}C; relative humidity = 55 ± 5% and light intensity = 450 μmol-m^{-2}.s^{-1} (Photosynthetically Active Radiation – PAR), under a light/dark cycle of 16 and 8 hours, within the growth chamber. TSWV transmission competences are reported to be temperature-dependent, at least in larvae of *Thrips tabaci* (Chatzivassiliou *et al.,* 2002) and *F. occidentalis* (Wijkamp & Peters 1993). Successful transmission of TSWV is thought to occur at 16^{o}C, with distinct patterns of virus accumulation, vector and host specificity for various thrips species (Paliwal 1976; Wijkamp *et al.,* 1995; Inoue *et al.,* 2002; Nagata *et al.,* 2002; Sakurai *et al.,* 2002). The specificity is also thought to be governed by other factors including vector sex (Wijkamp *et al.,* 1995; Van de Wetering *et al.,* 1998; Sakurai *et al.,* 1998, 2002), thrips development stage (Moritz 2002; Inoue *et al.,* 2002), and thrips vector preferences and performances on TSWV accumulation hosts (Allen & Broadbent, 1986; De Kogel, 2002; Chatzivassiliou *et al*., 2001). This shows the complexity of virus transmissions by thrips. In field situations, however, average summer temperatures in South-eastern Australia are similar to those used in this study. And it is in these areas where sporadic TSWV epidemics have occurred. Such discrepancy would suggest the involvement of additional factors in triggering and sustaining the observed infections in potato crops. Factors such as trade-offs in virus transmission fitness influenced by a previous host as a result of serial feeding on some hosts have been reported for *T. tabaci* population in experiments using leek (Chatzivassiliou *et al*., 1999). Additional evidence of this phenomenon have also been explained by the diversity resistance hypothesis in many other herbivore-virus-plant interactions, both theoretically (Thresh 1974, 1982; Fox & Morrow 1981; Futuyma and Moreno 1988; Jaenike 1990; Thompson 1994; Terry 1997; Holmgren & Getz, 2000; Kennedy *et al.,* 2002; Ye *et al.,* 2003b) and through experimental studies on preferences, associations and performance (Kirk 1985; Trichilo *et al.,* 1988; Yudin *et al.,* 1988; Teulon 1993; Bautista & Mau 1994; Wijkamp *et al*., 1995; Agrawal & Colfer 2000; Terry 1997; Ochoa *et al.*, 1999; Herrin & Warnok, 2002). These studies have provided insight into the potential constraints and selection pressures on the evolution of host range. In the current study, the use of thrips that were initially raised on beans and then given unlimited access to acquire the virus as first instar larvae on either TSWV infected tomato or potato or *D. stramonium* plants before being released in each appropriate cage and left for 14 days on the test plants may have affected their competency to transmit the virus under conditions tested.

Under field conditions, thrips normally have a diversity of hosts to feed on. This would mean that a phytophagous species like *T. tabaci*, has the ability to deal with secondary metabolites in many of these plants which would otherwise deter specialist herbivores. Offering thrips a choice of test plants in the current study after raising them on either TSWV-infected potato, tomato or *D. stramonium,* led to prefential feeding and reproduction on some test plants. Adult thrips survivorship and larval development was highiest when thrips were initially exposed to potato and lowest when exposed to *D. stramonium*. For adult thrips raised on potato, survivorship was highiest on *A. calendula* (45%), while that of larvae was on *S. nigrum* (40%) and not on potato. Similarly, thrips raised on tomato, performed marginally better on *A. calendula* and *S. nigrum* than on other test plants. No adult thrips, irrespective of which species of plant they were raised on, fed or survived on Bismark, Tasman and *C. album.* Adult thrips and larvae performance was relatively poor on Russet Burbank and Victoria, indicating that either morphological or chemical leaf constituents of these cultivars affected feeding, reproduction and/or development adversely.

Generally, the profiles of thrips preferences and performances on test plants under choice situations were similar to those observed in no-choice assays , although not identical (Figure 5.1 & 5.2). However, without a choice, thrips fed on the plants that they were offered, including Bismark, Tasman and *C. album,* which were avoided under choice conditions. Consequently, thrips performance was comparatively higher under non-choice

conditions. However, thrips did not reproduce on Bismark, Tasman and *C. album,* confirming non-preference for these plants. Similar to observations under choice conditions, the highiest thrips performance was again on *S. nigrum, A. calendula,* Shepody and Atlantic, with thrips previously exposed to potato and tomato having comparatively higher adult and larvae densities and causing more feeding damage than those from *D. stramonium*. This kind of thrips performances suggest the influence of experience on a previous host, which may have affected fertility and oviposition preference on subsequent hosts. Such a phenomenon has been reported in a number of phytophagous insects (Jaenike 1990; Joshi & Thompson, 1995; Cunningham *et al*., 1998; Chatzivassiliou *et al*., 1999; Dicke, 2000; Agrawal 2000; Groot *et al*., 2003). However, the performance of thrips raised on potato or tomato, which did not prefer potato or solanaceous plants over other host species under both choice and non choice conditions, indicate that *T. tabaci* females do not distinguish between plants of the same family, or that they preferably oviposit not only in potato, but in solanaceous plants in general. This would suggest that other factors may predominate in explaining such preferences and performances. This was, particularly, evident in the non preference and inability of adult thrips to survive and reproduce on some potato cultivars such as Bismark and Tasman, regardless of the plant they had previously fed on. The resistance observed in cvs. Bismark and Tasman, was primarily due to non-preference, as casual observations during experimentation, indicated that adult thrips avoided these test plants before landing on them. This would suggest that some non-contact cues involving antibiosis or antixenosis may also be involved. For *C. album,* the relative smooth surface of leaves which made it more difficulty for adult thrips to move, may account for the absence of thrips, feeding and oviposition on this host. Natural resistance to the virus in both *C. album* and Bismark is lacking (Chapter 2 & 3) and resistance to thrips means that these plants can relatively escape virus infection under field situations. From these results, it can be concluded that experience of female *T. tabaci* as larvae or early adults on a previous host affects preferences and performance on subsequent hosts. Additionally, biochemical cues may be involved in the determination of this preference and performance.

The above conclusions are further supported by observations of feeding damage. More feeding marks were recorded on Royal Blue, followed by *S. nigrum* and then *A. calendula,* a combined result of both adult and larvae feeding activities as indicated by the positive correlation (Table 5.1). Thrips did not, however, reproduce on Royal Blue, suggesting that while this host was prefered for feeding over other test plants, it was deemed unsuitable for oviposition and support for larvae development. Similar observations were made on this potato cultivar in field exposure trials (Chapter 3). This observation that *T. tabaci* females utilizes different hosts for very different aspects of performance is not unique to this Thysanoptera species. Brodbeck *et al*., (2002) have shown in their research that while females of *F. occidentalis* are generalists in their feeding behaviour, they are specialists in their choice for oviposition. However, *T. tabaci* has been reported to descriminate between vegetation more than *F. occidentalis* (Kumar *et al.*, 1995). The results from the current study, therefore, validates these observations and also prove that the female *T. tabaci* population used has some preferences for oviposition on *S. nigrum, A. calendula,* Shepody and Atlantic, and these plants can support larvae development, albeit, with different capabilities. Breeding populations of *T. tabaci* have previously been observed on potato foliage in field trials in which Shepody and Atlantic were included as treatments (Chapter 3; Wilson 2001). Over the entire observation period in this study, larval thrips were found almost exclusively on the underside of younger leaves, while the vertical distribution of adults among leaves was even in different parts of the plant canopy, suggesting preferences for younger leaves for ovoposition and early development and no specific orientation for adult feeding. Specialisation for oviposition sites may be needed for *T. tabaci* females to ensure sufficient provision of nutrients for immobile offsprings to complete their development on the host selected by the adult thrips (Courtney & Kibota 1990; Thompson 1996; Hobbs *et al.,* 1993; Bautista & Mau 1994; Terry 1997). Such specialisation on a host plant species or family is predicted when there is a positive correlation between preference and performance for a host (Via 1991; Fry 1996; Holmgren & Getz, 2000).

The contribution of *S. nigrum* and *A. calendula* to epidemics in potato crops was examined further in a series of no choice experiments by more closely determining the relationships between experience of thrips on these weeds that were systemically infected with TSWV and subsequent preference and performance on potato cultivars. The influence of experience of thrips on *S. nigrum* and *A. calendula* on the subsequent preferences and performances on potato cultivars were not significantly different ($P = 0.05$). The only positive correlation found was between larvae and thrips damage (Table 5.3), which was attributed to the slightly higher population density of larvae than by adult thrips, particularly on *S. nigrum*. This would indicate that the experience of thrips on these weeds has similar effects on the subsequent preference and performance of thrips on potato. Extrapolating results from this study to a field situation would suggest that a larger populations of *T. tabaci* can be expected in areas where there are large patches of *A. calendula* and *S. nigrum,* such as in South-eastern areas of South Australia, which in turn increases infestation chances in nearby potato fields. And since cvs. Shepody and Atlantic are both susceptible to TSWV and can support one or more generations of thrips prior to the end of the growing season, they would serve as good inoculum sources for secondary spread of the virus to susceptible crops and weeds. Reproducing and viruliferous populations of *T. tabaci* from such late-season summer potato crops or weeds, move into an autumn-spring germinating annual weed like *S. nigrum* or an autumn-winter germinating annual like *A. calendula* (Hyde-Wyatt & Morris, 1975; Auld & Medd, 1987), which become infected early in their development (early phenological stage) and harbour TSWV inoculum throughout the winter and spring into early summer. Hibernating thrips have also been shown to harbour TSWV through winter (Jenser *et al*., 2003). Viriluferous thrips emerging from infected weed hosts which mature in late spring, may act to move TSWV into

early potato crops. These implied patterns of movement allow for the completion of the cycle from an over-wintering annual host, concurrently supporting vector populations, to an early potato or other annual weed host, which will retain the infection until the following autumn/wintering season. TSWV-infected *S. nigrum* and *A. calendula* have been found, at the end of summer, within and adjacent to commercial potato crops during surveys (Chapter 2). However, the comparatively poor reproduction of *T. tabaci* on potato plants after experience on either *S. nigrum* and *A. calendula* is evidence that secondary TSWV spread from infected potato plants in the field, probably accounts for only a small part of the disease transmission in potato ecosystems, except when other conditions supporting the development and movement of large populations of vector thrips prevail.

The findings in this study expands on previous work (Chapter 2 & 3, Wilson 2001) documenting the relative potential of common annual weed species to contribute to the maintenance and spread of TSWV in potato crops in Australia, where these species commonly occur and *T. tabaci* appears to be the primary vector. The preferences and performances of *T. tabaci* in this study have indicated some of the complexities of host/plant interactions in TSWV epidemiology in potato crops. It should be noted, however, that unlike in Tasmania where *T. tabaci* is the sole vector thrips species, the situation in other states of Australia is that there are commonly more than one vector species. Therefore, the observed patterns of preferences and performances of the *T. tabaci* population used on the test plants would only represent the scenario of possible TSWV transmission in Tasmania. The implied infection cycle from potato to weed and back into potato, is expected to get altered to varying levels when other susceptible potato cultivars, weeds and vector thrips species are included in the ecosystem, as is the case in many parts of Australia. Consequently, using resistance to a single vector as a control strategy may not provide a full solution to compound vector species.

The general applicability of results from this study merits further investigations, bearing in mind that other factors such as plant age at the time of infestation, thrips population density, and growth conditions, all have a major effect on preferences and performance (Terry, 1997). The study, therefore, provides a framework for subsequent studies, particularly those relating to the identification of the chemical/nutritional basis driving thrips/host plant interactions (e.g in Bismark and Tasman), which is essential to the understanding of host plant resistance. The central biochemical/nutritional questions will have to addressed; What are the primary feeding and ovipositional cues in potato cvs Bismark and Tasman, which are most likely to determine thrips distribution? What are the comparative roles of biochemical/nutrients in potato cvs Bismark and Tasman, which determines thrips resistance? These question were not addressed in this study. Clear identification of difficulties in this line of research may lead to protocols that can assist in bringing these research efforts up to speed with the rapid advances being made in other thrips/crop systems.

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Chapter 6

ASSESSMENT OF EFFICACY OF INSECTICIDES FOR THE MANAGEMENT OF TSWV SPREAD

There is some question as to the efficacy of insecticide treatment for control of diseases caused by TSWV infection in potato, particularly if the major inoculum soyrces are external to the crop.

Materials and Methods

A series of field trials were established over the three years of the project to assess the efficacy of foliar and granular applied insectides to reduce TSWV spread within potato.

Season 2000 / 01

This trial was established at the University Farm, Cambridge, Tasmania. Potatoes of two cultivars (Atlantic – TSWV susceptible, and Russet Burbank –TSWV resistant) were planted in the first week of November 2000 in plots of three rows x five tubers (15 plants total) with 40cm betqween tubers. Each plot was adjacent to an infector row (consisting of plants of potato cv. Pink Eye with approx. 10% TSWV infection plated 20cm between tubers) with a 1m buffer between plots. Eight insecticide treatments were assessed, each replicated in eight plots per cultivar.

1. Control (no insecticide treatment)
2. Thimet (Phorate) granular insecticide applied with seed at planting
3. Spinosad (Success) applied as a triple foliar treatment at weekly intervals from first observation of TSWV
4. Lambda-cyhalothrin (Karate) applied as per Spinosad
5. Fipronil (Regent) applied as per Spinosad
6. Thimet granules + Spinosad foliar
7. Thimet granules + Lambda-cyhalothrin foliar
8. Thimet granules + Fipronil foliar

Industry standard fertiliser and irrigation schedules were used. No other pesticides (fungicides or inxsectiocides) were applied.
Presence of TSWV infections was monitoried by weekly visual inspection and montly ELISA testing. Tuber from plants succumbing to infection were to be harvested and tested by ELISA to determine seed infection rate.
Thrips numbers and species within the trial were monitored fortnighly using sticky yellow traps placed adjacent to the trial site.

Season 2002 / 03

Three field trials were established at the University Farm, Cambridge during 2002/03 season.
The trials aimed to assess the efficacy of foliar and granular insecticide treatments on the prevention of spread of TSWV from infector plants sited outside, but adjacent to treatment plots.
In the **first trial** the foliar treatments were applied weekly from emergence until senecence (to provide the greatest possibly chance of observing an effect).. In the **second trial** foliar treatments were applied as two strategically applied sprays (reflecting treatment frequency more likely to be used by industry) during January when thrips monitoring indicated thrips populations had sharply risen. Both these trials were planted on 20th November 2002. The **third trial** was planted on 15th January 2003, and aimed to assess the effect of late planting (to avoid major thrips flights) on TSWV spread.
In these trials TSWV-infected tomato plants (2 per plot) were used as inoculum sources
In all three trials the following insecticide treatment combinations were used:

1. Control (no insecticide treatment)
2. Thimet (Phorate) granular insecticide applied with seed at planting
3. Spinosad (Success) applied as a foliar treatment
4. Lambda-cyhalothrin (Karate) applied as a foliar treatment
5. Fipronil (Regent) applied as a foliar treatment
6. Thimet granules + Spinosad foliar
7. Thimet granules + Lambda-cyhalothrin foliar
8. Thimet granules + Fipronil foliar

All treatments were replicated four times in a randomised block design. Treatment plots consisted of 1 row of 10 plants each of cv.s Russet Burbank & Shepody.
TSWV infection anf thrips populations were monitored as before.

Season 2003 / 04

Two field trials were establised in 2003 / 04 season, one in Cambridge Tasmania, and one in South Australia at a site distant from current commercial crops but were TSWV epidemics had previously occurred. TSWV-infected tomato plants (2 per plot) were used as an inoculum source.
Both trials were planted in November 2003. Treatments included in both trials were:

1. Control (no insecticide treatment)
2. Thimet (Phorate) granular insecticide applied with seed at planting
3. Confidor (Gaucho) applied as soil treatment (in furrow)
4. Spinosad (Success) applied as a foliar treatment
5. Pyrethrin (Dominex) applied as a foliar treatment
6. Confidor (Gaucho) applied as a foliar treatment
7. Thimet granules + Success foliar
8. Thimet granules + Dominex foliar
9. Thimet granules + Confidor foliar
10. Confidor soil treatment + Success foliar
11. Confidor soil treatment + Dominex foliar
12. Confidor soil treatment + Confidor foliar

In addition, the Cambridge trial included:

1. Fipronil (Regent) foliar
2. Thimet granules + Regent foliar
3. Confidor soil treatment + Regent foliar

Foliar treatments were applied as two sprays in January when monitored thrips numbers indicated a rapid rise in thrips activity. All treatments (both sites) were replicated four times in a randomised block design. Treatment plots consisted of 1 row of 10 plants each of cv. Shepody.

Results

Season 2000 / 01

In the first season trials no infection were recorded within the thrial plots. This is despite plentiful inoculum present in infector rows (cv. Pink Eye) and large numbers of vector thrips recorded. We believe the infector source (potato cv. Pink Eye) may have been unsuitable for acquisition by the thrips vectors. Iot is possible this cultivar does not support breeding population of thrips as has been demonstrated for some varieties in chapter 5 of this report.

Season 2002 / 03

Again despite plentiful thrips observed within the trial on monitoring cards, and plentiful TSWV inoculum in the form of infected tomato plants interspersed around the trial plots, very little infection was observed within the trials. The first trial had only nine individual infected plants, the second trial only 11 individual infected plants, and the third trial no infections recorded. This level of infection meant meaningful; comparisons of treatment effects were impossible.
However interesting trends were observed. Fig 6.1. Shows the infections observed in trial 1. The majority of which were in control treatments indicating a possible effect of granular and weekly foliar insecticide treatments on the rate of infection. Fig 6.2. Shows the infections observed in plots with and without Thimet (phorate) granules irrespective of foliar treatments across trials 1 & 2. Again this shows and interesting trend that plots with Thimet were less affected than plots without Thimet.
That trial 3 failed to show any infections indicates that late planting (avoiding major thrips flights) may indeed have an effect on reducing virus infection levels.

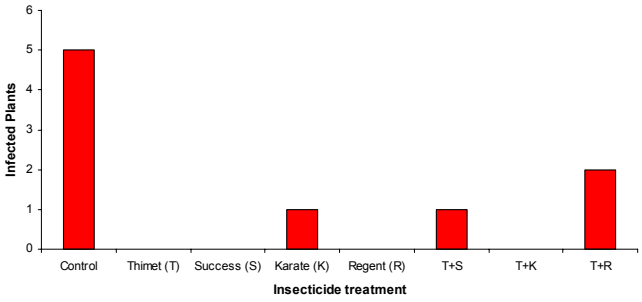


Fig 6.1. Effect of granular and foliar applied insecticides on TSWV infections (trial 1 2002/03)

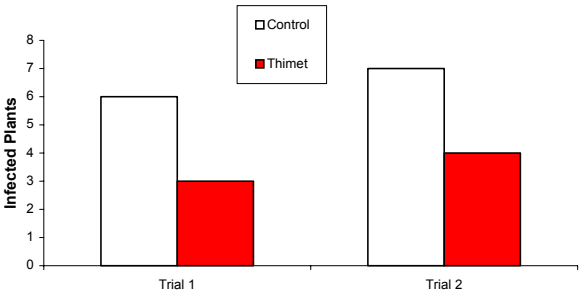


Fig 6.2. Effect of Thimet granules on observed infections (trials 1 & 2 2002/03)

Season 2003 / 04

Low levels of TSWV were again found in both this seasons trials.
In Tasmania eight infections were recorded, and in South Australia 16 infections confirmed (Table 6.1)

Table 6.1 Infections noted within 2003 / 04 insectidie trial treatments

Treatment	Infections	
	Tasmania	South Australia
Control (no insecticide treatment)	**4**	**2**
Thimet (Phorate) granular insecticide applied with seed at planting	0	**2**
Confidor (Gaucho) applied as soil treatment (in furrow)	0	**2**
Spinosad (Success) applied as a foliar treatment	**3**	0
Pyrethrin (Dominex) applied as a foliar treatment	**1**	**1**
Confidor (Gaucho) applied as a foliar treatment	0	**1**
Thimet granules + Success foliar	0	0
Thimet granules + Dominex foliar	0	**2**
Thimet granules + Confidor foliar	0	**1**
Confidor soil treatment + Success foliar	0	0
Confidor soil treatment + Dominex foliar	0	**4**
Confidor soil treatment + Confidor foliar	0	**1**
Fipronil (Regent) foliar	0	-
Thimet granules + Regent foliar	0	-
Confidor soil treatment + Regent foliar	0	-
TOTAL	**8**	**16**

- = not tested

The results from Tasmania show a similar trend to those observed in previousl seasons, with most infections occuring within the untreated control, and most (with the possibly exception of spinosad foliar treatment) insecticide treatments appearing to offer some level of control. Confidor (as a soil treatment) appeared equally as effective as Thimet.
In South Australia, however, observed trends were less clear. Infections were scattered throughout most treatments. Those three treatments which included Spinosad (Success) were the only three not to posses any infections.

Discussion

The outcomes of this series of insecticide trials was a little dissapointing. Despite our best efforts (and signifcant epidemics in nearby crops), realtively little virus infection was recorded across the trials. This is however typical of the sporadic nature of this disease and highlihts the difficulty facing researchers working with this pathosystem. Examination of the trends observed across the trials, did show some encouraging results however. In Tasmania (where onion thrips is the only known TSWV vector present in potato crops), almost all crop insecticde treatments appeared to reduce infection with TSWV. This is notable as the external inoculum

sources (infected tomato plants) from which thrips would have aquired the virus were not treated (simulating the natural situation where control and treatment of external TSWV sources is beyond the scope of the grower). One would expect that insecticide treatment of inoculum sources would provide the greatest effect on TSWV spread, as the thrips must remain associated with the plant for a generation (2-3 weeks) in order to successfully aquire the virus. In contrast thrips only need to feed for a matter of seconds – minutes on the recipient plants (the potato crop) in order the transmit the virus. That insecticide treatments appeared to provide some protection when applied to the potato crop suggests that the chemicals may have deterrred thrips alighting or feeding on the treated plants, as thrips population control within the crop would have little effect on virus spread.
In the one trial run in South Australia (where both onion and tomato thrips TSWV vectors are found in potato crops), the outcomes were less clear. Little obvious benefit of granular or foliar treatments were shown (although the limited infection level restricts our ability to intrepret the data beyond the broadest context). This suggests either tomato thrips are less easily deterred by these treatments, or under (slightly) higher inoculum pressure, the limited benefit of insectiocde treatments is lost.

It will be important to continue such trials (perhaps as strips within commercial crops) in order to determine how these treatments perform under greater inoculum pressure.

Thrips must breed on a TSWV-infected plant in order to aquire the virus as in only the 1st or 2nd instar larvae can TSWV successfully passage from the gut into the insect haemocyle an into the salaviary glands (REF). Experimental evidence available at the start of this project indicated that virus acquisition from potato was limited. For example, in a field trial in northern Tasmania (cv. Russet Burbank) planted with 50% infected seed, no infections of healthy plants were recorded during the life of the trial. In contrast, iobservations within a crop (cv. Atlantic) in NSW showed spatial patterns of TSWV infection which sugegsted virus spread from infected potato sources. Following outcomes from Chapter 5 we now know that Russet Burbank (and other varieties) appear not to support breeding population of onion thrips which would explain this lack of virus spread (in absence of external TSWV sources)., and in contrast cv.s like Atlantic do support breeding populations and thus have the potential to act as inoculum sources. This disparency may be important in concluding the importance of insecticde use in commerical crops. Obviously TSWV infected plants within a field of Russet Burbank are of little consequence for further spread, and controlling thrips populations within the crops may have limited benefit. In contrast infected plants within a crop of Atlantic or Shepody could act as a source of infection.

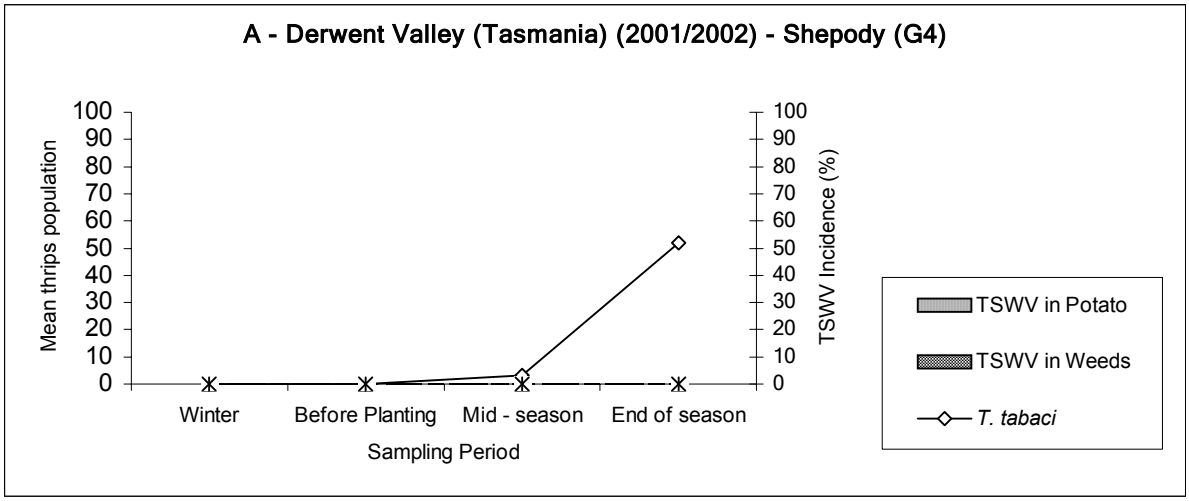
Recommendations for future activity

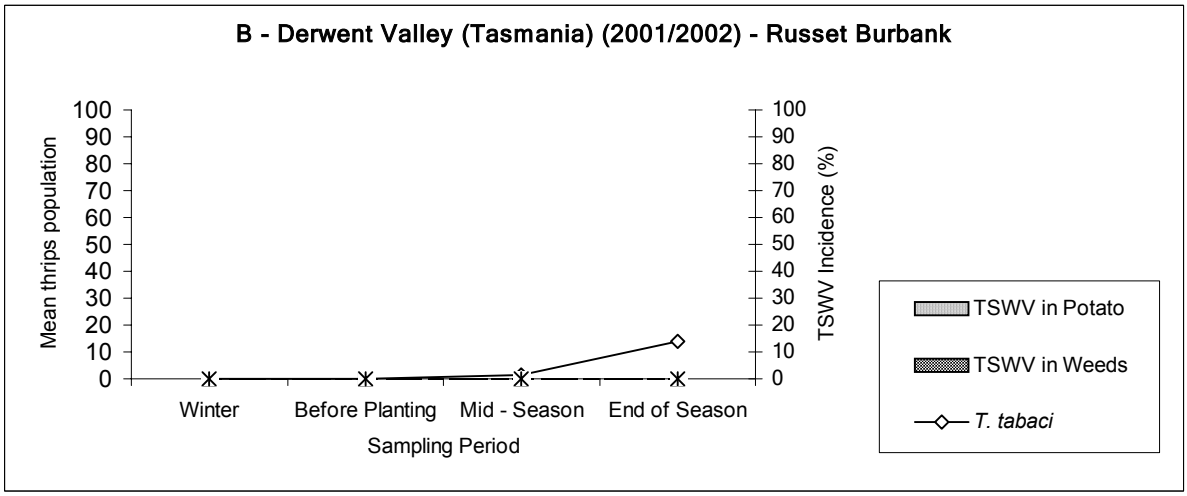
This study has advanced our knowledge of the TSWV – potato pathosystem and has indicated several options for disease intervention.
A great deal more needs to be done however before an integrated management strategy can be developed. Areas we feel additional work is required include:

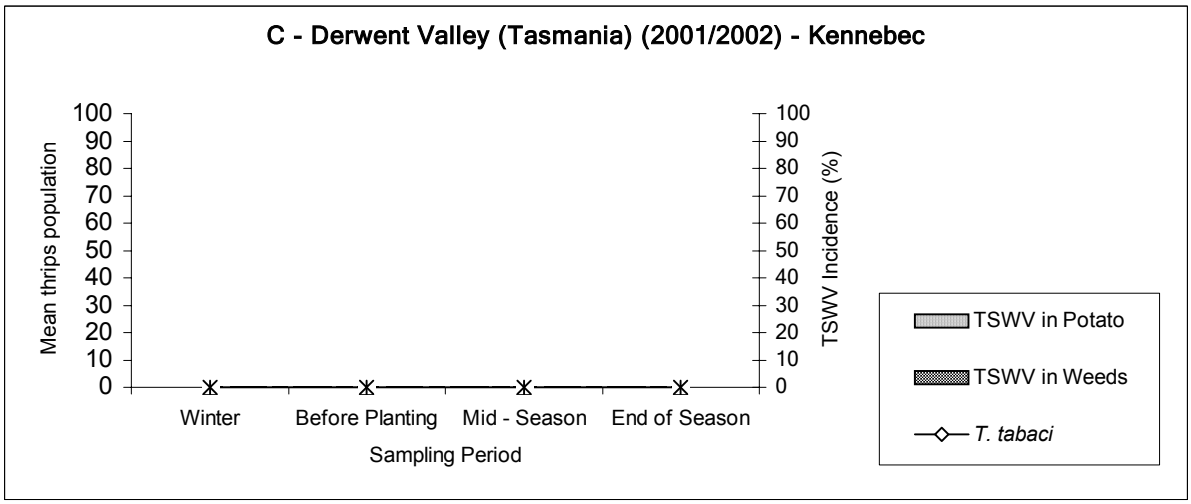
1) Further field monitoring of epidemics, and associations with inoculum sources and vectors. In this project we have developed tool to assist in prediction vector thrips populations dynamics within potato fields. This can be used to indicate potential risk periods for TSWV transmission (transmission events if they happen occur during peaks in thrips population activity). However, thrips themselves are not the driving force behind TSWV epidemics (rather the timing only). Presence of inoculum sources is more likely to determine the extent of disease. Thus we need additional information on prediction of inoculum levels in the vicinity of the crop. This may be most easily determined through development of a test for presence of TSWV within migratory thrips. Thus we would link thrips population with virus presence.
2) Cultivar screening has revealed several lines more tolerant of virus infection than others. Greater screening of a wide range of genotypes within the potato breeding program would allow us to determine the scope and extent of resistance within current germplasm, and indicate useful parents for cultivar development in further breeding experiments.
3) We have shown temperature influences TSWV infection rate, systemic movement in infected plants, and symptom expression. Further work could concentrate on effect of diurnal fluctuations of temperature and thus determine how weather patterns from different cropping regions in Australia may influence virus epidemiology, and tuber infections.
4) Resistance to thrips preference and breeding has been shown. This trait offers avoidance of the disease and is thus a highly useful approach to resistance. Further more, presence of a thrips deterring volatile from potato foliage offers a potential chemical approach to disease management (targeting thrips deterrence rather than population reduction which is more likely to be effective in reducing virus transmission). Additional work examining the chemical basis of thrips deterrence, and development of gene markers to these resistance trait would be a valuable approach to take.
5) Insecticide treatments appear to show some promise for disease control, but further testing is required. The data collected from trials to date has been obtained under low inoculum pressure. Insecticide treatments may preform quite differently under high inoculum pressure. Thus further trials should be undertaken

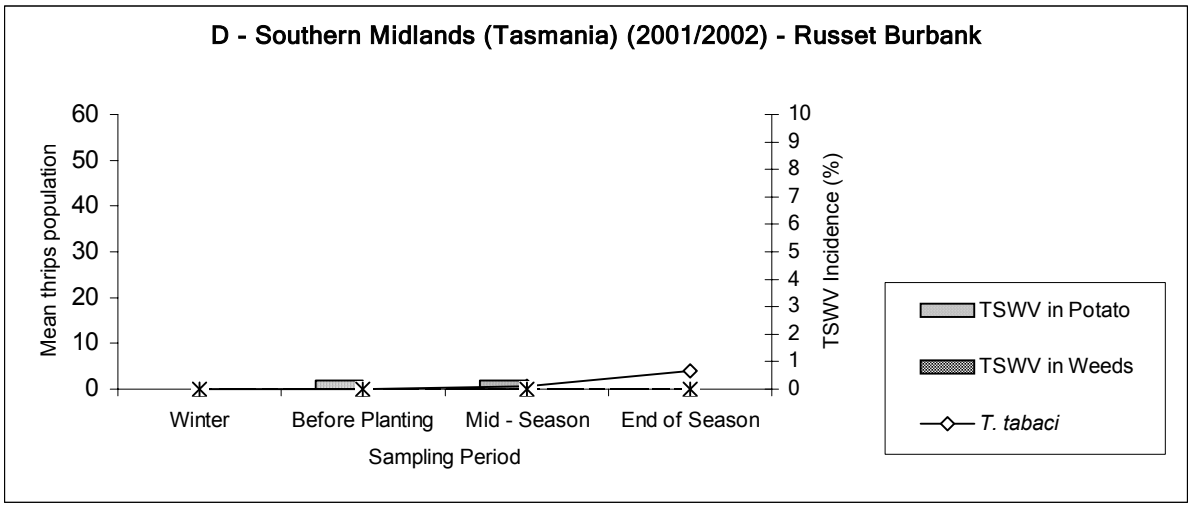
Appendices

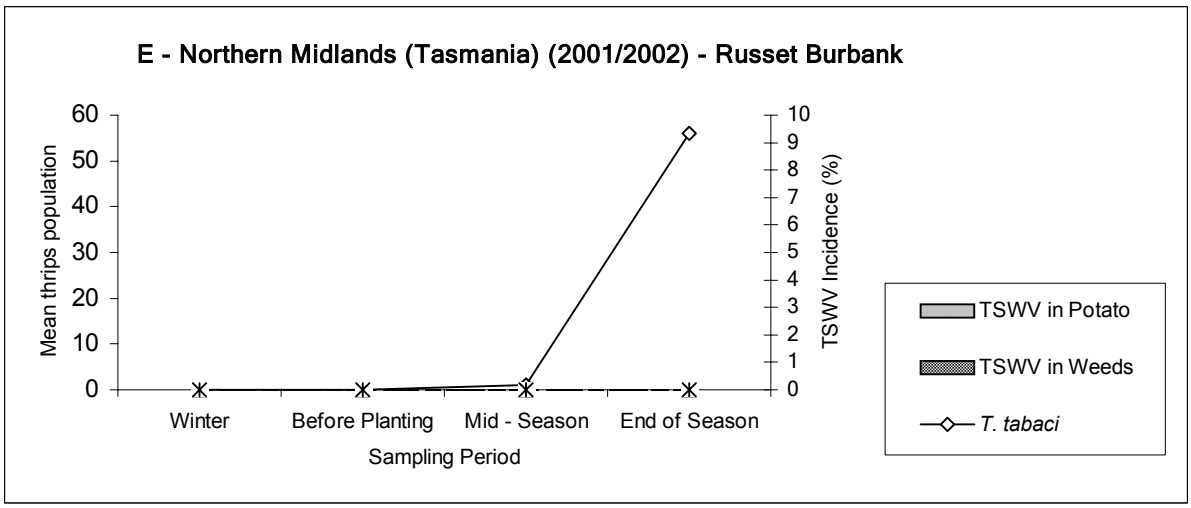
Appendice 1 Relationship between population dynamics of known vector thrips and incidence of TSWV in potato cultivars and weeds in Tasmania, Victoria and South Australia during 2001/2002 and 2002/2003 seasons

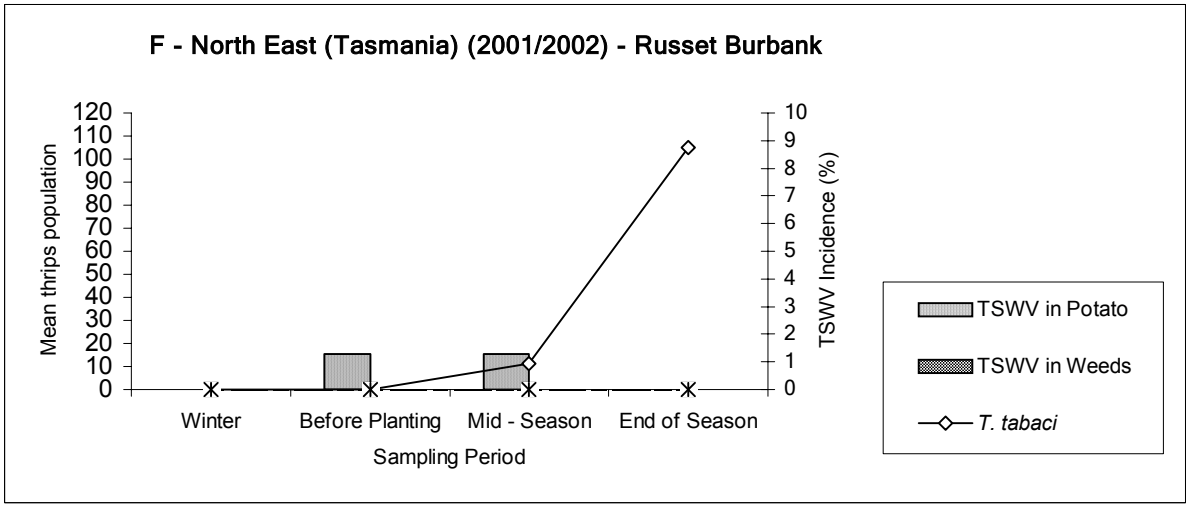


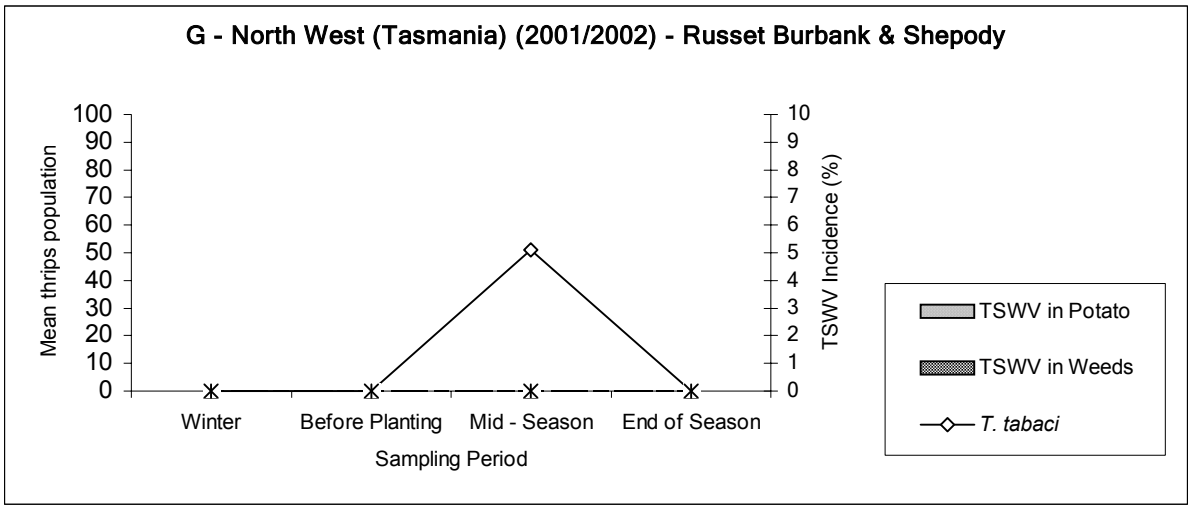


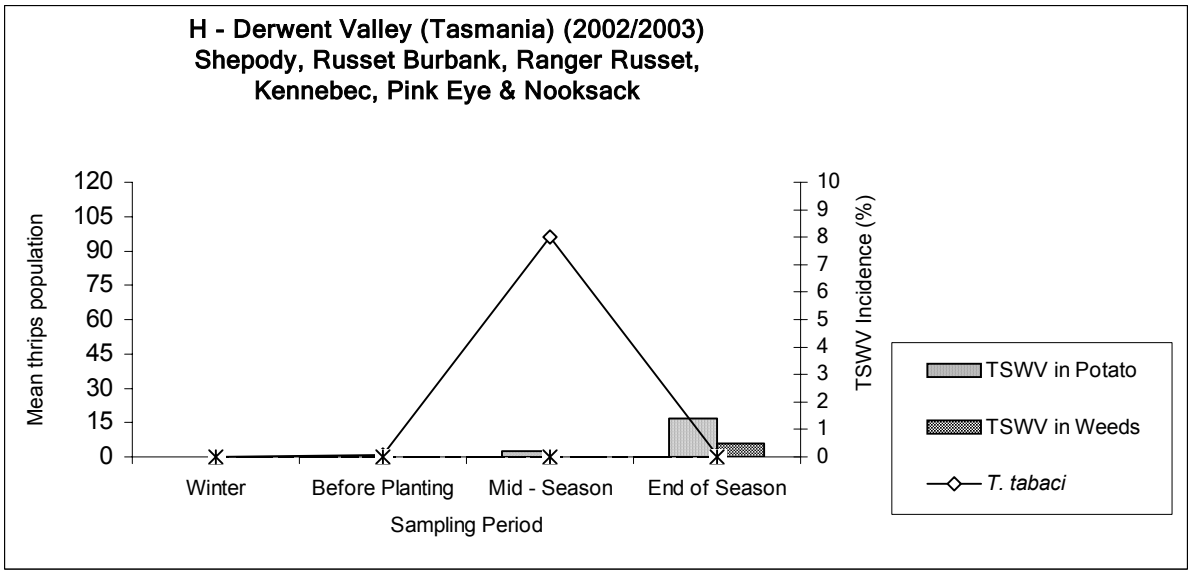


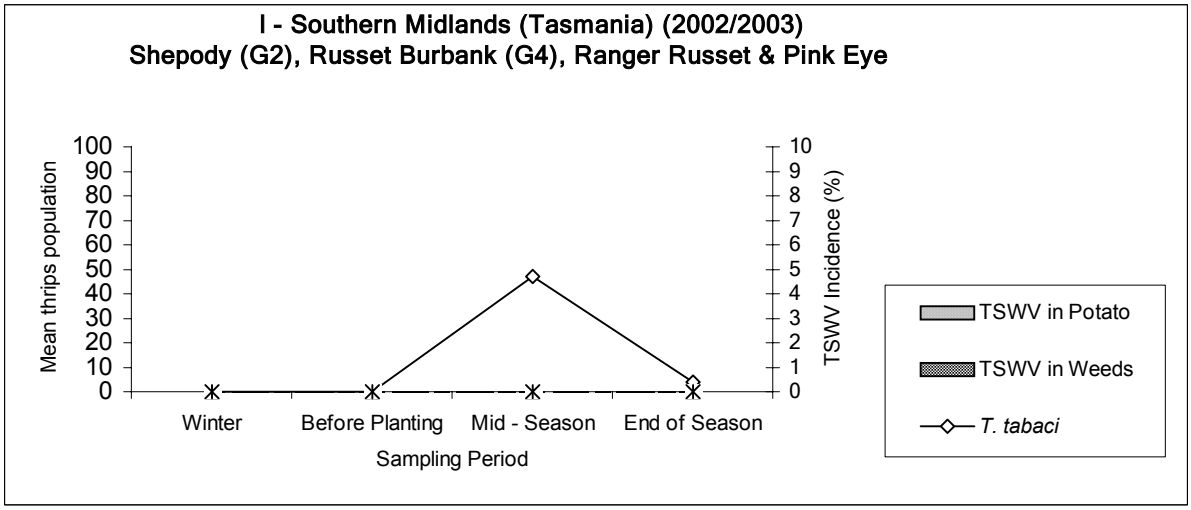

D - Southern Midlands (Tasmania) (2001/2002) - Russet Burbank
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

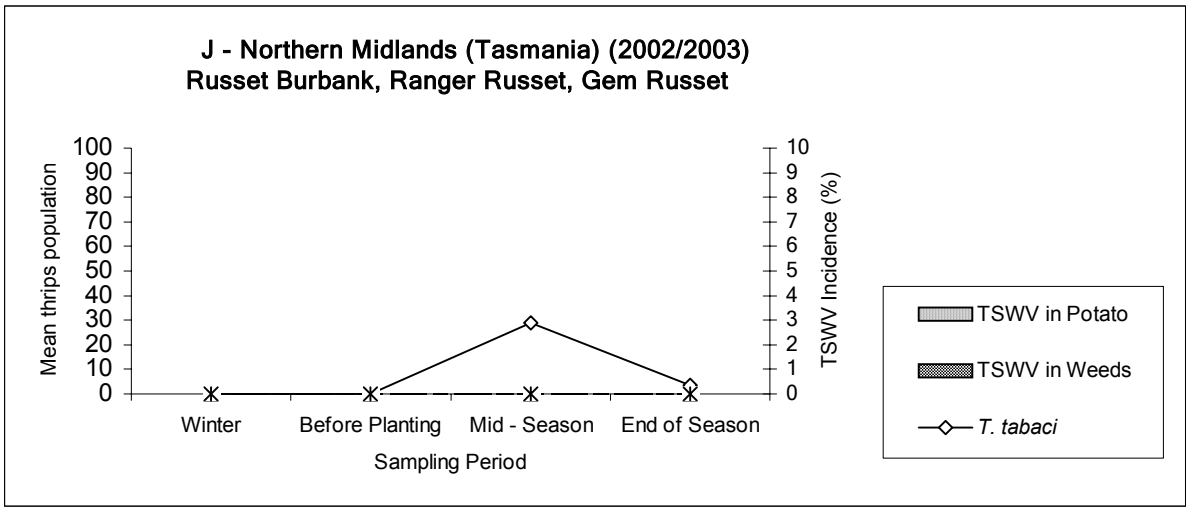

E - Northern Midlands (Tasmania) (2001/2002) - Russet Burbank
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

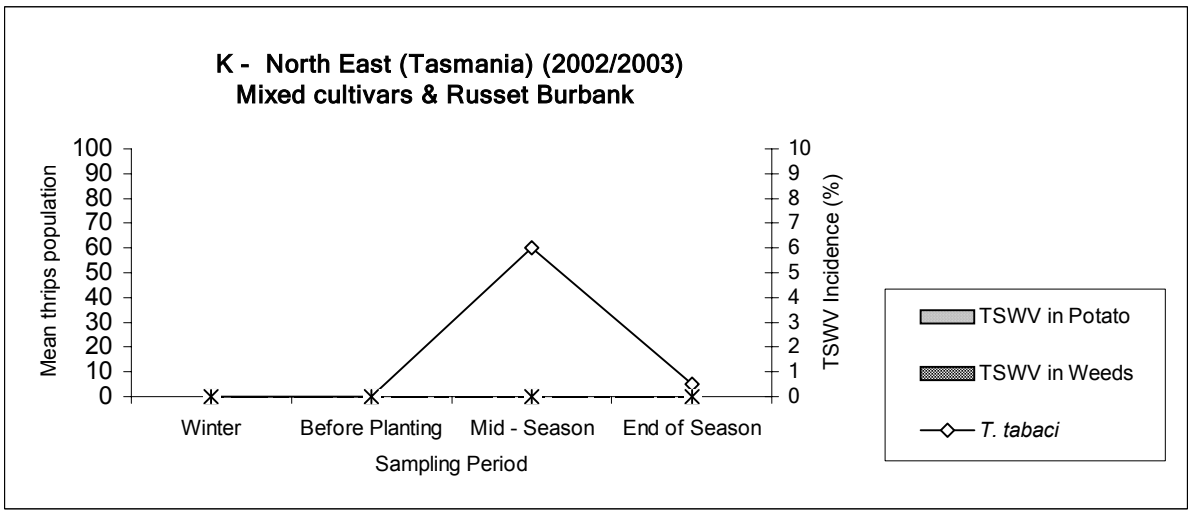

F - North East (Tasmania) (2001/2002) - Russet Burbank
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

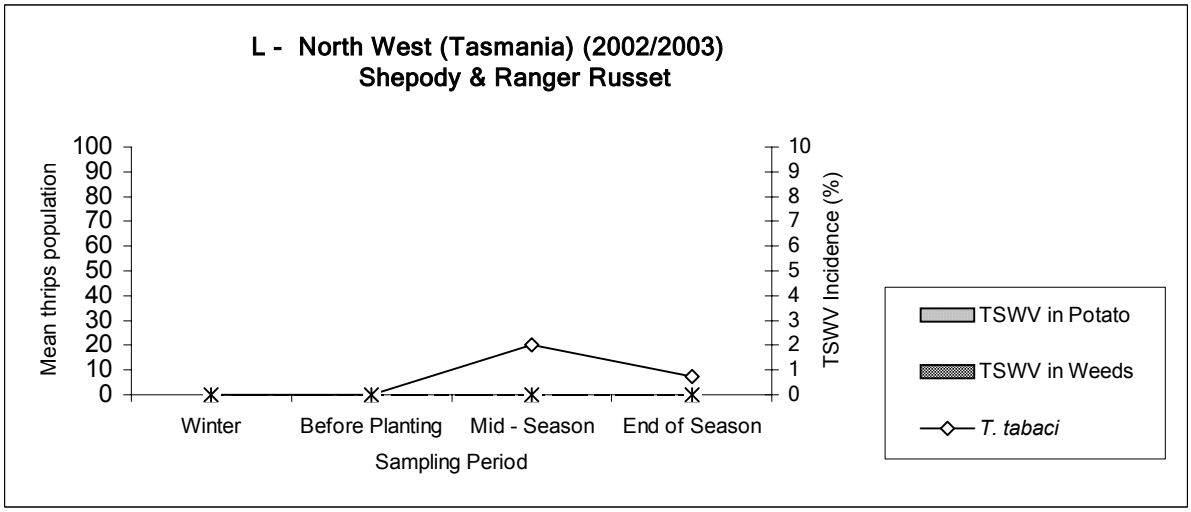

G - North West (Tasmania) (2001/2002) - Russet Burbank & Shepody
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

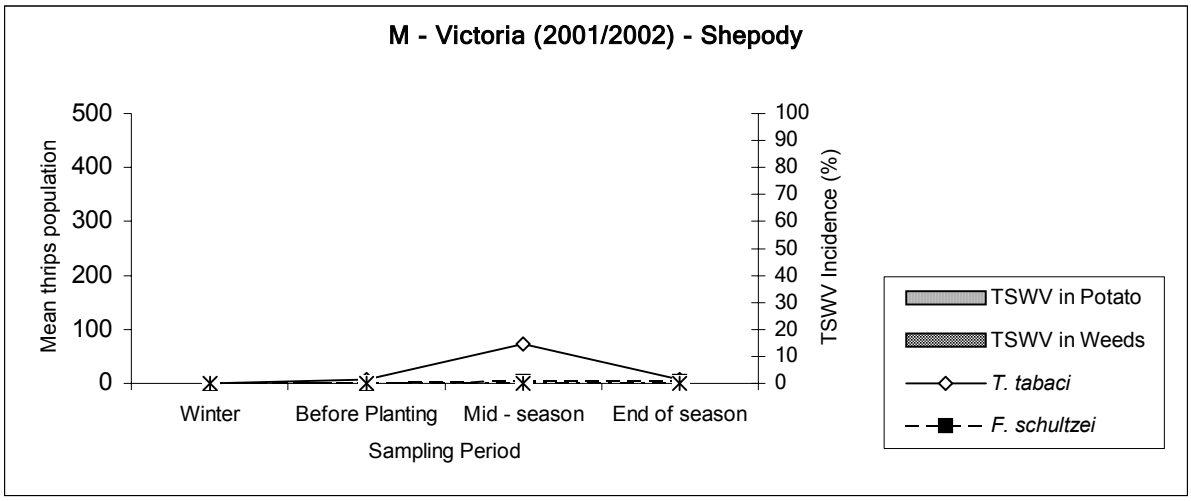

H - Derwent Valley (Tasmania) (2002/2003)
Shepody, Russet Burbank, Ranger Russet,
Kennebec, Pink Eye & Nooksack
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

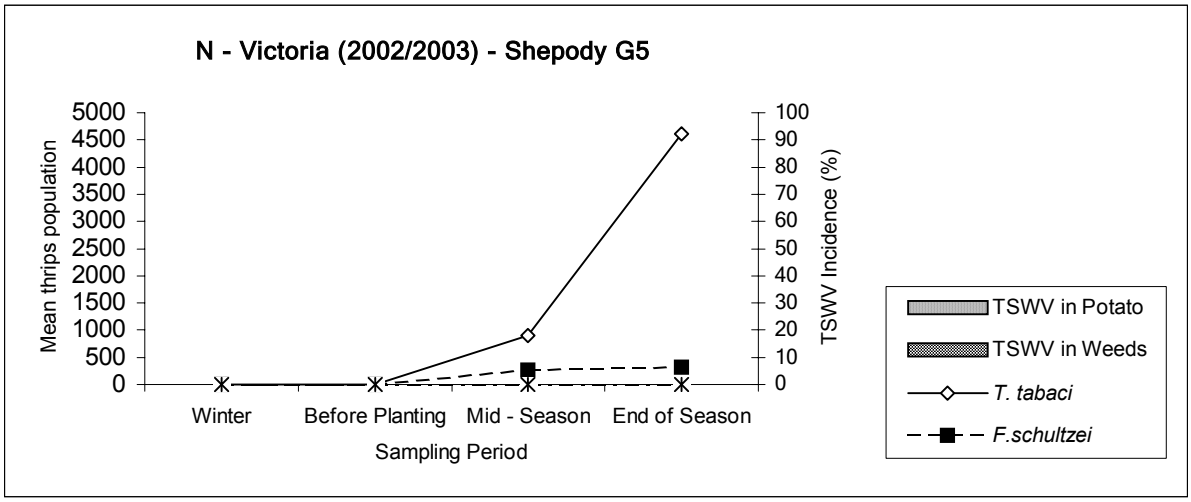

I - Southern Midlands (Tasmania) (2002/2003)
Shepody (G2), Russet Burbank (G4), Ranger Russet & Pink Eye
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

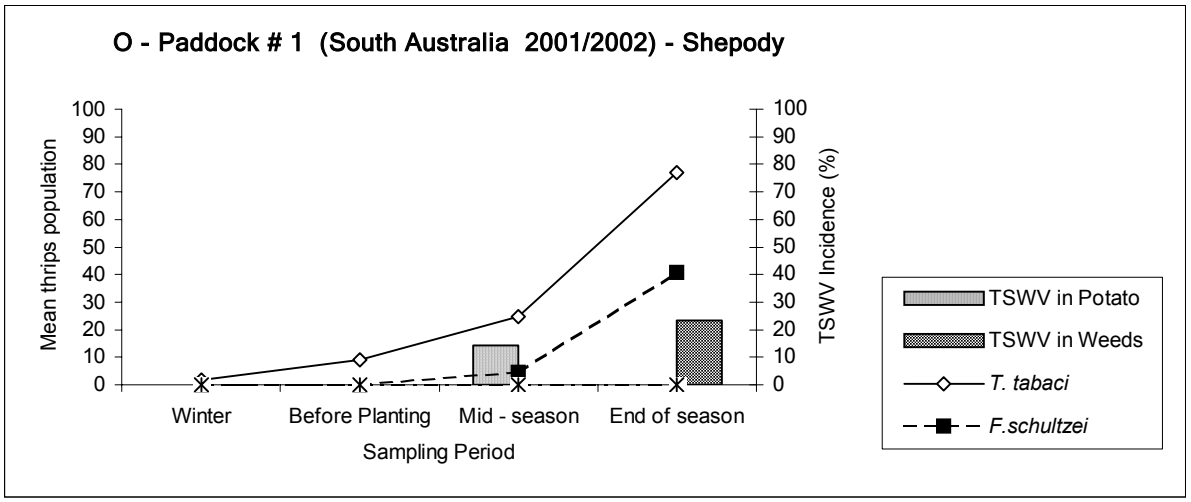

J - Northern Midlands (Tasmania) (2002/2003)
Russet Burbank, Ranger Russet, Gem Russet
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

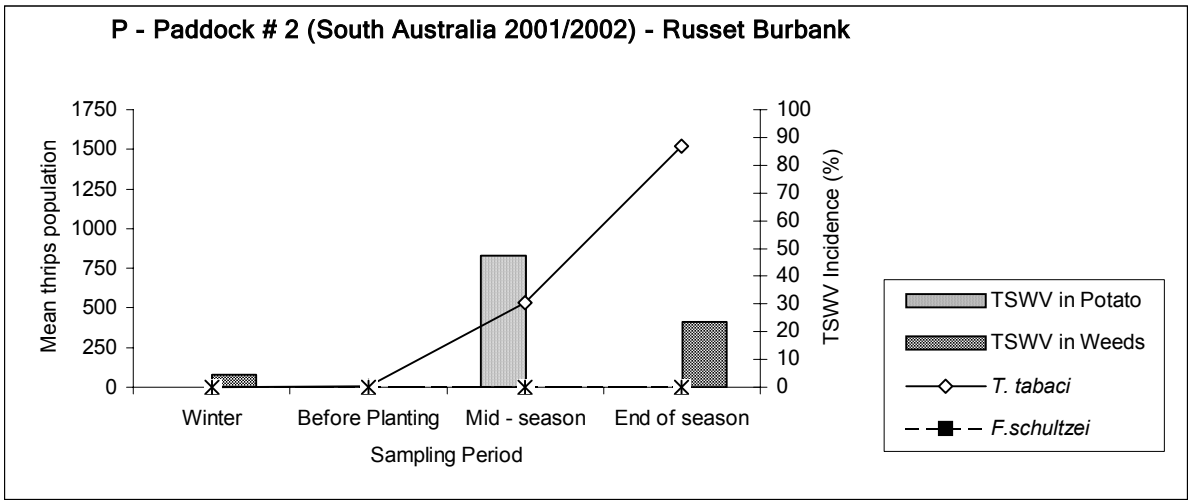

K - North East (Tasmania) (2002/2003)
Mixed cultivars & Russet Burbank
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

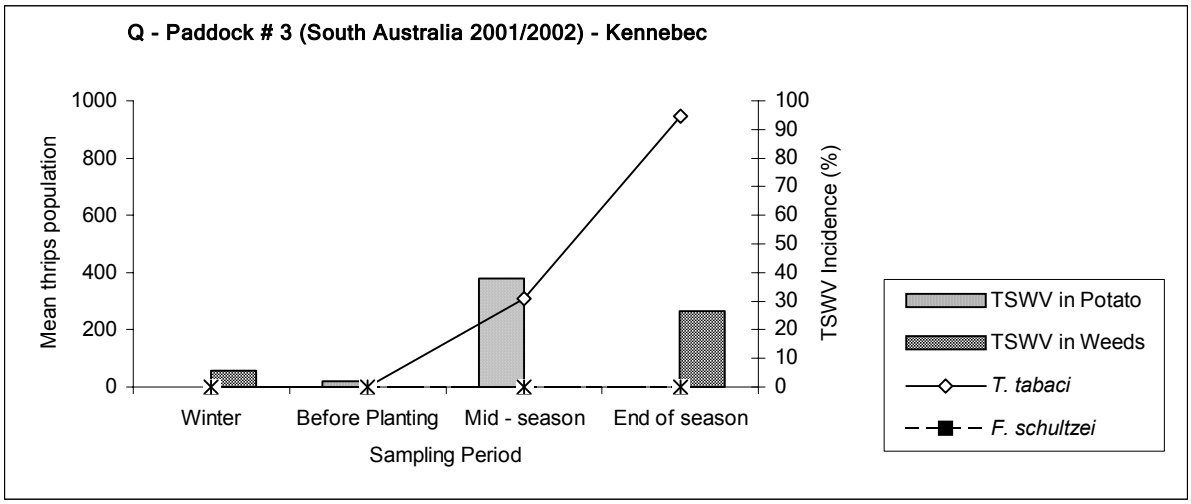

L - North West (Tasmania) (2002/2003)
Shepody & Ranger Russet
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci

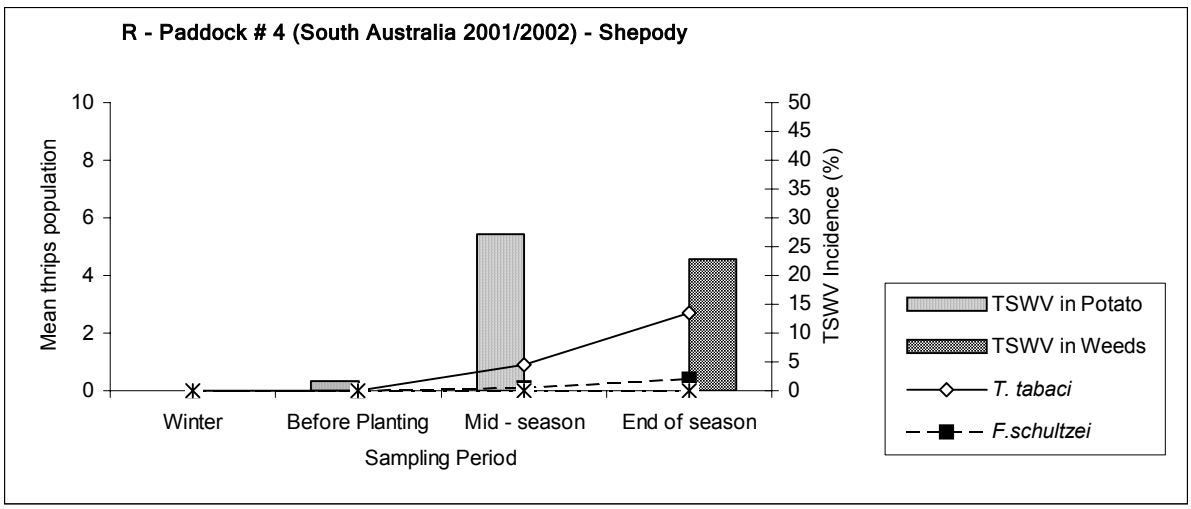

M - Victoria (2001/2002) - Shepody
Mean thrips population
TSWV Incidence (%)
500
400
300
200
100
0
100
90
80
70
60
50
40
30
20
10
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F. schultzei

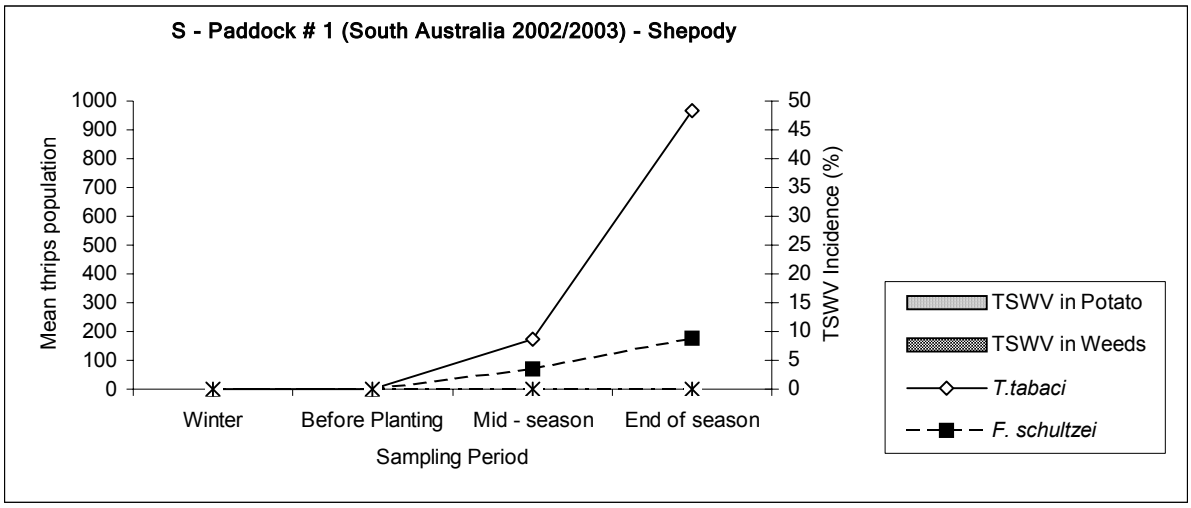

N - Victoria (2002/2003) - Shepody G5
Mean thrips population
TSWV Incidence (%)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
100
90
80
70
60
50
40
30
20
10
0
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F.schultzei

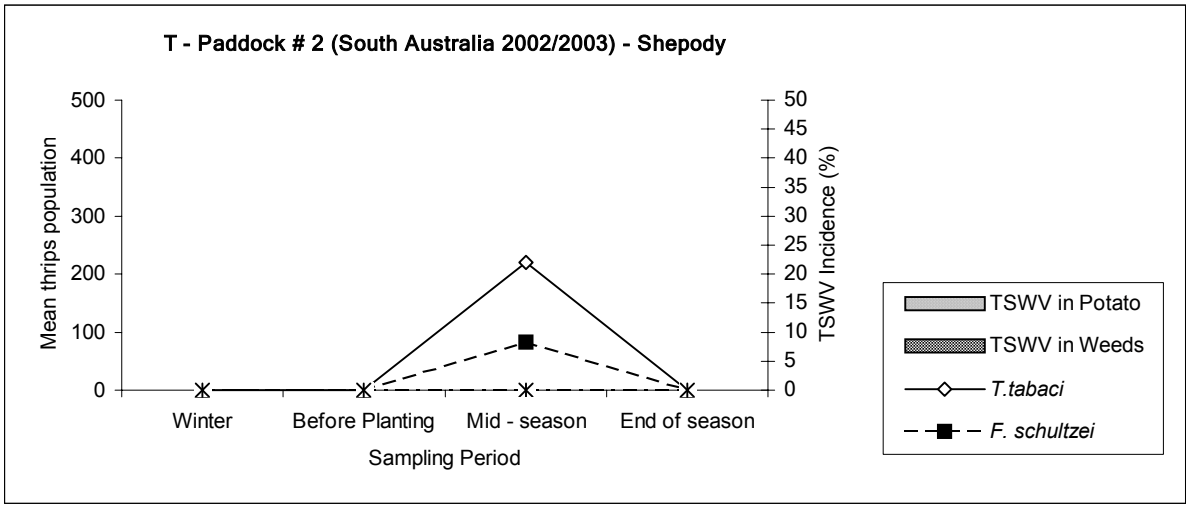

O - Paddock # 1 (South Australia 2001/2002) - Shepody
Mean thrips population
TSWV Incidence (%)
100
90
80
70
60
50
40
30
20
10
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F.schultzei

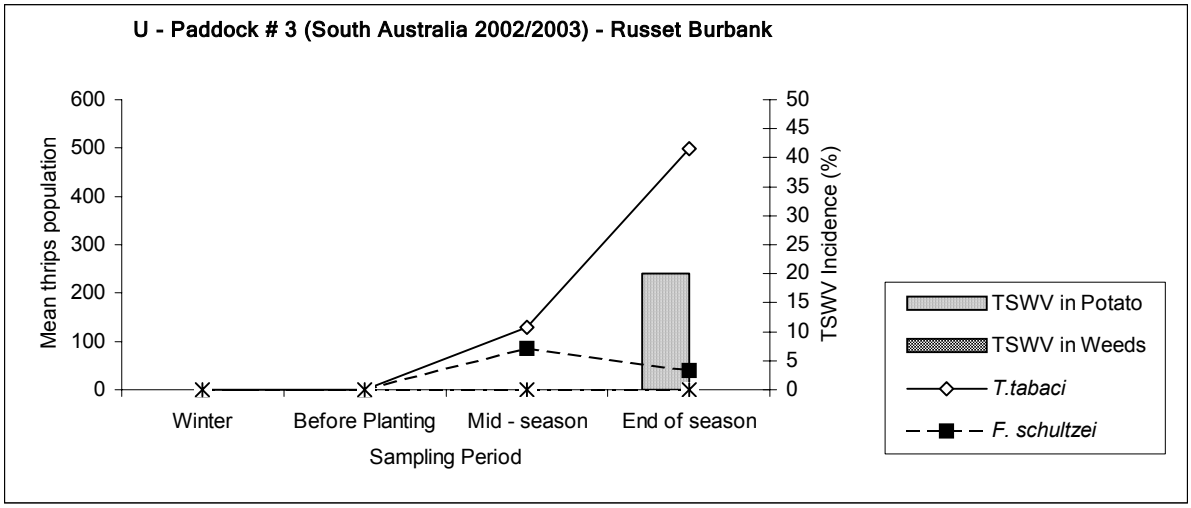

P - Paddock # 2 (South Australia 2001/2002) - Russet Burbank
Mean thrips population
TSWV Incidence (%)
1750
1500
1250
1000
750
500
250
0
100
90
80
70
60
50
40
30
20
10
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F.schultzei

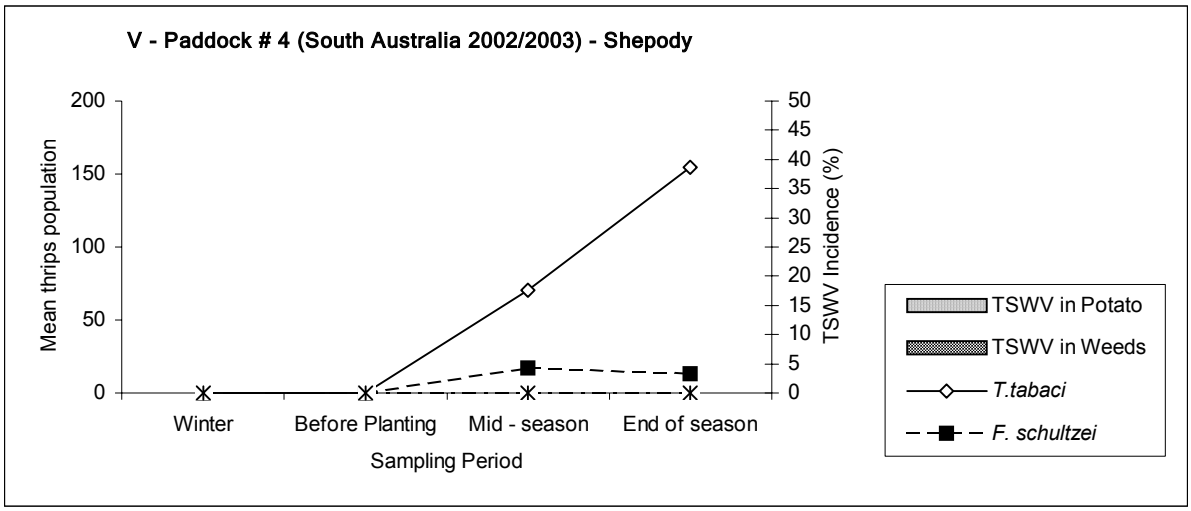

Q - Paddock # 3 (South Australia 2001/2002) - Kennebec
Mean thrips population
TSWV Incidence (%)
1000
800
600
400
200
0
100
90
80
70
60
50
40
30
20
10
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F. schultzei

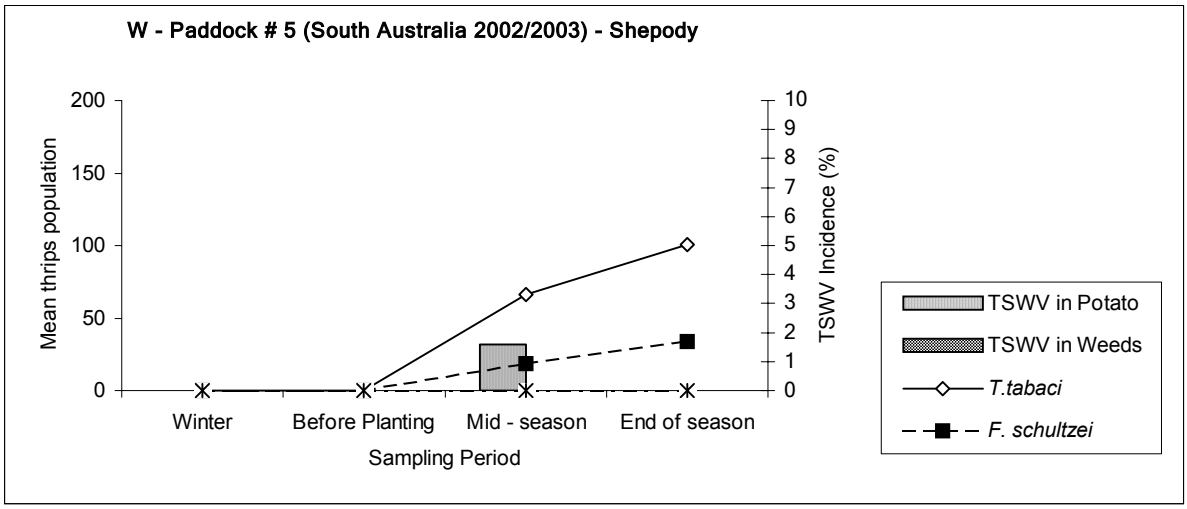

R - Paddock # 4 (South Australia 2001/2002) - Shepody
Mean thrips population
TSWV Incidence (%)
10
8
6
4
2
0
50
45
40
35
30
25
20
15
10
5
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T. tabaci
F.schultzei

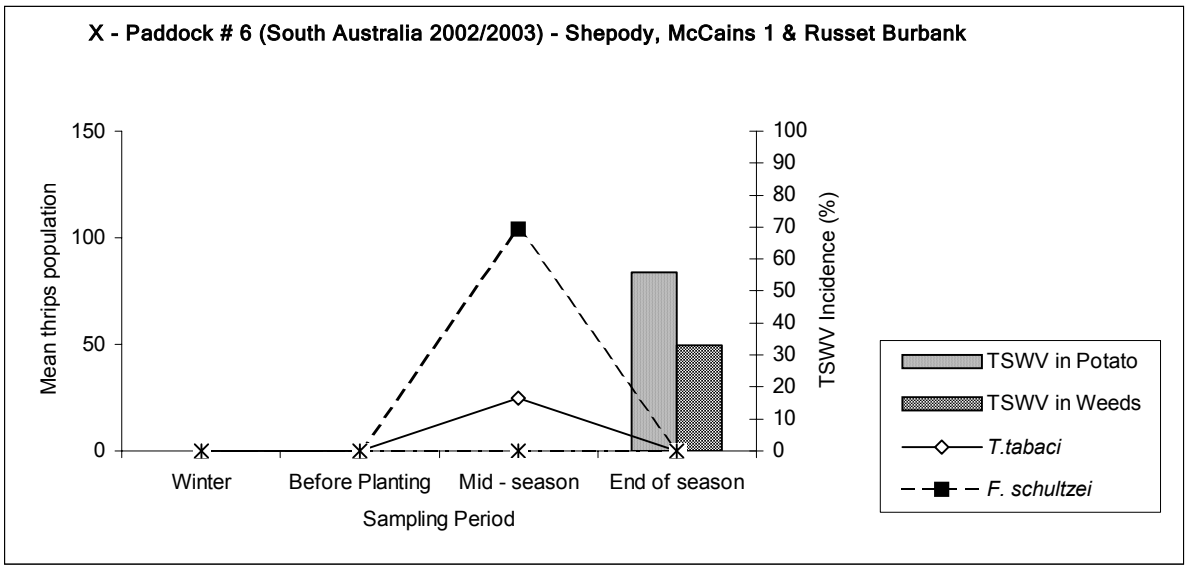

S - Paddock # 1 (South Australia 2002/2003) - Shepody
Mean thrips population
TSWV Incidence (%)
1000
900
800
700
600
500
400
300
200
100
0
50
45
40
35
30
25
20
15
10
5
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei

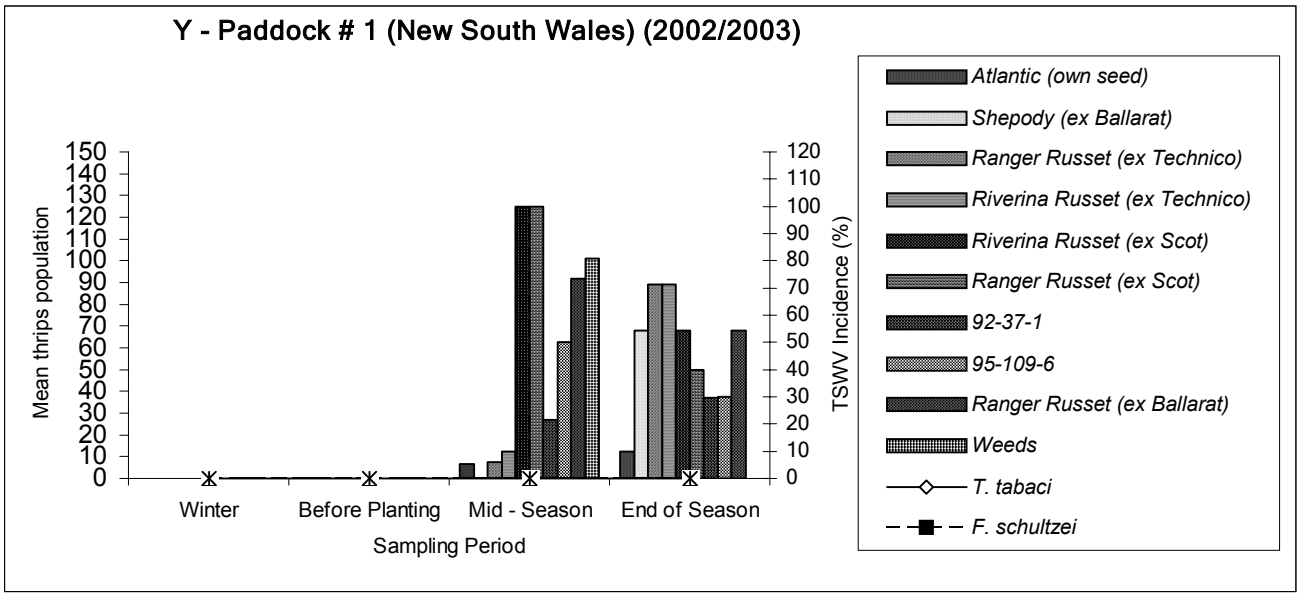

T - Paddock # 2 (South Australia 2002/2003) - Shepody
Mean thrips population
TSWV Incidence (%)
500
400
300
200
100
0
50
45
40
35
30
25
20
15
10
5
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei


U - Paddock # 3 (South Australia 2002/2003) - Russet Burbank
Mean thrips population
TSWV Incidence (%)
600
500
400
300
200
100
0
50
45
40
35
30
25
20
15
10
5
0
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei


V - Paddock # 4 (South Australia 2002/2003) - Shepody
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei


W - Paddock # 5 (South Australia 2002/2003) - Shepody
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei


X - Paddock # 6 (South Australia 2002/2003) - Shepody, McCains 1 & Russet Burbank
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - season
End of season
Sampling Period
TSWV in Potato
TSWV in Weeds
T.tabaci
F. schultzei


Y - Paddock # 1 (New South Wales) (2002/2003)
Mean thrips population
TSWV Incidence (%)
Winter
Before Planting
Mid - Season
End of Season
Sampling Period
Atlantic (own seed)
Shepody (ex Ballarat)
Ranger Russet (ex Technico)
Riverina Russet (ex Technico)
Riverina Russet (ex Scot)
Ranger Russet (ex Scot)
92-37-1
95-109-6
Ranger Russet (ex Ballarat)
Weeds
T. tabaci
F. schultzei

Appendice 2: Gompertz curve = $Y = A + Ce^{-e^{-B(X-M)}}$

Where: Y_1 = *T. tabaci*, Y_2 = *F. Schutzei*, A = Constant, In this analysis C<0 and A+C =0, therefore, $Y_1 = C*EXP(-EXP(-B*(X-M)))$, $Y_2 = C*EXP(-EXP(-B*(X-M)))$

State	Season	Parameter	Regression equation	R^2	F. pr	V.R.	P (0.05)
Tasmania	2001/2002 †	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -1.59*EXP(-EXP(-0.549*(X + 19.58)))$	0.17	0.210	3.92	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -1.635*EXP(-EXP(-7.046*(X + 9.42)))$	0.61	0.102	9.00	NS
		Mean Daily Precipitation (mm)	$Y_1 = -0.9584*EXP(-EXP(+3.364*(X + 4.20)))$	N/A	0.380	1.78	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -1366*EXP(-EXP(+0.3135*(X + 35.55)))$	N/A	0.267	2.89	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -81.07*EXP(-EXP(-2.574*(X + 22.56)))$	0.37	0.083	4.76	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -380.6*EXP(-EXP(-7.433*(X + 12.72)))$	0.58	0.037	7.89	S
		Mean Daily Precipitation (mm)	$Y_1 = -64.48*EXP(-EXP(+9.331*(X + 1.385)))$	N/A	0.234	2.18	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -65.6*EXP(-EXP(+1*(X + 52)))$	N/A	0.204	2.45	NS
Victoria	2001/2002 †	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -583.1*EXP(-EXP(-0.16721*(X + 6182)))$	0.17	0.005	6.30	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -3934*EXP(-EXP(+1.64E-02*(X + 2.28E+02)))$	0.32	0.001	8.79	S
		Mean Daily Precipitation (mm)	$Y_1 = -0.527*EXP(-EXP(-0.3916*(X + 31.42)))$	N/A	0.051	3.22	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -1.171*EXP(-EXP(+1039*(X + 50.5)))$	0.12	0.008	5.61	S
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = -1.157*EXP(-EXP(-2.293*(X + 22.26)))$	0.87	<.001	60.26	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = -1.718*EXP(-EXP(-0.719*(X + 10.688)))$	0.75	<.001	28.52	HS
		Mean Daily Precipitation (mm)	$Y_2 = -0.5418*EXP(-EXP(+10.98*(X + 1.368)))$	0.04	0.047	3.32	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -1.059*EXP(-EXP(+1.262*(X + 50.25)))$	0.84	<.001	47.37	HS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -3.141*EXP(-EXP(-2.672*(X + 23.30)))$	0.53	<.001	20.77	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -7.857*EXP(-EXP(-0.2258*(X + 15.45)))$	0.29	0.002	12.99	S
		Mean Daily Precipitation (mm)	$Y_1 = -2.21*EXP(-EXP(+4.22E-03*(X + 1.37E+03)))$	N/A	0.018	6.20	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -3.21*EXP(-EXP(+0.075*(X + 44.3)))$	N/A	0.008	8.18	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = -2.574*EXP(-EXP(-1.121*(X + 24.105)))$	0.62	0.001	20.49	S
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = -2.86*EXP(-EXP(-0.427*(X + 11.15)))$	0.23	0.007	8.68	S
		Mean Daily Precipitation (mm)	$Y_2 = -17.4*EXP(-EXP(+1.923*(X –0.9078)))$	N/A	0.041	4.43	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -2.69*EXP(-EXP(+0.132*(X +39.80)))$	0.26	0.006	9.10	S
South Australia	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -1088*EXP(-EXP(-12.58*(X + 14.79)))$	0.86	<.001	30.16	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -735.4*EXP(-EXP(-0.011*(X + 110)))$	N/A	0.478	0.90	NS
		Mean Daily Precipitation (mm)	$Y_1 = -21456*EXP(-EXP(+0.076*(X -59.59)))$	N/A	0.459	0.95	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -4124*EXP(-EXP(+0.5720*(X -52.61)))$	0.09	0.153	2.24	NS
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = -12621*EXP(-EXP(-1.744*(X +19.54)))$	N/A	0.261	1.58	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = -23.77*EXP(-EXP(-2.189*(X +9.74)))$	0.41	0.025	5.11	S
		Mean Daily Precipitation (mm)	$Y_2 = -26.01*EXP(-EXP(-0.9809*(X +12.66)))$	0.40	0.026	5.03	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -3.653*EXP(-EXP(+0.8078*(X +72.90)))$	0.10	0.205	1.87	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -327*EXP(-EXP(-0.652*(X - 24.48)))$	0.54	0.012	8.92	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -341.3*EXP(-EXP(-9.955*(X + 11.07)))$	0.87	<.001	36.56	HS
		Mean Daily Precipitation (mm)	$Y_1 = -266*EXP(-EXP(+2.81*(X + 0.902)))$	N/A	0.173	2.34	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -330*EXP(-EXP(+2.664*(X + 44.92)))$	0.42	0.024	6.71	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = -105.7*EXP(-EXP(- 4.301*(X +23.68)))$	0.32	0.078	3.78	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = -124.3*EXP(-EXP(- 2.04*(X +11.288)))$	0.28	0.093	3.43	NS
		Mean Daily Precipitation (mm)	$Y_2 = -83.50*EXP(-EXP(+10.88*(X +1.311)))$	0.04	0.202	2.10	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -129011*EXP(-EXP(+0.3673*(X +21.66)))$	0.92	<.001	44.86	HS

R^2 = Adjusted coefficient of multiple determination
V.R = Variance Ratio
† = Data transformed (Log_{10})
N/A = Residual variance exceeds variance of response variate
NS = Not significant (P=0.05)
S = Significant (P=0.05)
HS = Highly Significant (P=0.05)

Appendice 3: Logistic curve $Y_1 = A + C/(1 + e^{-B(X-M)})$

Where: Y_1 = *T. tabaci*, Y_2 = *F. Schutzei*, A = Constant

State	Season	Parameter	Regression equation	R^2	F. pr	V.R	P (0.05)
Tasmania	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.1898 + 1.003/(1 + EXP-2.455*(X - 17.91))$	N/A	0.788	0.40	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 0.2320 + 1.380/(1 + EXP-5.839*(X - 9.375))$	11.1	0.577	1.17	NS
		Mean Daily Precipitation (mm)	$Y_1 = 0.6095 + 10.63/(1 + EXP-2.631*(X - 0.1452))$	N/A	0.961	0.08	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 1.562 - 0.9343/(1 + EXP-23.82*(X - 58.93))$	N/A	0.884	0.21	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = - 4.390 + 97.43/(1 + EXP-1.769*(X - 22.40))$	0.08	0.448	1.18	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 14.24 + 7044/(1 + EXP-2.064*(X - 14.71))$	0.12	0.424	1.27	NS
		Mean Daily Precipitation (mm)	$Y_1 = -16.60 + 38.34/(1 + EXP-38.24*(X - 1.319))$	N/A	0.799	0.34	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 66.77 - 26.26/(1 + EXP-20.63*(X - 48.54))$	N/A	0.978	0.06	NS
Victoria	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.2415 + 1.190/(1 + EXP- 4.273*(X - 22.41))$	0.38	0.017	4.67	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 0.2960 + 1.394/(1 + EXP- 3.767*(X - 9.975))$	0.31	0.036	3.70	S
		Mean Daily Precipitation (mm)	$Y_1 = 0.6667 - 0.2212/(1 + EXP- 6.339*(X - 0.9181))$	N/A	0.957	0.10	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 0.2539 - 0.8779/(1 + EXP- 2.186*(X - 50.84))$	0.21	0.090	2.61	NS
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = -0.0041 + 1.155/(1 + EXP- 5.30*(X - 22.092))$	0.87	<.001	42.66	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = -0.041 + 2.44/(1 + EXP- 0.679*(X - 10.97))$	0.74	<.001	17.97	HS
		Mean Daily Precipitation (mm)	$Y_2 = 0.02136 + 0.5309/(1 + EXP- 13.51*(X - 0.4002))$	N/A	0.507	0.81	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -0.0008 + 1.059/(1 + EXP- 2.661*(X - 50.45))$	0.83	<.001	30.96	HS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.4811 + 2.788/(1 + EXP- 1.105*(X - 23.23))$	0.28	0.166	2.29	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -0.05228 + 2.522/(1 + EXP- 5.936*(X - 6.721))$	N/A	0.565	0.73	NS
		Mean Daily Precipitation (mm)	$Y_1 = 4.326 - 2.388/(1 + EXP- 5.560*(X - 1.999))$	N/A	0.743	0.42	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 1.350 + 1.988/(1 + EXP- 0.2160*(X - 39.58))$	N/A	0.661	0.55	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 0.5431 + 2.093/(1 + EXP- 4.257*(X - 24.45))$	0.52	0.045	4.56	S
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 2.013 - 1.321/(1 + EXP- 12.81*(X - 10.18))$	N/A	0.496	0.88	NS
		Mean Daily Precipitation (mm)	$Y_2 = 2.434 - 1.101/(1 + EXP- 25.29*(X - 1.836))$	N/A	0.829	0.29	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = 0.9976 + 1.891/(1 + EXP- 0.8387*(X - 35.10))$	0.13	0.298	1.49	NS
South Australia	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -26.10 - 10959/(1 + EXP- 1.939*(X - 16.43))$	0.56	0.022	5.72	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 2519 - 2324/(1 + EXP- 16.95*(X - 9.865))$	N/A	1.000	0.00	NS
		Mean Daily Precipitation (mm)	$Y_1 = 352 - 156/(1 + EXP- 12*(X - 12.33))$	N/A	1.000	0.00	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 71.24 + 9548/(1 + EXP- 0.1941*(X - 41.55))$	N/A	0.477	0.91	NS
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 0.1860 + 2.750/(1 + EXP- 87.50*(X - 14.78))$	N/A	0.573	0.71	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 0.7797 + 313.5/(1 + EXP- 0.7701*(X - 13.95))$	0.35	0.095	3.01	NS
		Mean Daily Precipitation (mm)	$Y_2 = -2.220 + 195.2/(1 + EXP- 0.1011*(X - 40.59))$	0.08	0.337	1.31	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -0.5054 + 4.529/(1 + EXP- 0.2270*(X - 72.92))$	N/A	0.713	0.47	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = -14.63 + 301/(1 + EXP- 1.647*(X - 22.79))$	0.47	0.114	3.33	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -2.174 + 333.1/(1 + EXP- 5.496*(X - 10.92))$	0.70	0.029	7.25	S
		Mean Daily Precipitation (mm)	$Y_1 = 95.01 + 177.3/(1 + EXP- 27.17*(X - 0.6288))$	N/A	0.661	0.57	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 57.04 + 261.5/(1 + EXP- 1.108*(X - 44.84))$	0.08	0.394	1.22	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 1.93 + 504/(1 + EXP- 1.055*(X - 27.39))$	0.98	<.001	156.93	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 1.4 + 120.9/(1 + EXP- 4.4*(X - 11.092))$	0.13	0.344	1.40	NS
		Mean Daily Precipitation (mm)	$Y_2 = 77.26 - 80.01/(1 + EXP- 4.941*(X - 1.50))$	N/A	0.806	0.33	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -12.08 + 4074/(1 + EXP- 0.2241*(X - 25.99))$	0.81	0.009	12.54	S

R^2 = Adjusted coefficient of multiple determination
V.R = Variance Ratio
† = Data transformed (Log_{10})
N/A = Residual variance exceeds variance of response variate
NS = Not significant (P=0.05)
* = Significant (P=0.05); ** =Highly Significant (P=0.05)

Appendice 4: Exponential $Y_1 = A + BR^X$

Where: Y_1 =Expected *T. tabaci* population, Y_2 = Expected *F. Schutzei* population, A = Constant, X = Explanatory variable, In this analysis R>1 and A+B =0, therefore, $Y_1 = BR^X$, $Y_2 = BR^X$

State	Season	Parameter	Regression equation	R^2	F. pr	V.R	P (0.05)
Tasmania	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.0028 * 1.342^X$	0.42	0.060	8.29	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 0.060 * 1.320^X$	0.37	0.068	7.50	NS
		Mean Daily Precipitation (mm)	$Y_1 = -0.8165 * 2.417E-08^X$	N/A	0.208	2.77	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = 8.165E-17 * 7.901^X$	N/A	0.208	2.77	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 8.34E-15 * 4.904^X$	0.53	0.018	9.94	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 1.204E-14 * 18.51^X$	0.48	0.024	8.68	S
		Mean Daily Precipitation (mm)	$Y_1 = - 49.5 * 0.0005^X$	N/A	0.189	2.36	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = - 49.5 * 1^X$	N/A	2.34	0.192	NS
Victoria	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.0210 * 1.178^X$	0.22	0.001	9.99	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 0.0173 * 1.486^X$	0.35	<.001	13.81	HS
		Mean Daily Precipitation (mm)	$Y_1 = 0.251 * 1.49^X$	N/A	0.092	2.76	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -0.5265 * 0.3598^X$	N/A	0.018	5.14	S
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 0.00355 * 1.2581^X$	0.59	<.001	22.29	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 0.00869 * 1.551^X$	0.75	<.001	41.04	HS
		Mean Daily Precipitation (mm)	$Y_2 = 1.191E-16 * 5169^X$	N/A	0.141	2.20	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = - 0.3193 * 0.3583^X$	N/A	0.060	3.32	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.113 * 1.1302^X$	0.26	<.001	17.98	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 0.418 * 1.188^X$	0.36	<.001	21.35	HS
		Mean Daily Precipitation (mm)	$Y_1 = 59 * 1.02^X$	N/A	0.092	3.14	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -2.208 * 0.2816^X$	N/A	0.004	10.46	S
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 0.0147 * 1.2056^X$	0.49	<.001	21.08	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 0.210 * 1.219^X$	0.30	0.002	14.30	HS
		Mean Daily Precipitation (mm)	$Y_2 = 0.51 * 2.18^X$	N/A	0.217	1.82	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -1.521 * 0.3016^X$	N/A	0.013	7.27	S
South Australia	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 1.33E-11 * 8.12^X$	0.65	<.001	15.49	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = -228 * 0.56^X$	N/A	0.237	1.67	NS
		Mean Daily Precipitation (mm)	$Y_1 = -186 * 0.073^X$	N/A	0.549	0.64	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -197.7 * 0.5447^X$	N/A	0.270	1.50	NS
	2001/2002	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 1.99E-11 * 5.72^X$	0.05	0.120	2.64	NS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 2.41E-13 * 27^X$	0.47	0.007	8.53	S
		Mean Daily Precipitation (mm)	$Y_2 = 0.000 * 4.5^X$	0.47	0.007	8.54	S
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = -2.206 * 0.5351^X$	N/A	0.254	1.58	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_1 = 0.211 * 1.316^X$	0.51	0.005	12.05	S
		Mean Daily Minimum Air Temperature (°C)	$Y_1 = 4.4 * 1.402^X$	0.47	0.007	10.89	S
		Mean Daily Precipitation (mm)	$Y_1 = 159.5 * 8.269E-13^X$	N/A	0.111	3.06	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_1 = -151.7 * 4.13E-01^X$	N/A	0.105	3.16	NS
	2002/2003	Mean Daily Maximum Air Temperature (°C)	$Y_2 = 0.000 * 2.136^X$	0.99	<.001	450.74	HS
		Mean Daily Minimum Air Temperature (°C)	$Y_2 = 1.62 * 1.388^X$	0.18	0.080	3.70	NS
		Mean Daily Precipitation (mm)	$Y_2 = -52.03 * 8.269E-13^X$	N/A	0.266	1.61	NS
		Mean Daily Relative Humidity at 3pm (%)	$Y_2 = - 49.40 * 0.3955^X$	N/A	0.260	1.64	NS

R^2 = Adjusted coefficient of multiple determination
V.R = Variance Ratio
† = Data transformed (Log_{10})
N/A = Residual variance exceeds variance of response variate
NS = Not significant (P=0.05)
* = Significant (P=0.05), ** = Highly Significant (P=0.05)