The Epidemiology and Control of Capsicum Viruses in Natal

by Krzysztof Budnik

Submitted in partial fulfilment of the requirements

for the Degree of Master of Science in the

Department of Microbiology and Plant Pathology

Faculty of Science

University of Natal

Pietermaritzburg

February 1995

Declaration

I hereby certify that, unless specifically indicated in the text, this research is the result of my own investigation.

K. Budnik

Contents

Acknowledgements

I wish to express my sincere gratitude to the following who made this thesis possible:

- Robertsons (Pty) Ltd for their financial support over the last two years of this work. In particular I wish to thank Mr. Damien Marshall and Mr. Will Wallace for their expertise which kept this project on track.
- Professor J.V. da Graça and Mr. M.D. Laing for their supervision and academic support.
- Mr. Yagambaram Gounder (Dan) for sacrificing his land, workforce, time and money, to allow many of the field trials to be carried out.
- Sunshine Seedlings nursery for providing most of the pepper plants used in the trials, free of charge.
- ProSeed cc who provided their land and several hundred of breeding lines for evaluation.
- Several postgraduate students at the Department of Microbiology and Plant Pathology who provided the necessary workforce in setting up the virus control trials.
- Brian Garman (the hairy chap in Fritz's office) for intelligent advice.
- My parents whose financial support during the "early years" provided the means to reach my current level.
- My wife, Zoë, without whose gentle PRODDING and a lot of patience this thesis may have never materialised.

Abstract

Virus diseases pose a serious threat to commercial pepper (*Capsicum annuum* L.) production in Natal. Following a survey of the principal capsicum-producing areas, potato virus Y (PVY) was found to be the predominant virus infecting peppers, often reaching 100% incidence. Currently, TSWV incidence and CMV levels are insignificant with respect to pepper crops in Natal. Thus, the diverse ecological and epidemiological factors which determine PVY infection of peppers were investigated.

The potential host range of PVY was established in a glasshouse study. Seed from solanaceous weeds commonly occurring in vegetable-producing areas of Natal was collected. Seedlings were mechanically inoculated with a pepper strain of PVY and assayed for infection using double-antibody sandwich ELISA. *Nicandra physaloides* L., *Solanum elaegnifolium* Cav., *S. nigrum* L., *S. velosum* L. and *S. aculeastrum* L. were found to be susceptible to PVY infection. In addition, a field survey of over 100 samples of commonly occurring weed species growing in or adjacent to capsicum crops in the Pietermaritzburg and South Coast regions of Natal was carried out. Several weed species were found to be naturally infected with PVY, including *Acanthospermum hispidum* DC., *Bidens pilosa* L., *N. physaloides* and *S. nigrum*.

The spread of PVY into a pepper crop on the Natal South Coast was monitored during 1993. Virus spread was rapid, with PVY first detected in pepper seedling one week after planting, suggesting a nearby source of the virus. A survey of the wild vegetation prior to planting of the crop, revealed that populations of *N. physaloides* may be the primary sources of PVY infecting the crop. Large virus-infected *S. nigrum* populations appeared later in the season, suggesting its role in maintaining high levels of PVY during periods when no pepper cultivation takes place.

In addition to identifying possible virus reservoirs, several virus control measures were investigated, demonstrating ways of avoiding or minimising infection. The effects of insecticides, oil sprays (Virol), insect repellents (Azatin™), yellow polyethylene traps and plastic mulches on virus incidence within peppers were evaluated in field experiments.

Results of weekly sprays of the insecticide mercaptothion at 5%, increased virus incidence in peppers by 15% when compared to the untreated control. Similarly, the effects of insecticide applications on pepper yields and quality were negative. Results of applications of Virol at 1% and Azatin™ at 1.5% did not differ from those of the unsprayed control. Mulching was most effective by reducing virus incidence in treated plots by 50% and resulted in a yield increase of 62% and a 40% increase in fruit quality. The use of yellow sticky traps reduced virus incidence by 35%, with a yield increase of 25% and a 24% improvement in fruit quality, when compared to the untreated control. Both mulching and the use of yellow sticky traps reduced the number of aphids trapped within the plots.

In order to assist the development of capsicum cultivars resistant to PVY infections, a screening method was developed to determine susceptibility levels of a breeding population. Two rating procedures were investigated based on disease severity of the whole plant and on the fruit (chilli pods). The technique was effective in detecting small incremental increases in susceptibility within a breeding population, provided that an adequate positive selection pressure is applied. Using this technique breeders may be able to define a large breeding population to those parents exhibiting a genetic base most suitable for resistance development and eliminate those which exhibit low frequencies of resistance genes.

Based on the results obtained, an integrated virus management strategy is suggested, including the elimination of virus sources and the use of cultural practices which facilitate a reduction in virus spread.

CHAPTER 1

Plant Virus Epidemiology - A literature review

1.1 Introduction

... there have been epidemics of plant virus diseases only because we have taken into cultivation a lot of annuals and other plants that have no record of ecological dominance in nature.

J.E. Vanderplank (1963)

The extensive cultivation of annuals has introduced a basic element of instability in agriculture. This has been further enhanced by the impact of breeding policies and many of the cultural practices introduced to increase yields or extend cropping areas and growing seasons (Thresh, 1982). Thus, virus disease epidemics are a recurring feature of many annual crops that can be almost totally infected within a few weeks of sowing. Control is often difficult to achieve, and in some regions, plant viruses largely determine which crops or varieties can be grown successfully and where or when they can be produced.

Plant pathologists have responded to this situation in different ways, and an important development has been the attempt to explain the underlying causes of major epidemics (Thresh,1983). There has been much emphasis on the frequency of epidemics in crops compared with natural vegetation and this has led scientists in various disciplines to the view that disease epidemics are largely the result of human interference in the "balance of nature" (Harlan, 1976).

The various ways in which plants avoid serious virus disease can be expressed diagramatically (**Figure 1.1.1**). The main possibilities are evasion, some form of inherent ability to tolerate infection, or the ability to prevent infection through resistance to the virus and/or its vector(s) (Thresh, 1982). Each feature can be effective alone or when combined with others, but the situation is in a state of flux. Escape is unlikely to be effective in seasons when spread is unusually rapid or when the host population persists longer than usual or exceeds a critical density. Similarly, levels of resistance or tolerance may be inadequate if there is an increase in host abundance or increase in virulence of the virus, or if

the environmental conditions become highly favourable for disease spread. Thus many cropping practices, such as crop stand uniformity and the trend to monoculture, extended growing seasons, crop sequences and the introduction of crops to new areas, can be interpreted as having an effect on the balance between host and pathogen.

The plant pathologist's principal objective is to devise disease management systems such that crop stands acquire some of the stability and resistance of natural vegetation. Since this is unlikely to be achieved by reverting to outmoded cropping systems or by simulating the enormous diversity found in nature and in primitive agriculture, it is necessary to understand the ecology and epidemiology of the causal virus and to determine the infection route between the virus and the host (Tomlinson, 1987). This necessitates the identification of the virus, a knowledge of how the virus survives, and is introduced into the crop and an understanding of the life cycles, and disease transmission roles of vectors. Only then can the weakest link in the chain of events that results in infection be modified or prevented.

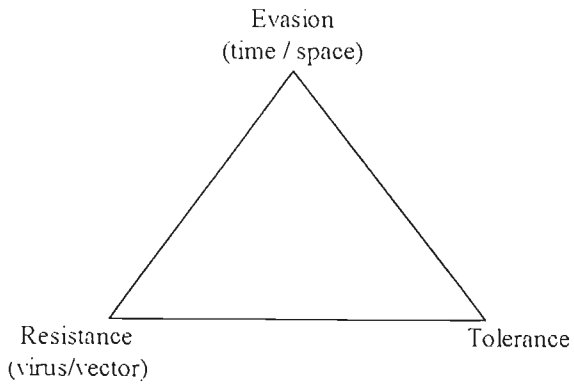

Figure 1.1.1 Diagrammatic representation of the ways in which plants avoid the harmful effects of plant viruses.

1.2 The Ecological Model

1.2.1 The Systems Theory

Systems theory probably began in Germany with the concept of ***gestalt***, which means ***an organised whole*** (holistic). Gestalt theory treats a system as an organised whole that has properties additional to, and different from, those of its components considered separately (Robinson, 1987). The systems theory postulates that inquires should proceed from the top down, rather than from the bottom up. For example, viewing separate pieces of a picture

puzzle would reveal little. An inquiry from the top down, however, would examine the whole picture first. Only then should individual pieces be examined to determine how this overall or holistic effect was achieved.

One of the conclusions associated with the systems concept is that the fundamental structure of science is the ***pattern***. A pattern is an arrangement of units just as a word is a pattern of letters. A system is hierarchy of patterns of patterns, and each pattern of patterns is called a systems level. A book is a system. At the top systems level, a book is a pattern of subsystems called chapters. At the next system level, each chapter is a pattern of secondary subsystems called paragraphs. Each of these is a pattern of tertiary subsystems called sentences, and so on down. In the sense of gestalt, the book as a whole has properties that are additional to, and different from, those of its components (Robinson, 1987).

The concept of systems levels is particularly important in the study of biologic systems, such as ecosystems and pathosystems. A clear recognition of differences in systems levels greatly facilitates our comprehension of complex systems.

1.2.2 Pathosystems

An ***ecosystem*** is the interaction of all biotic factors (i.e., living organisms) and abiotic factors (i.e., climate, topography, geology) within a defined area (Robinson, 1987). Increasingly, crops are being studied as ecosystems. A ***pathosystem*** is a subsystem of an ecosystem and is defined by the phenomenon of parasitism (Robinson, 1987). A plant pathosystem is one in which the host is a plant. The parasite may be an insect, mite, nematode, bacterium, fungus or virus.

There are three categories of plant pathosystem (Robinson, 1987):

The Wild Plant Pathosystem

A wild plant pathosystem is one in which people are not involved. It is a subsystem of a natural ecosystem and is in a state of dynamic equilibrium and natural systems balance. It is also a stable system. There are three fundamental components in a wild pathosystem: the host population, the parasite population, and the environment. Each apex of a two-dimensional triangle represents one of these components, and each side of the triangle

represents the interaction between two of the components (**Figure 1.1.1**). Because all three components are considered to be of equal importance, the triangle is equilateral.

The Crop Pathosystem

The crop pathosystem is derived from the wild pathosystem but it differs fundamentally because of a fourth component, people, who have changed the other three components (**Figure 1.2.1**). The crop pathosystem has lost many of the self-stabilising properties of the wild pathosystem. It is often unstable and the losses caused by parasites can be high.

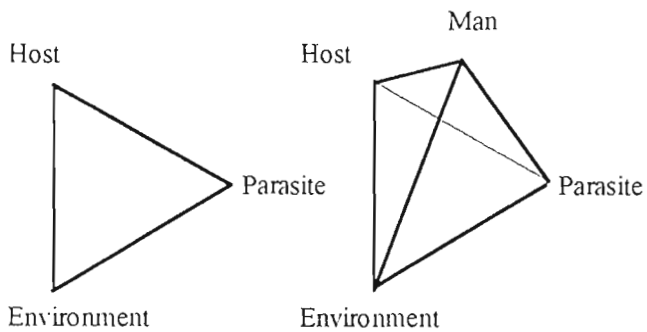

Figure 1.2.1 The wild plant pathosystem triangle and the crop pathosystem tetrahedron.

The Weed Pathosystem

The weed pathosystem is also a result of agricultural activities. Weeds are considered to be a component of the crop pathosysem when they serve as alternative hosts of crop parasites, or harbour vectors of crop parasites.

Vector Pathosystems

The above plant pathosystems include the possibility of vector pathosystems that involve a third species, the vector, in addition to the host and parasite. The vector is any organism that carries the parasite to the host.

1.2.3 The Virus Pathosystem

A plant pathosystem where the parasite is a virus, is known as a ***virus pathosystem***. To survive, a virus must have plants in which to multiply and persist, and a means of spreading from infected to healthy plants, via ***vectors***. The virus pathosystem is a group of complex interactions which include the vector pathosystem. The vector element compounds the complexity of the relationship between pathogen, host, environment and man (see **Figure**

1.2.1) by demanding an understanding of the interactions between the pathogen and its vectors and between the vectors and the host plant of the virus (Raccah and Irwin, 1988). Plants themselves, including wild ones, may "actively" participate in the vector pathosystem through contact or by their natural propagation material including true seeds. Man himself has been actively engaged in spreading viruses with crop propagation material and in germplasm.

Furthermore, in the virus pathosystem, the hosts are differentiated into "target" crops and sources of infection. The ***crops*** have to be protected from ***viruses***. Viruses may require completely different ways of control, since not only do they differ in form and size but also in ecological relationships. Besides the vectors, crops and viruses are the ***sources of infection***. These may be:

(1) similar crops, or closely or remotely related crops,

(2) individual plants within the crop to be protected, introduced by infected seed or planting stock, or

(3) uncultivated plants in or near the crop, or far away (Bos, 1981).

Moreover, the ***biological environment***, including sources of infection and the vectors or other means of spread, is distinguished from the ***physical environment*** of soil, water and climate (Bos, 1983). Growing conditions greatly influence susceptibility and sensitivity of crops and wild plants to virus infection, as well as vector behaviour. ***Crop micro-climate*** is considerably affected by the presence of wild plants within the crop territory (Bos, 1981).

Figure 1.2.2 summarises the groups of factors involved in virus ecology and displays the dynamic interrelationships within the virus pathosystem as developing with time. An appreciation of the virus pathosystem lies not in understanding all the properties of a virus which favour survival and spread but mainly those which guarantee survival from one year to the next (Tomlinson, 1987). From information on the ecology of a particular virus, epidemiological models can be constructed from which control strategies can be considered.

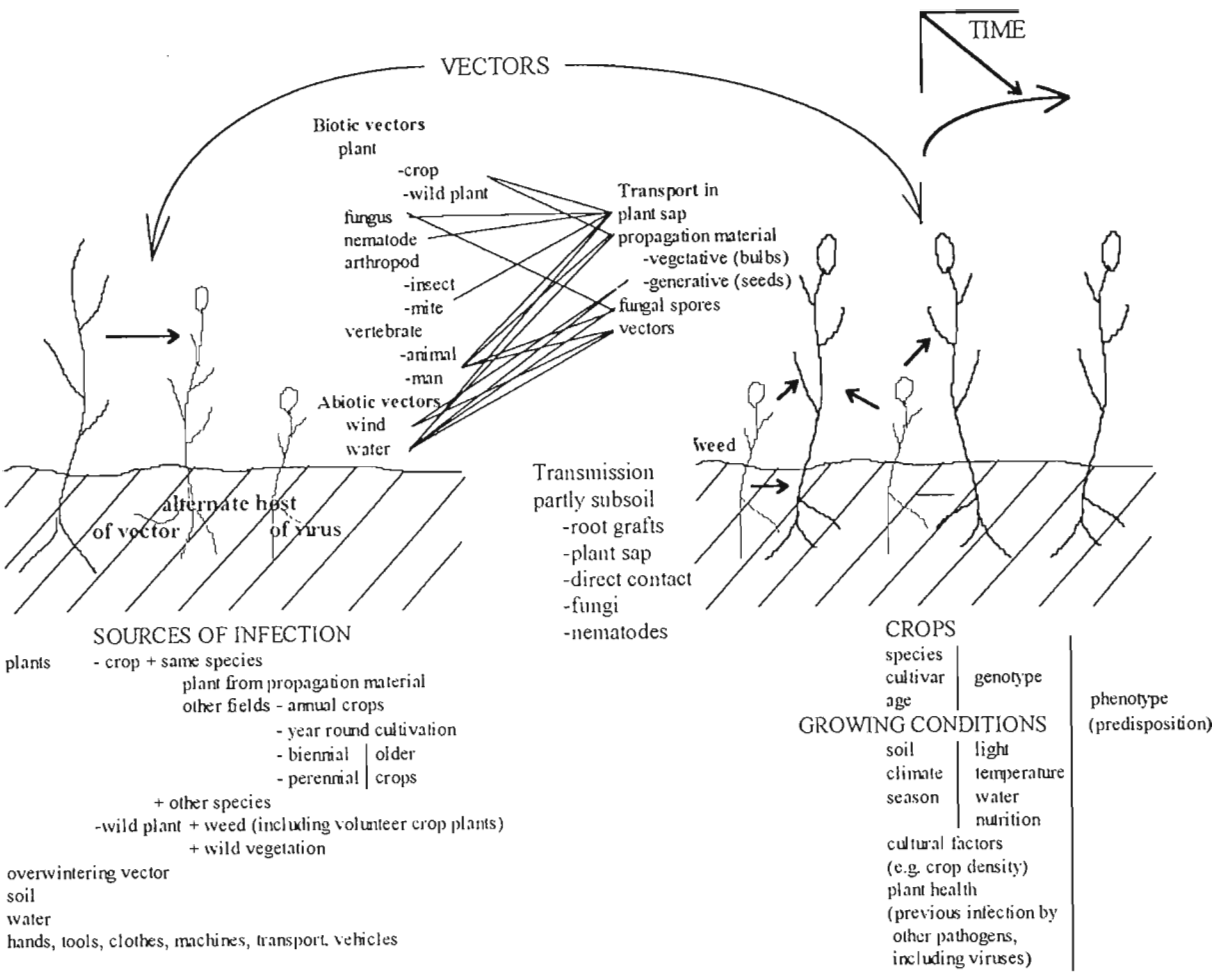

Figure 1.2.2 A generic scheme of the virus pathosystem, of the groups of factors involved, and of their interrelationships. After Bos (1981).

1.3 Plant Pathosystem Management

The function of pathosystem analysis is to understand pathosystems in order to manage them. And the function of pathosystem management is to stabilise the pathosystem and to minimise the losses caused by crop parasites. Each of the four main components of the crop pathosystem as described by Robinson (1987) (see **Figure 1.2.1**), constitutes a separate aspect of crop pathosystem management (**Figure 1.3.1**).

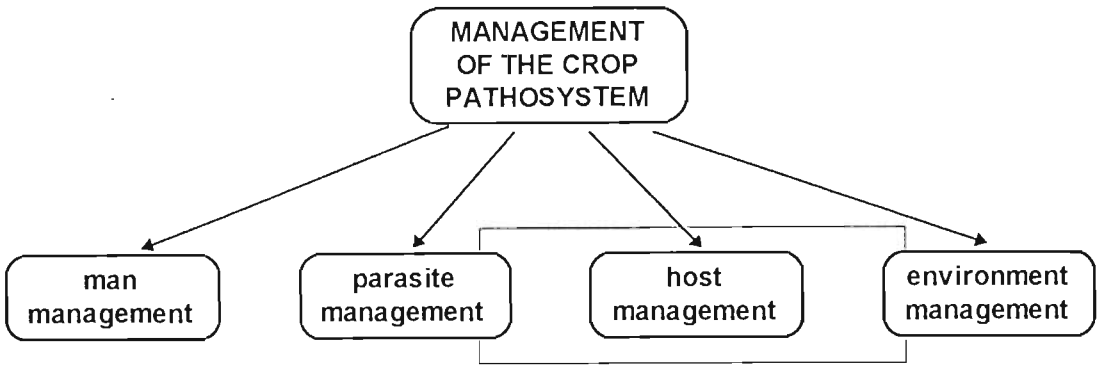

Figure 1.3.1 Pathosystem management. Management of a crop pathosystem involves the four components of the crop pathosystem tetrahedron.

Man management involves education in the form of extension work to improve farmers' expertise. ***Environment management*** involves practices such as irrigation, the use of fertilisers, cultivation, and weeding. ***Parasite management*** consists of a direct assault on the parasite itself. This usually means studying the life cycle of the parasite and then hitting it hard at its most vulnerable stage, usually through the use of insecticides or fungicides. ***Host management*** has two components. The first is non-genetic and concerns various crop management practices such as crop rotation. The second component is genetic and involves plant breeding for resistance to crop parasites.

Traditionally, the management of the crop pathosystem has consisted almost exclusively of parasite management. It is a control which is neither complete nor perfect. As a result, the losses caused by crop parasites are often high and the crop pathosystem is frequently an unstable system (Robinson, 1987).

Five major drawbacks to current parasite management have been identified by Robinson (1987). The first drawback is that the effects of parasite management are short-lived. Second, many of the tools of parasite management appear to be within the capacity for microevolutionary change of the parasite. Simply put, the parasite produces a new strain that is unaffected by that tool. A third and very real problem is expense, which adds considerably to the cost of production. The fourth drawback is related to man management and concerns the need for considerable technical expertise on part of the farmer. Lastly,

some parasite management tools may be environmentally hazardous, or dangerous to human health, or both.

De Ponti (1983) has made the useful observation that most parasite management methods aim at increasing the death rate of the parasite. Host management methods aim at decreasing the birth rate of the parasite. The latter is the more sensible approach, and with major improvements, parasite management may even become unnecessary.

Because chemotherapy of virus diseases is not yet possible, management of a virus pathosystem has to rely on mechanisms other than parasite management by use of chemical pesticides. Management of the virus pathosystem therefore has to be achieved through host, man, environment and vector management systems (**Figure 1.3.2**). Vector management is particularly important since it may be related to man, host and environment management elements. Host management includes the management of sources of infection, in addition to crop management (genetic and non-genetic).

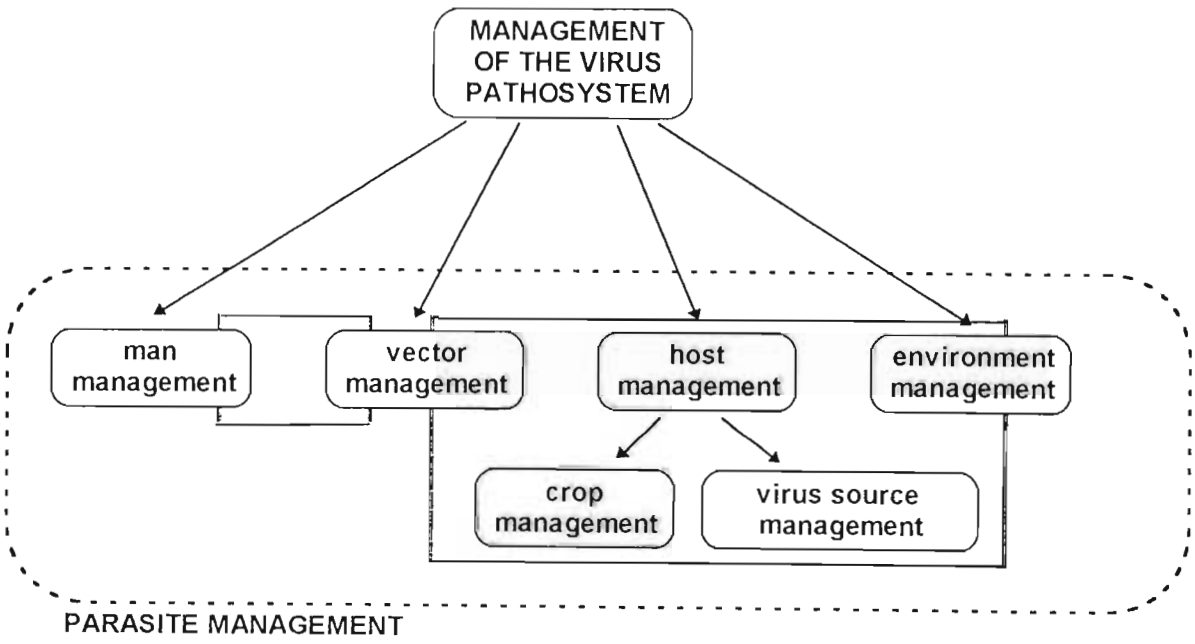

Figure 1.3.2 Virus pathosystem management. Parasite management is achieved through the integrated management of all components.

1.4 Host Management

1.4.1 Crop Management

Disease patterns are to a great extent a product of our plant-breeding and agricultural practices (Simmonds, 1962). The crop plants of the world are a diverse assemblage of species that have been developed for various reasons and at different times and places from a range of habitats with markedly contrasting climate and vegetation (Harlan, 1981). Many such plants are herbaceous annuals which are not good competitors in nature and are seldom prominent in closed communities (Hawkes, 1969). As a result, pests and pathogens which have evolved the ability to survive in sparsely distributed stands of annual plants in natural habitats, thrive and cause severe damage to such plants when grown widely as crops (Thresh, 1982). Crop management aims to prevent the damage caused by pathogens rather than control them (Robinson, 1987), via resistance breeding strategies or cultural methods. Essentially, crop management can be divided into genetic and non-genetic management.

(a) Genetic management

Damaging epidemics of virus disease can occur only if many plants are infected and they are seriously affected. Thus the overall susceptibility and sensitivity of host populations have a crucial influence on the losses sustained (Thresh, 1982). These can be enhanced or avoided by growers according to their choice of variety. Since the dawn of agriculture, farmers have selected their crops to increase yield and thereby have often selected those that escape disease or suffer minimal effects from it (Gibbs *et al.*, 1986). Since the acquisition of scientific knowledge about breeding techniques, resistant varieties have been widely used, when available.

Russel (1978), distinguished six different kinds of resistance:

Immunity - it is generally the case when no infection is possible.

Resistance to virus infection - plants are susceptible but show a propensity to escape from virus infection.

Resistance to virus spread - plants show infection which is generally limited to a few cells surrounding the entry point of the virus, e.g. hypersensitivity.

Resistance to virus multiplication - only a low concentration of virus particles occurs in the infected plant.

Tolerance - there are several kinds of tolerance. Most commonly, there is either no apparent symptom or no yield decrease despite a high virus titre.

Resistance to the vector - three kinds can be distinguished:

non-preference of the vector for the host plant;

antibiosis or decreased vector growth and multiplication on the host, and;

host plant tolerance to mild infestation by the vector.

The aim is to introduce one or several of these resistances into a selected variety to protect it against the main virus or viruses which might infect it; and as far as possible, these introduced resistances must be durable (Quiot *et al.*, 1982).

An important first step is to determine if the crop/virus system being studied has coevolved or if it is the result of a recent encounter (Buddenhagen, 1977). This requires consideration of the origins and spread of crops and of their viruses and vectors. Many crop virus diseases are the result of new encounters arising from man's exploration and colonisation (Buddenhagen, 1983). Coevolution or recent encounter in genetic crop management is crucial in considering strategies for resistance breeding. For coevolved systems the centre of origin can be expected to be a source of coevolved genes for vertical resistance to vector and/or virus. Genes for tolerance are also likely to occur at the centre of origin, which should slow epidemics and reduce the severity of infection (Buddenhagen, 1981). It should therefore be useful to search for individuals with these characteristics in the regions where coevolution occurred.

For non-coevolved systems it is likely that resistance will be found in material genetically quite different from that in the affected area. Such "accidental" resistance may act vertically or horizontally, but one would not expect such resistance to break down, even if the hosts are immune (Buddenhagen, 1983). This is because no strains and no gene-for-gene relationships have evolved for the crop/virus system.

One might expect that vertical resistance of plants to viruses is unlikely to be durable in the field. In practice, however, much vertical resistance to viruses has usually been stable, even

even when controlled by only one or two genes, such as those for hypersensitivity of tobacco (*Nicotiana* sp.) to tobacco mosaic virus, and immunity of raspberry (*Rubus* sp.) to tomato black ring virus (**Table 1.4.1**) (Harrison, 1981). In several instances, resistance-breaking strains of viruses are known but are not prevalent, suggesting a lack of fitness. A new strain of raspberry ringspot virus in Scotland virulent against newly developed resistant cultivars has characteristics which militate against its survival (Harrison, 1981). Similarly, strains of potato virus X which cause systemic mottling in a resistant potato (*Solanum tuberosum* L. cv. King Edward) are easily controllable in crops (Harrison, 1978).. The evolution and selection *in situ* of resistance-breaking virus strains does not appear to be common, possibly because the virus has so few genes, and these genes are so finely tuned for survival, that any change reduces fitness (Buddenhagen, 1983).

Table 1.4.1 Examples of stable resistance of crop plants to viruses

Host Plant	Virus	Genetic Control	Resistance-breaking strain known
Tobacco	Tobacco mosaic	Gene N	No
Raspberry	Tomato black ring	Gene I_{tb}	No
Potato	Potato X	Gene N_x	Yes
Raspberry	Raspberry ringspot	Gene I_{rr}	Yes

With plant pathogens the epidemiological cycle can be broken before the epidemic or early or late in the epidemic, and the pre-epidemic stage is most vulnerable to attack. With viruses of annual crops this may mean consideration of alternate weed hosts, and development of resistance in the crop host should then take into account the strains and vectors surviving in the weeds. It is probable that not only immunity but also all aspects of resistance to vectors, including non-preference, as well as resistance to virus infection, decreased susceptibility to virus invasion and increased tolerance, can be useful (Buddenhagen, 1983). If the virus is transmitted by the vector to its progeny and no weed hosts occur, then resistance to insects, non-preference and resistance to infection should become the primary targets of resistance breeding. If viruses survive through seed, then breeding for non-seed-transmissibility becomes a logical and simple target.

In recent years, as genetic engineering evolved as a tool for introducing genes into agronomically important plants and thus confer novel phenotypes, genetically engineered virus resistance became an option. A number of plant virus nucleic acid sequences, including those encoding virus coat proteins, have been found to be especially useful in the development of virus-resistant plants (Beachy *et al.*, 1990). Resistance conferred by the expression of coat protein genes, ***coat protein-mediated resistance***, has been described for plant viruses in seven different virus groups. Although the actual mechanism of coat protein-mediated protection is unknown, many laboratories around the world are engineering transgenic plants to express the coat protein of one or many different viruses in the hope that plants will exhibit cross-protection against subsequent virus infection in the field (de Zoeten, 1991).

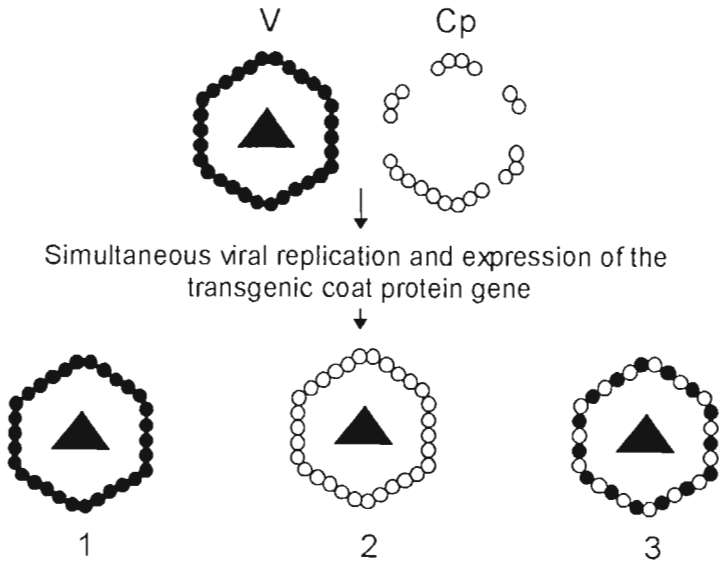

Figure 1.4.1 Possible combinations of viral RNA and capsid proteins in the progeny virus in a virus-infected coat-protein-transgenic plant. Shown are the infecting virus (V) and its identical offspring (1), the coat protein (Cp) for which the plant is transgenic, and the possible capsid combinations when transcapsidation (2) or phenotypic mixing (3) occurs (After Rochow, 1977).

However, when we take into consideration that the coat protein of several important plant viruses plays a role in determining virus transmissibility by insect vectors, two replicative

phenomena of plant viruses need to be considered, which could lead to a creation of an apparently "new" disease (**Figure 1.4.1**). ***Transcapsidation*** of virus RNAs in mixed infections occurs in nature and has led to altered virus/vector specificities (Creamer and Falk, 1990), and ***template switching*** or recombination of viral RNAs giving rise to new viruses with altered vectors and host ranges and new combinations of genes (Bujarski and Kaesberg, 1986). Although it is quite obvious that plants expressing viral coat protein are exhibiting potentially agronomically useful levels of disease resistance, the problems which may be created which will negate these developments (de Zoeten, 1991).

(b)Non-genetic management

To control diseases, practice careful seed selection and crop rotation and do not plant when the moon is full or the sun has a halo,...

Ayamara Indians of Peru (Thurston, 1990)

In the frequent event when virus-resistant crop varieties are unavailable, non-genetic crop management strategies have to be adopted. Often, these management strategies have been used for generations by traditional farmers and are only now appreciated as having a role in virus disease management (Thurston, 1990).

Parasite escape involves cultivation of the crop at a time when the parasite population is largely inactive (Robinson, 1987). Differences in planting date can have large effects on the spread of viruses in several crops. Almeida and Corso (1991), for example, found that by delaying the sowing time of soybean (*Glycine max* Merr.) crops it was possible to drastically reduce the incidence of tobacco streak virus.

Crop rotation with non-susceptible plants to reduce build-up of inoculum source and/or vectors has been used with good effect by Cho *et al.* (1989) to manage tomato spotted wilt virus (TSWV) of several vegetable crops. By reducing cultivation growers reduced losses caused by TSWV from 60-65 % to 10%.

Strip intercropping is being re-examined as insect resistance to pesticides and general public sensitivity to toxic pesticide use increases (Chaney, 1993). Essentially it consists of cultivating the crop in-between strips of, for example, trap plants which attract aphids or

insectary plants which attract beneficial insects. In addition, crop biodiversity is increased, thereby shifting the imbalance within the crop pathosystem back towards an equilibrium.

Raised fields, raised beds, ridges, and mounds were used widely for millennia by traditional farmers. Planting in soil raised above the surrounding area was a significant disease management practice, especially for soil-borne pathogens (Thurston, 1990).

Crop placement to avoid planting susceptible crops adjacent to each other has been used successfully by farmers to reduce losses caused by TSWV by as much as 80% (Cho *et al.*, 1989).

Cross protection is a mechanism which consists of infecting a plant with a mild virus strain to protect it from the severe effects of a second, related virus. Cross protection has been used on a large scale to control citrus tristeza virus (CTV). In Brazil, the number of protected sweet orange trees (*Citrus sinensis* (L.) Osbeck.) exceeded 8 million in 1980, and no breakdown in protection was observed (Costa and Muller, 1980). Similarly, control of papaya ringspot virus in Taiwan has been achieved by cross protection (Yeh *et al.*, 1988).

Planting virus-free seed or propagative organs can significantly reduce virus disease. The use of highly specific serological tests permits the detection of seed borne viruses. If the main source of the virus is the seed, efficient virus control can be achieved through the use of healthy seed (Quiot *et al.*, 1982). When a vegetatively propagated species is susceptible to a given virus, progressive infection of the whole species is generally observed, unless sanitary selection measures are used. The use of virus free clones is necessary to achieve control (Quiot *et al.*, 1982).

Successful implementation of many of the abovementioned management practices, however, hinges on two factors:

(1) Management is not totally effective if virus and vector occurrence is high throughout an area. During these periods, it makes little sense to continue planting susceptible crops.

(2) Areawide co-operation of growers is essential, particularly when dealing with insect-borne viruses due to their substantial movements. Growers must control alternative hosts

of the virus and vector, use virus free seedlings, avoid sequential planting and plough harvested and abandoned crops immediately.

1.4.2 Virus Source Management

Virus sources can be located in the crop itself, when the virus is seedborne, or in surrounding areas, if weeds, volunteers or other plants are infected.

(a) Seed

Although the use of virus-free seeds has already been discussed as a crop management strategy, the fact that approximately 20% of the known viruses are seed-transmitted (Tomlinson, 1987) warrants further discussion. Seed-borne virus diseases, which may subsequently be transmitted in the crop by aphids, beetles or nematodes, can be the major initial source of infection (Mandahar, 1981), thus establishing randomly distributed infection foci throughout the crop.

Two kinds of seed transmission can be distinguished (Bennett, 1969). Some viruses are retained at the surface of the seed or in parts thereof besides the embryo, in which cases a mechanism allowing for infection of the new seedling at germination is necessary. Other viruses infect the embryo inside the seed directly, and a young plant issued from an infected seed is always diseased. In the latter case, every virus infected plant detected in the field will correspond to an infected seed.

For some viruses such as tobacco mosaic virus in tomatoes (*Lycopersicum esculentum* Mill.), which are carried on the surface of the seed, disinfection with chemicals (e.g. sodium phosphate, sodium hypochlorite, hydrochloric acid, etc.) can significantly reduce the rate of infected seeds (Bennett, 1969). Heat therapy can sometimes eliminate virus without affecting germination. Fletcher *et al.* (1969) observed that cucumber green mottle virus can be eliminated from seeds by heat treatment at 70° C for more than one day.

Control of viruses which infect the seed embryo is more difficult. The test of commercialised seeds for virus transmission presents some technical problems: what rate of infected seed may be acceptable risk for the grower, how to separate with accuracy the

good and bad seed lots, which test must be used, what size sample must be analysed, and so on (Quiot *et al.*, 1982). In addition, traditional farmers are used to producing their own seed without virus testing.

Thus, widespread seed certification programmes and grower education is the only solution to efficient seed-borne virus management. Early removal of infected plants (roguing) can sometimes be effective, by decreasing the numbers of virus sources, thus slowing virus spread. This is effective particularly for monocyclic viruses or polycyclic viruses with low rates of disease increase.

(b) Cultivated plants

Similar or closely related crops often serve as the main virus source. For example, in the USA and Europe, infected mature crops of sugar beet (*Beta vulgaris* L.), including sugar beet seed crops, are probably the most important source of the two yellows viruses (beet yellows and beet western yellows viruses) for newly planted beet crops (Tomlinson, 1987). Other important virus reservoirs are infected plant debris, roots and volunteer plants carried over from the previous season. Improved cultural practices (e.g. immediate ploughing after crop harvest) and awareness of virus host range assist in managing virus disease.

(c) Weeds and non-cultivated plants

The importance of weeds in providing foci of infection for the subsequent spread of viruses within crops was probably first established with cucumber mosaic virus (CMV) in cucurbit crops (Doolittle and Walker, 1925). In many spring plantings, the first infected plants occur in scattered groups around infected overwintered plants of the perennial mayweed (*Asclepias media* L.) which regenerated between the rows of plants. In years since, an ever increasing number of species have been recognised as potential hosts for plant viruses. Douine *et al.* (1979 cited by Green and Kim, 1991) reported 775 species belonging to 86 families as susceptible to CMV.

Weeds and wild plants also act in ways other than as sources of infection of the virus. They have a much more diverse impact on the virus pathosystem (Bos, 1981). Wild plants allow or greatly assist in virus survival through adverse environmental periods. They may play a

role in virus spread from one field to another and even to geographically distant areas, and in virus establishment after introduction into new regions. They may also indirectly act as refuges and sources of virus vectors, even if they are exempt from infection by the viruses concerned.

Virus survival. Several crops are short lived and absent from the field during winter or dry summer. They may also be absent for long periods of time in crop rotations, and intervening crops may be immune. In such cases, wild alternative hosts may be essential for virus survival (overwintering, oversummering, perennation) (Bos, 1981). Several weeds in crops and around fields may be short lived as well, but may have growing periods that overlap those of crops and may play a critical role as bridging hosts between crops.

Virus spread. Where viruses pass through seed to the offspring of infected plants, virus spread in weed seeds, for instance by wind, can do much to spread virus locally, from region to region, or even country to country (Bos, 1981). For example, in many vegetable crops CMV is not seed transmissible. However, in chickweed (*Stellaria media* (L.) Gyr.) it is seed transmitted and because of its large number of seeds, it probably contributes to outbreaks of CMV in vegetable crops where *S. media* is a common weed (Tomlinson, 1987).

Virus establishment. Even the strictest international quarantine cannot absolutely prevent introduction of new viruses through commercial propagation material (Bos, 1981). A diverse natural vegetation at the site of introduction greatly enhances the chances of newly introduced alien viruses or virus strains becoming established. Once a virus has been introduced, there may be a slow build-up of infection in the wild vegetation, where the virus may remain hidden. Simons (1959) presented an example of the establishment of potato virus Y in wild vegetation with the advent of commercial potato production. Peppers (*Capsicum annuum* L.) and tomatoes were severely damaged in those areas where potatoes were grown in previous years.

Refuges and sources of virus vectors. Besides harbouring crop viruses and other pathogens, wild plants act as important refuges and sources of insects, mites, nematodes,

and fungi that may directly damage crops and may be essential in the ecology of vectors (Bos, 1981). Certain wild species may be indispensable to a vector as its alternative host. Even though itself not susceptible to infection by the virus, such a species is then an essential intermediary in the virus pathosystem.

Wild plants are now recognised as important sources of crop viruses and of vectors of viruses (Bruckart and Lorbeer, 1976; Conti *et al.*, 1979; Cho *et al.*, 1986; Stobbs *et al.*, 1992) Their removal eliminates sources of infection, reduces virus spread in seeds (if seed transmitted) and prevents vectors from breeding on them. Simons (1959) found that weed elimination in an area in Florida, USA, before planting peppers was more effective than insecticides on the crop itself, provided all growers in the region co-operated in weed control.

However, virus infection in wild hosts is often symptomless, and the role of weeds may remain obscure but for the ultimate effect of infection in the crop. Such sources may be hard to detect and require comprehensive studies within an area.

1.5 Vector Management

1.5.1 Man As Vector

The ease of spread of very stable viruses such as tobacco mosaic virus (TMV) on clothes, hands and tools is well known. TMV was the only virus prevalent in pepper crops planted in plastic tunnels, where spread by contact was facilitated by frequent handling and the use of the same propagative sites for successive plantings (Conti and Masenga, 1977). Sterilisation of farming tools and minimal handling of plants are sufficient to limit spread of mechanically transmitted viruses.

Much more important and far-reaching is the involvement of man as a vector through traffic in plant propagules (Bos, 1983). To illustrate this, Kahn and Monroe (1970) held 551 samples of imported vegetative wild and cultivated *Solanum* spp. in quarantine at the US Department of Agriculture's Plant Introduction Station, and tested them over a period of

ten years. Of the 445 samples of cultivated material, 73% proved to be infected with virus and of the 106 samples of wild material, 39%. Thus 66% of the imported specimens were reported to contain viruses, and only one third showed virus symptoms. Besides the risk of spreading a great number of viruses, vectors and host weeds world-wide, the growing development of international exchanges raises the possibility of new strains occurring by the mechanism of pseudo-recombination, in the case of viruses having multipartide genomes (Quiot *et al.*, 1982).

Inspection at import and export, especially of propagation material, is an important means of reducing the international dissemination of viruses and other harmful organisms. By international agreement, emphasis is on certification by the plant protection service of the exporting country (Bos, 1983). This mainly involves field inspection during the growing season, but is always supplemented by bulk inspection at export. Such systems are not foolproof for viruses, however, and should not substitute fully for detailed tests on arrival or quarantine.

1.5.2 Arthropod Transmission of Plant Viruses

Transmission of plant viruses by arthropods is a complex phenomenon involving interactions between plant and virus, plant and vector, virus and vector, and plant and virus and vector (Maramorosch and Harris, 1981). Arthropod vectors can transmit viruses in a nonpersistent, persistent, or semipersistent manner (**Table 1.5.1**). The type of transmission greatly influences the patterns of virus spread that occur and the tactics that are effective in managing virus spread in crop plants (Kennedy, 1986). Aphids and leafhoppers are capable of spreading viruses over long distances, but the distance a particular virus is spread from a source is related to both the dispersal of the vector and the persistence of the virus.

Table 1.5.1 Characteristics of nonpersistent, persistent and semipersistent transmission of plant viruses.

Category	Characteristics
Nonpersistent	Virus acquired in seconds
	No latent period in vector
	Virus inoculated in seconds
	Retained for minutes to hours
	Noncirculative in the vector
Persistent	Virus acquired in minutes
	Latent period in vector
	Virus inoculated in minutes
	Retained through moult
	Circulative
	Some replicate in vector; most do not
Semipersistent	Virus acquired in minutes
	No latent period in vector
	Inoculated in minutes
	Retained for hours to days
	Noncirculative in vector

Although aphids can disperse over large distances (Taylor, 1979) on wind systems, nonpersistently transmitted viruses are retained by the vector for a limited period of time and thus the distance over which they are spread from particular foci is generally limited (Thresh, 1976). In contrast, persistently transmitted viruses are retained by their vectors for long periods, often the life of the vector. They can be successfully carried as far as the vector disperses. In addition, by virtue of the processes involved in nonpersistent transmission (Harris, 1977) and the manner in which aphids select their host plants (brief probes following random landing on hosts and nonhosts alike), the nonpersistent viruses are not restricted in their host range to plants colonised by their vectors (Irwin and Goodman, 1981). In contrast, persistently transmitted viruses require prolonged probes for acquisition and inoculation, and are generally restricted in host range to plants within the host range of their vectors (Kennedy, 1986).

A chain of events must occur for a plant pathogenic virus to infect a host plant. In simplified terms, the following sequence seems reasonable (Irwin and Ruesink, 1986):

1) A virus source exists (usually an infected plant, but may be an overwintering vector);

2) the vector comes in contact with the virus infected source plant;

3) the vector probes or feeds on the source plant;

4) the vector acquires the virus from the source;

5) the vector moves to a non-infected plant;

6) the vector inoculates the plant (through probing, feeding, or some other fashion); and

7) the plant becomes infected.

Each of these steps is essential for spread. The relationships between virus and host are manifested in Steps 1) and 7). Those relationships between virus and vector mainly involve Steps 4), 5), and 7), and those between vector and virus host are manifested in Steps 2), 3), 6), and 7). Further factors affecting spread, are environmental conditions which impinge on the above parameters (Raccah and Irwin, 1988). Most important environmental effects are on vector activity. For aerial vectors, the crucial factors are temperature, wind speed and direction, and degree of air turbulence (Harrison, 1981). Simons and Eastop (1970) indicated that at low temperature (10° C), aphids could acquire, but not inoculate, virus. Similarly, the effect of temperature increase was more pronounced on inoculation than on acquisition. In Florida, a primary infection of watermelon mosaic virus 2 was recorded upwind from non-infected plants (Alderz, 1978). Shivanathan, (1983) found that wind often initiated whitefly movements therefore facilitating disease spread within chilli peppers, tomato, and other vegetable diseases in Sri Lanka.

The intimate understanding of virus-vector-plant relationships, the virus transmission process, and the epidemiology of arthropod-borne viruses can contribute to the development of sound conceptual basis for approaching vector management. Crop resistance to vectors offers promise of limiting the spread of plant viruses, especially in the case of those that are persistently transmitted (Kennedy, 1986). It can suppress virus spread by reducing the vector population or by reducing the frequency of vector-plant contacts. There are various other procedures for reducing the spread of arthropod transmitted plant viruses. These include, for example, the use of insecticides to reduce vector populations,

reflective mulches to repel aphids, oil sprays to interfere with inoculation, and repellent sprays. These are discussed in detail in Chapter 4.

1.5.3 Aphids as virus vectors

The disease distribution potential of the aerial migrant aphid is formidable. Aphids combine seemingly simple search behaviour with enough reproductive capacity to saturate large areas of terrain by their wind-borne host finding flights (Taylor, 1986). This attribute combined with their ability to transmit nonpersistent viruses, the largest group of plant viruses with over 100 viruses included (Harris, 1983), warrants further discussion.

Nonpersistent viruses have several characteristics which affect their epidemiology. They are acquired and inoculated in a relatively short time and retained by their vectors for a short time, generally not exceeding a few hours (Pirone and Harris, 1977). The specificity of these viruses for aphid vectors is low, and many aphid species are able to acquire and transmit various viruses (Harris, 1983). The potato virus Y group (potyviruses) is the largest and most widespread group of virus diseases known, and its importance is magnified by the large role aphids play in virus dissemination. Of 87 viruses and 15 strains in this group, Edwardson (1974) listed 72 which are transmitted in a nonpersistent manner. Other viruses which are transmitted in a nonpersitent manner are those belonging to the cucumber mosaic virus group (cucomoviruses), carlaviruses, caulimoviruses and alfalfa mosaic virus (Zitter, 1977).

Aletes (winged forms of aphids) are most important for the transport of virus from one plant to another (Raccah, 1986). Crowding (Lees, 1966) and nutrition (Raccah *et al.*, 1973) was found to affect wing formation in aphids. Spread of nonpersistent viruses is greatly affected by the flight behaviour of their aphid vectors. Within the flight behaviour pattern (**Figure 1.5.1**), one may refer to takeoff, forms of flight, landing, and settling on the host (Raccah, 1986).

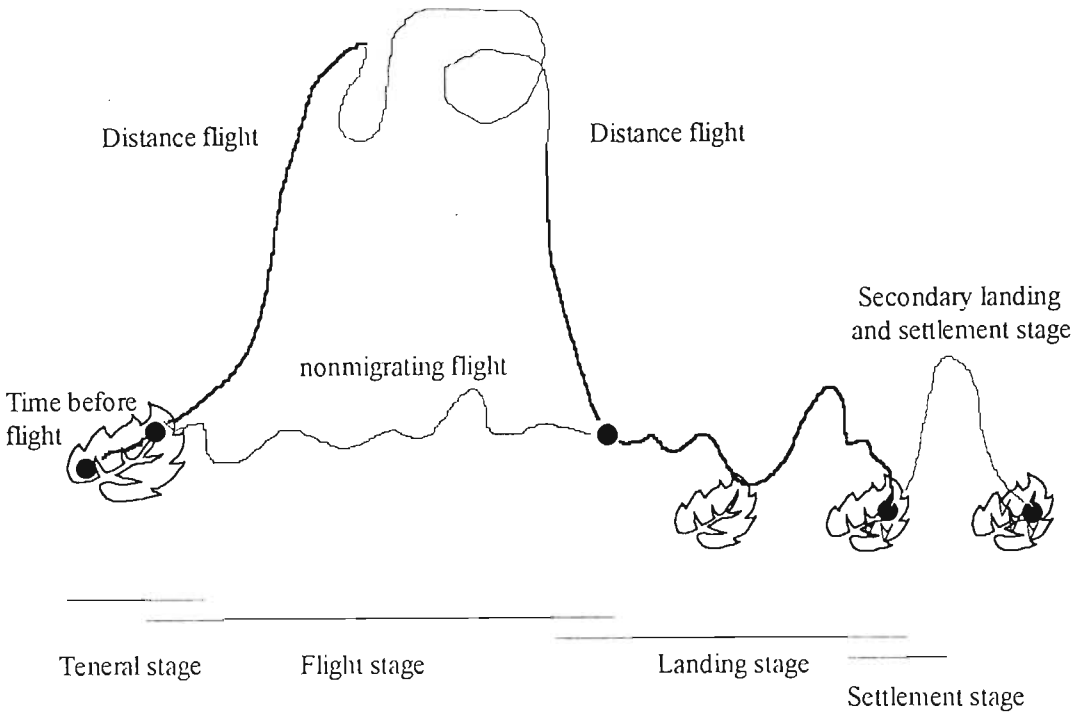

Figure 1.5.1 Schematic representation of the four behavioural stages of the winged aphid (After Moericke, 1955).

a) Takeoff

Taylor (1957) defined the term ***teneral period*** as the time between the moulting of an alate and its takeoff. The teneral stage varies in different aphid species and lasts at least until the wings and body harden after the moult (Raccah, 1986). Takeoff is affected by environmental conditions such as light and temperature, hence the overlap between the teneral stage and flight stage when flight-ready aphids are prevented from flight because of unfavourable conditions (Raccah, 1986; Kring, 1972).

b) Flight

There is a difference between migrating (distance flight) and nonmigrating aphids in the mode of flight. The former will fly toward the sky while the latter will fly at a low level above ground or among plants (Bodeheimer and Swirski, 1957). Where the transmission of nonpersistent viruses is concerned, only the nonmigrating flight behaviour of aphids is effective and that only if the aphid lands on a susceptible host (Kennedy, 1986). This is because, typically, the spacial gradients of infection for nonpersistently transmitted viruses

are steep (**Figure 1.5.2**) as a result of short retention times of the virus in the aphid (Irwin and Goodman, 1981).

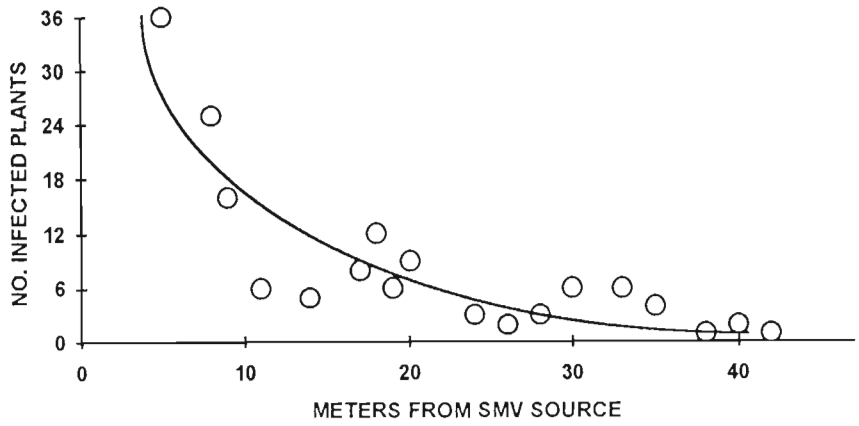

Figure 1.5.2 Gradient of downwind spread of a nonpersistent aphid borne virus, soybean mosaic virus (SMV), during a single growing season. (after Irwin and Goodman, 1981)

c) Landing

Many factors govern the active selection of the landing surface. During flight a primary distinction between sky and ground is made by aphids on the basis of wavelength (Raccah, 1986). A secondary distinction of soil and plants may also be possible. The influence of transmitted light on aphid landing behaviour has been shown to have a marked effect on aphid rejection or acceptance of the host.

Short-wave light. Aphids approaching a white surface that reflects short-wave light or an aluminium surface that reflects most light, will often fly along the edge of the surface, turn away, or fly in bobbing flight over the surface (Moericke, 1955). Whether this is a result of repellency to sunlight or reflected sky light or a stronger attraction to the contrasting radiation from the soil and nearby plants is not known. However, this response has been used with great effect in vector management strategies (Greennough *et al.*, 1990; Marco, 1993).

Response to yellow. Reflected or transmitted yellow light, on the other hand, induces aphid landing behaviour and favours settling (Moericke, 1955). This too can be used to our advantage as a means of vector management. Cohen and Marco (1973) reduced the spread

of aphid transmitted viruses in peppers by using sticky sheets of yellow polyethylene located outside the field with the aim of trapping winged aphids.

d) Settling

Settling is that stage during which the aphid accepts a plant as a feeding and reproductive site. Upon landing, aphids probe the epidermal cell by inserting their stylets to a depth of less than 10μm (Loebenstein and Raccah, 1980). A small amount of sap, probably containing the virus, is ingested by the aphid, filling the food canal. Infection occurs probably when a viruliferous aphid egests the contents of its food canal while probing on another plant. More probing attempts are found to be made on hosts than on nonhosts (Raccah, 1986). The probing behaviour of aphids is the successful means by which nonpersistent viruses are acquired and inoculated (Raccah, 1986).

1.6 Discussion

In recent years, changes in agricultural practices have had a profound effect on the incidence of virus diseases. Some management practices have brought together new and large communities of crop plants and weeds associated with their cultivation. Plants, insect vectors, and potential vectors have been transported into new areas and insects already present in these newly cultivated areas have been able to spread into the new crops. In addition, the effects of repeated cultivation of a single crop species (monocropping) has allowed for a marked increase of aerially and soil-borne viruses (Tomlinson, 1987). Epidemics of nonpersistent viruses, in particular, are more common today than in the first half of the twentieth century (Raccah, 1986).

With a better knowledge of the virus pathosystem, man is able to interfere with nature and recognise his own harmful activities (Bos, 1983). There are various ways of avoiding the harmful effects of viruses:

1. increasing crop resistance,
2. altering crop management strategies so as not to facilitate virus spread,
3. removing or avoiding sources of infection,
4. decreasing spread through vector control.

The losses resulting from infection by virus depend on crop susceptibility and sensitivity (Bos, 1883). These in turn depend on the crop genotype being grown (Buddenhagen, 1983). The potential for influencing the system by altering the crop genotype is very great. Understanding the details of plant virus survival is a great aid in developing strategies for breeding new cultivars which will be less damaged and restrict virus spread. It is important that breeders, plant pathologists and epidemiologists maintain close contact, so as to prevent the development of cultivars that appear to develop "new" virus diseases (i.e. enhance existing, undetected ones), or convert existing virus diseases of minor importance to serious problems by changing levels of tolerance. Field surveys of disease and feedback to the breeder are necessary, as are efforts to make "occasional" diseases prevalent in breeders' plots, so as to facilitate selection.

While breeding resistant varieties is a long-term solution, knowledge gained from the understanding of virus pathosystems can be utilised immediately through changes in farming practices to manage disease. The trend to year-round cultivation involving overlapping cultivation and prolonged harvesting procedures of susceptible crops which facilitates epidemics has given way to modern rotation and planting schedules, with resultant yield increases (Duffus, 1983).

The importance of weeds as sources of infection varies widely. It depends on:

1. the virus and its pathogenicity and way of spread
   (= aggressiveness or infectivity + virulence),
2. crop vulnerability (= susceptibility + sensitivity),
3. vector behaviour, efficiency and abundance,
4. other sources of infection than weeds,
5. the weeds themselves as reservoirs of infection, their susceptibility, sensitivity, number, time of availability and distance from the sensitive crop, and
6. growing conditions (Bos, 1981).

Points 4 and 5 require further explanation. The relative contribution of weeds as sources of infection depends on the absence of other sources of infection, such as plants developing from infected propagation material, or other infected crops nearby. Therefore, the cleaner

the propagation material, the more critical is the absence of external sources of infection. Efficiency of weeds as sources of infection, also depends on their infected number and thus on their abundance and rate of infection, which depends on virus pathogenicity, and on susceptibility and chances of survival of the weed and so on tolerance to infection, the host's lifespan and possible virus transmission through seed. An important quantitative factor is distance between weed sources of infection and crop. It has to be short if nonpersistent insect transmission is required. Similarly, proximity is a determinant for soil transmission. With nematode borne viruses, the immediate presence of various weed hosts makes up for the low mobility of the vector (Bos, 1981).

In the virus/vector/plant chain, it is the vector which acts as the disseminating element allowing for the spread of the virus; so it can be tempting to try to break the chain at this link (Quiot *et al.*, 1982). It is now widely recognised that effective control measures depend on a thorough knowledge of vector ecology and epidemiology (Tomlinson, 1987).

The activities employed in producing agricultural commodities modify the ecology of an agroecosystem (Herzog and Funderburk, 1986). Frequently, these practices can be deliberately modified in ways that minimise crop loss. Such cultural control tactics can be used in the management of the virus pathosystem, can be compatible with other pest control tactics and can be low cost. Additionally, few would have undesirable ecological consequences. An in-depth understanding of the processes involved in virus/vector/plant interactions facilitates such modifications, including those modifying plant species in crop and non-crop habitats, those facilitating vector management and those operations used to plant, maintain, and harvest specific crops. It is unrealistic to say that many of the agricultural practices that play a role in the successful management of the virus pathosystem have evolved through the guidance of ecological theory. Nonetheless, many of the management approaches that have proven effective are consistent with those that emerge from a consideration of the relevant systems theory.

1.7 Literature Cited

Alderz, W.C. 1978. Watermelon mosaic virus 2 epidemics in Florida 1967-1977. J. Econ. Entomol. 71 : 596-587.

Almeida, A.M.R. and Corso, I.C. 1991. Effect of sowing time on the incidence of bud blight in soybean (*Glycine max* L. Merr.). J. Phytopathol. 132 : 251-257.

Beachy, N., Loesch-Fries, S., and Tumer, N.E. 1990. Coat protein-mediated resistance against virus infection. Ann. Rev. Phytopathol. 28 : 451-474.

Bennett, C.W. 1969. Seed transmission of plant viruses. Adv. Virus Res. 14 : 221-261.

Bodeheimer, F.S. and Swirski, E. 1957. The Aphidoidea of the Middle East. Weizmann Science Press, Jerusalem. 378 pp.

Bos, L. 1981. Wild plants in the ecology of virus diseases. In: Plant diseases and vectors: Ecology and epidemiology. K. Maramorosch and K.F. Harris (Eds.). Academic Press, New York. pp. 1-33.

Bos, L. 1983. Plant virus ecology; the role of man, anf the involvement of governments and international organisations. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T. Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 7-23.

Bruckart, W.L. and Lorbeer, J.W. 1976. Cucumber mosaic virus in weed hosts near commercial field of lettuce and celery. Phytopathology 66 : 253-259.

Buddenhagen, I.W. 1977. Resistance and vulnerability of tropical crops in relation to their evolution and breeding. Ann. NY Acad. Sci. 287 : 309-326.

Buddenhagen, I.W. 1981. Conceptual and practical considerations when breeding for tolerance or resistance. In: Plant disease control. R.C. Staples and G.H. Toenniessen (Eds.) John Wiley, New York. pp. 221-234.

Buddenhagen, I.W. 1983. Crop improvement in relation to virus diseases. In: Plant virus epidemiology :The spread and control of insect-borne viruses. R.T Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 25-37.

Bujarski, J.J. and Kaesberg, P. 1986. Genetic recombination between RNA components of a multipartite plant virus. Nature 321 : 528-531.

Chaney, B. 1993. The IPM Practitioner. Vol XV pp. 1-11.

Cho, J.J., Mau, R.F.L. and Mitchell, W.C. 1986. Reservoir weed hosts of tomato spotted wilt virus. Plant Dis. 70 : 1014-1017.

Cho, J.J., Mau, R.F.L., German, T.L., Hartmann, R.W., Yudin, L.S., Gonsalves, D. and Provvidenti, R. 1989. A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Dis. 73 : 375-383.

Cohen, S. and Marco, S. 1973. Reducing the spread of aphid-transmitted viruses in peppers by trapping the aphids on sticky yellow polyethylene sheets. Phytopathology 63 : 1207-1209.

Conti, M. and Masenga, V. 1977. Identification and prevalence of pepper viruses in northwest Italy. J. Phytopathol. 90 : 212-222.

Conti, M., Caciagli, P. and Casetta, A. 1979. Infection sources and aphid vectors in relation to the spread of cucumber mosaic virus in pepper crops. Phytopath. Medit. 18 : 123-128.

Costa, A.S. and Muller, G.W. 1980. Tristeza control by cross protection: A U.S.-Brazil cooperative success. Plant Dis. 64 : 538-541.

Creamer, R. and Falk, B.W. 1990. Direct detection of transcapsidated barley yellow dwarf luteoviruses in doubly infected plants. J. Gen. Virol. 23 : 41-49.

De Ponti, O.M. 1983. Resistance to insects promotes the stability of integrated pest control. In: Durable resistance in crops. F. Lamberti, J.M. Waller and N.A. van der Graaff (Eds.). Plenum Press, New York. pp. 211-225.

de Zoeten, G.A. 1991. Risk assessment: Do we let history repeat itself ? Phytopathology 81 : 585-586.

Doolittle, S.P. and Walker, M.N. 1925. Further studies on the overwintering and dissemination of cucurbit mosaic. J. Agric. Res. 31 : 1-58.

Douine, L., Quiot, J.B., Marchoux, G. and Archange, P. 1979. Recensement des espèces vègètales sensibles au virus de la mosaïque du concombre (CMV). Etude bibliographique. Ann. Phytopathologie 11 : 439-475. (in French)

Duffus, J.E. 1983. Epidemiology and control of aphid-borne virus diseases in California. In: Plant virus epidemiology :The spread and control of insect-borne viruses. R.T Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 221-228.

Edwardson, J.R. 1974. Host ranges of viruses in the PVY-group. Florida Agric. Exp. Sta. Monograph Series. No.5, 225 pp.

Fletcher, J.T., George, A.J. and Green, D.E. 1969. Cucumber green mottle mosaic virus its effect on yield and its control in the Lea Valley England. Plant Pathol. 18 : 16-22.

Gibbs, A., Skotnicki, A. and Skotnicki, M. 1986. Plant virus control strategies: future prospects. In: Plant virus epidemics: monitoring, modelling and predicting outbreaks. G.D. McLean, R.G. Garret and W.G. Ruesink (Eds.). Academic Press, Sydney. pp. 513-524.

Greenough, D.R., Black, L.L. and Bond, W.P. 1990. Aluminium-surfaced mulch: An approach to the control of tomato spotted wilt virus in Solanaceous crops. Plant Dis. 74 : 805-808.

Harlan, J.R. 1976. Diseases as a factor in plant evolution. Ann. Rev. Phytopathol. 14 : 31-51.

Harris, K.F. 1977. An ingestion-egestion hypothesis of noncirculative virus transmission. In: Aphids as Virus Vectors. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp. 166-220.

Harris, K.F. 1983. Sternorrhynchous vectors of plant viruses: Virus-vector interactions and transmission mechanisms. Adv. Virus Res. 28 : 113-140.

Harrison, B.D. 1978. The groups of nematode transmitted plant viruses, and molecular aspects of their variation and ecology. In: Plant disease epidemiology. P.R. Scott and A. Bainbridge (Eds.). Blackwell Scientific Publications, Oxford. pp. 255-264.

Harrison, B.D. 1981. Plant virus ecology: ingredients, interactions, and environmental influences. Ann. appl. Biol. 99 : 195-209.

Hawkes, J.G. 1969. The ecological background of plant domestication. In: The domestication and exploitation of plants and animals. P.J. Ucko and G.W. Dimbleby (Eds.). Duckworth, London. pp. 17-29.

Herzog, D.C. and Funderburk, J.E. 1986. Ecological basis for habitat management and pest cultural control. In: Ecological theory and integrated pest management practice. M. Kogan, (Ed.). John Wiley & Sons, New York. pp. 217-250.

Irwin, M.E. and Goodman, R.M. 1981. Ecology and control of soybean mosaic virus. In: Plant diseases and vectors: Ecology and epidemiology. K. Maramorosch and K.F. Harris (Eds.). Academic Press, New York. pp. 182-220.

Irwin, M.E. and Ruesink, W.G. 1986. Vector intensity: A product of propensity and activity. In: Plant virus epidemics: Monitoring, modelling and predicting outbreaks. G.D. McLean, R.G. Garrett and W.G. Ruesink (Eds.). Academic Press, Sydney. pp 13-34.

Kahn, R.P. and Monroe, R.L. 1970. Virus infection in plant introductions collected as vegetative propagations. FAO Plant Prot. Bull. 18 : 97-102.

Kennedy, G.G. 1986. Plant-plant-pathogen-insect interactions. In: Ecological theory and integrated pest management practice. M. Kogan (Ed.). John Wiley & Sons, New York. pp. 203-216.

Kring, J.B. 1972. Flight behaviour of aphids. Ann. Rev. Entomol. 17 : 461-492.

Lees, A.D. 1966. The control of polymorphism in aphids. Adv. Insect Physiol. 3 : 207-277.

Loebenstein, G. and Raccah, B. 1980. Control of non-persistently transmitted aphid-borne viruses. Phytoparasitica 8 : 221-235.

Mandahar, C.L. 1981. Virus transmission through seed and pollen. In: Plant diseases and vectors: Ecology and epidemiology. K. Maramorosch and K.F. Harris (Eds.). Academic Press, New York. pp. 241-292.

Maramorosch, K. and Harris, K.F., Eds. 1981. Plant diseases and vectors: Ecology and epidemiology, Academic Press, New York.

Marco, S. 1993. Incidence of nonpersistently transmitted viruses in pepper sprayed with whitewash, oil and insecticide, alone or combined. Plant Dis. 77 : 1119-1122.

Moericke, V. 1955. Über die lebensgewohnheiten der gefügelten blättlause (under besonderer berücksichtigung des verhaltens beim landen). Z. Agnew. Entomol. 37 : 29-91.

Pirone, T.P. and Harris, K.F. 1977. Nonpersistent transmission of plant viruses by aphids. Ann. Rev. Phytopathol. 15 : 55-73.

Quiot, J.B., Labonne, G., and Marrou, J. 1982. Controlling seed and insect-borne viruses. In: Pathogens, vectors and plant diseases: approaches to control. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp. 95-122.

Raccah, B. 1986. Nonpersistent viruses: epidemiology and control. Adv. Virus Res. 31 : 387-429.

Raccah, B. and Irwin, M.E. 1988. Techniques for studying aphid-borne virus epidemiology. In: Experimental techniques in plant disease epidemiology. J. Kranz and J. Rotem (Eds.). Springer-Verlag, Berlin. pp. 209-222.

Raccah, B., Applebaum, S.W. and Tahori, A.S. 1973. The role of folic acid in the appearance of alate forms in *Myzus persicae*. J. Insect Physiol. 19 : 1849-1855.

Robinson, R.A. 1987. Host management in crop pathosystems. Macmillan Publishing Company, New York. 263pp.

Rochow, W.F. 1977. Dependent virus transmission from mixed infection. In: Aphids as virus vectors. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp. 253-276.

Russel, G.E. 1978. Plant breeding for pest and disease resistance. Butterworths, London.

Shivanathan, P. 1983. The epidemiology of three diseases caused by whitefly-borne pathogens. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 323-328.

Simmonds, N.W. 1962. Variability in crop plants, its use and conservation. Biol. Rev. 37 : 442-465.

Simons, J.N. 1959. Potato virus Y appears in additional areas of pepper and tomato production in south Florida. Pl. Dis. Reptr. 43 : 710-711.

Simons, J.N. and Eastop, V.F. 1970. Temperature effects on aphid transmission of nonpersistent viruses with notes on morphological variation within clones of aphids with differing vector efficiencies. J. Econ. Entomol. 63 : 484-490.

Stobbs, L.W., Broadbent, A.B., Allen, W.R. and Stirling, A.L. 1992. Transmission of tomato spotted wilt virus by the western flower thrips to weeds and native plants found in southern Ontario. Plant Dis. 76 : 23-29.

Taylor, L.R. 1957. Temperature relations of teneral development and behaviour in *Aphis* fabae. J. Exp. Biol. 34 : 189-208.

Taylor, L.R. 1979. The Rothamsted insect survey-an approach to the theory and practice of synoptic pest forecasting in agriculture. In: Movement of highly mobile insects: Concepts and methodology in research. R.L. Rabb and G.G. Kennedy (Eds.). University Graphics, North Carolina State Univ. pp. 2-65.

Taylor, L.R. 1986. The distribution of virus disease and the migrant vector aphid. In: Plant virus epidemics: monitoring, modelling and predicting outbreaks. G.D. McLean, R.G. Garrett and W.G. Ruesink (Eds.). Academic Press, Sydney. pp 35-58.

Thresh, J.M. 1976. Gradients of plant virus diseases. Ann appl. Biol. 82 : 381-406.

Thresh, J.M. 1982. Cropping practices and virus spread. Ann. Rev. Phytopathol. 20 : 193-218.

Thresh, J.M. 1983. Plant virus epidemiology and control: current trends and future prospects. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T. Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 349-360.

Thurston, H.D. 1990. Plant disease management practices of traditional farmers. Plant Dis. 74 : 96-102.

Tomlinson, J.A. 1987. Epidemiology and control of virus diseases of vegetables. Ann. appl. Biol. 110 : 661-681.

Vanderplank, J.E. 1963. Plant Diseases: Epidemics and control. Academic Press, New York. 349pp.

Yeh, S.-D., Gonsalves, D., Wang, H.-L., Namba, R., and Chiu, R.-J. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72 : 375-380.

Zitter, T.A. 1977. Epidemiology of aphid-borne viruses. In: Aphids as virus vectors. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp. 385-412.

CHAPTER 2

The Capsicum Viruses

2.1 Introduction

...the indigenous population, whatever their social rank, live on the 'green' chilli pepper, which is prepared in all sorts of different ways. They divide the chilli pepper into seven types, according to the degree of hotness: cococ (hot), cocopatic (very hot), cocopetzpatic (very, very hot), cocopetztic (exceedingly hot), cocopetzquanitl (extremely hot), and cocopalatic (supremely hot)...

Spanish monk, Bernardino de Sahagún, following the conquest of Mexico by Hernando Cortéz, 1519

(Anon. 1992)

Although several species of peppers, members of the Solanaceae family, originated from South America, in the region of southern Peru and Bolivia, according to current knowledge the original home of the most important species, *Capsicum annuum* L., is Central America, where it has probably been cultivated since around 8,000 B.C. Together with maize (*Zea mays* L.) and pumpkin (*Cucurbita pepo* L.), the chilli or Spanish pepper formed the basic diet of the original inhabitants and, primarily because of its vitamin and mineral content, was of particular importance. The chilli was also highly prized by the Aztecs, as evidenced by the fact that they even demanded it as a tribute from the peoples they subjugated. They also used it for medicinal purposes, e.g., as a pain killer (Anon., 1992). Another species grown commercially is *C. frutescens* L., from which Tabasco sauce is produced. About 16 other species of peppers have been identified (Heiser and Smith, 1953). Five distinct species have been domesticated with the diversity of fruit shape, size, colour, flavour, aroma, pungency, and plant growth habit defying description (Villalón, 1981).

Before Columbus' first voyage to the New World, peppers were unknown to the civilised world. Later, many different types of sweet and hot peppers were discovered throughout Mexico and Central and South America. These were introduced into Spain, all of Europe, and eventually the Orient, where they became very popular, with each area developing its own type. Thus peppers became the first New World food item commercially used in Europe (Heiser and Smith, 1953). Today, the green and red, sweet bell fruit types are used

in salads and in cooked dishes. Processed spices such as cayenne pepper, paprika and curry powder are made from dry fruit (mostly the pungent chilli type). Certain red chilli varieties are also used to provide natural colourings in the food industry.

Capsicums are now grown world-wide under various environmental and climatic conditions, covering an area of nearly one million hectares (Martelli and Quacquarelli, 1983). From an economic point of view, pepper yield is often low and variable. Virus diseases are an important factor contributing to low yields and reduced fruit quality. One hundred percent losses of marketable fruit have been reported (Marte and Wetter, 1986), and in some areas infection with viruses has rendered the growing of peppers uneconomical, causing whole fields to be abandoned prior to harvest (Greenleaf, 1986).

Symptoms of virus infection vary greatly in expression and severity, and include mild mottle, mosaic, veinbanding, ringspots, various types of necroses, leaf discoloration, deformation and blistering and severe stunting of the whole plant (Green and Kim, 1991). Leaves, stems and flowers, as well as fruits, may be affected.

Virus identification should never be based on symptoms alone because symptoms vary with the strain of the virus, the host cultivar, the age of the host, environmental conditions and possible co-infection with other viruses (Sherwood *et al.*, 1986). Furthermore, different viruses may cause similar symptoms, and insect damage, particularly by thrips and mites, may mimic virus symptoms. Certain herbicides, such as 2.4-D, and growth hormones may also cause reactions in the plant which resemble virus symptoms (Green and Kim, 1991). Exact identification of pepper viruses should be based on differential host plant tests, confirmed by serological tests (Marco and Cohen, 1979) or vice versa, and if possible supplemented by electron microscopic characterisation of the virus particle and virus-induced inclusions and by vector transmission tests. A more recent technique applicable to virus diagnosis is the use of hybridisation and PCR (polymerase chain reaction) technology (Hull, 1993). Using defined nucleic acid probes and suitable conditions it is possible to have systems for the detection of either viruses or individual virus strains. The most important feature of this technique is its sensitivity. Most plant viruses occur in such high concentrations that this is irrelevant (Hull, 1993). However, for

some viruses, and especially those that are phloem-limited, it can be difficult to develop a reliable detection system. Theoretically, PCR can detect a single molecule of a target nucleic acid.

Some 45 viruses have been reported to infect Capsicums (Green and Kim, 1991; **Appendix 1**). Of these, more than half are aphid-transmitted. Potato virus Y (PVY), tobacco mosaic virus (TMV) and tomato spotted wilt virus (TSWV) were listed by Gorter (1977) as infecting Capsicums in South Africa. Most pepper viruses are distributed world-wide with the exception of chilli veinal mottle virus, pepper severe mosaic virus, pepper veinal mottle virus, pepper mild mosaic virus and pepper mottle virus.

2.2 Commercial Production of *Capsicum* spp. in South Africa

In recent years, as a result of low profit margins of some crops and overproduction of others, farmers have begun to experiment with new crop types (Roodeplaat Bulletin, 1990). In particular, expansion in the paprika industry was most notable. The South African access to the paprika market may be attributable to two factors. Firstly Spain, the market leader, had to retreat from the market as a result of high outstanding loans to paprika farmers. Secondly, the birth of the "Green" movement is seen as a contributing factor in the increasing demand for natural colourings and taste enhancers (Roodeplaat Bulletin, 1990). The industry responded well with the construction of African Oil's first oleo resin extraction plant (Sunday Times, Business Times, 1992). The Thabazimbi-based plant had a projected turnover of R25-million in the first year, processing 20 tons of raw paprika daily, thus adding value to regional paprika crops which have mostly been exported for processing to Europe.

The fresh capsicum market has also been lucrative. In 1994, for the period of January-November, over R22-million worth of sweet peppers has been sold on the fifteen national

fresh produce markets at an average price of R2063.81 per tonne. In contrast to 1993 this indicates in an almost two-fold increase in the average price of sweet peppers (**Table 2.2.1**). Similarly, over R6-million worth of chillies was sold at an average price of R3288.60 per tonne as opposed to R1494 per tonne in the previous year (Statistics on Fresh Produce Markets, 1994). This price increase indicates a growing demand for capsicum products and thus an incentive to produce capsicums in Natal on a commercial scale.

Table 2.2.1 Mass, value, and average prices of peppers and chillies sold on the fifteen national fresh produce markets for the period 1990-1994.

	Peppers			Chillies		
	Tonne	Rand	R/Tonne	Tonne	Rand	R/Tonne
1990	7,397	13,205,604	1,785.27	2,074	4,165,903	2,008.88
1991	8,753	11,492,247	1,313.00	1,964	4,462,257	2,272.52
1992	8,523	15,492,514	1,817.71	2,042	5,258,470	2,574.83
1993	10,957.15	14,706,283	1,342.16	2,965.54	4,430,520	1,494.00
1994*	8,585.31	22,354,496	2,603.81	1,912	6,287,869	3,288.60

* For the period January-November

2.3 Some Important Capsicum Viruses

2.3.1 Introduction

This review of some of the most common Capsicum viruses was combined with infection studies carried out in the glasshouse at the University of Natal, Pietermaritzburg. Sweet pepper cv. Carousel seedlings were raised in an insect proof cage (**Figure 2.3.1**) to ensure no mixed infections. Six plants (2 per pot) were used for each virus type. The following viruses were used in the infection studies:

(1) PVY - isolated from a field infected pepper plant showing mosaic symptoms;

(2) CMV - obtained courtesy of Dr. G. Pietersen of the Plant Protection Institute in Pretoria;

(3) TSWV - isolated from a garden variety of a *Dahlia* sp. courtesy of Prof. M.M. Martin;

(4) TMV - tobacco strain maintained at the University of Natal.

For the prevalence of these viruses in other crops in South Africa, refer to Gorter (1977).

Figure 2.3.1 Insect proof cage where pepper seedlings were grown to prevent mixed virus infections.

2.3.2 Potato Virus Y

Potato Virus Y (PVY) is the most common potyvirus infecting peppers. It occurs world-wide although it appears to be more important in warmer areas (Mills and Abdul-Magid, 1987). Disease incidence may be as high as 100% in some areas, resulting in considerable crop loss (Sharma *et al.*, 1989). Although mosaic, veinclearing and yellowing are typical symptoms of infection by PVY (**Figure 2.3.2**), other symptoms such as leaf crinkling, leaf distortion and stunted plant growth (**Figure 2.3.3**) are also common, depending on the virulence of the strain and the host-pathogen interaction (Sharma *et al.*, 1989). Fruit quality is severely affected (**Figure 2.3.4**).

PVY is transmitted in a nonpersistent (stylet-borne) manner by aphids. The green peach aphid (*Myzus persicae* Sulz.) is considered to be the single most important vector, although several other aphid species such as *Aphis gossypii* (Glov.), *Macrosiphum solani* (Kaltenb.), *M. pisi* (Koch.) and *A. spireacola* (Patch) are also known to transmit this virus (Raccah *et al.*, 1985). Optimal acquisition feeding time is from 15 to 60 seconds and most feeding aphids cease to transmit the virus within 1 hour (de Bokx and Huttinga, 1981). PVY is not known to be seed-transmitted in peppers (Raccah *et al.*, 1985).

Figure 2.3.2. Virus symptoms on pepper (*Capsicum annuum* L.) plants.

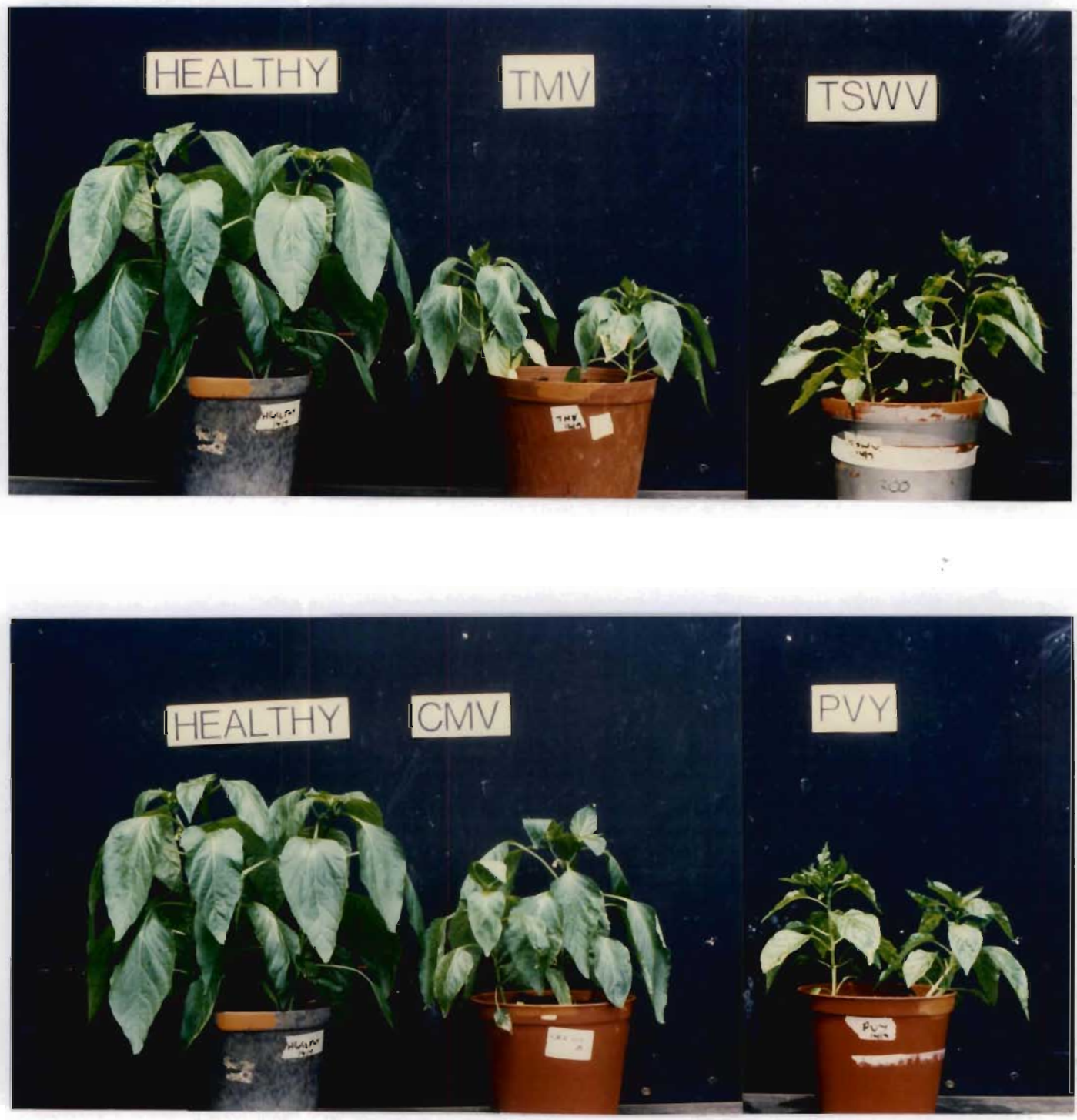

Figure 2.3.3 Effect of virus infection on plant growth.

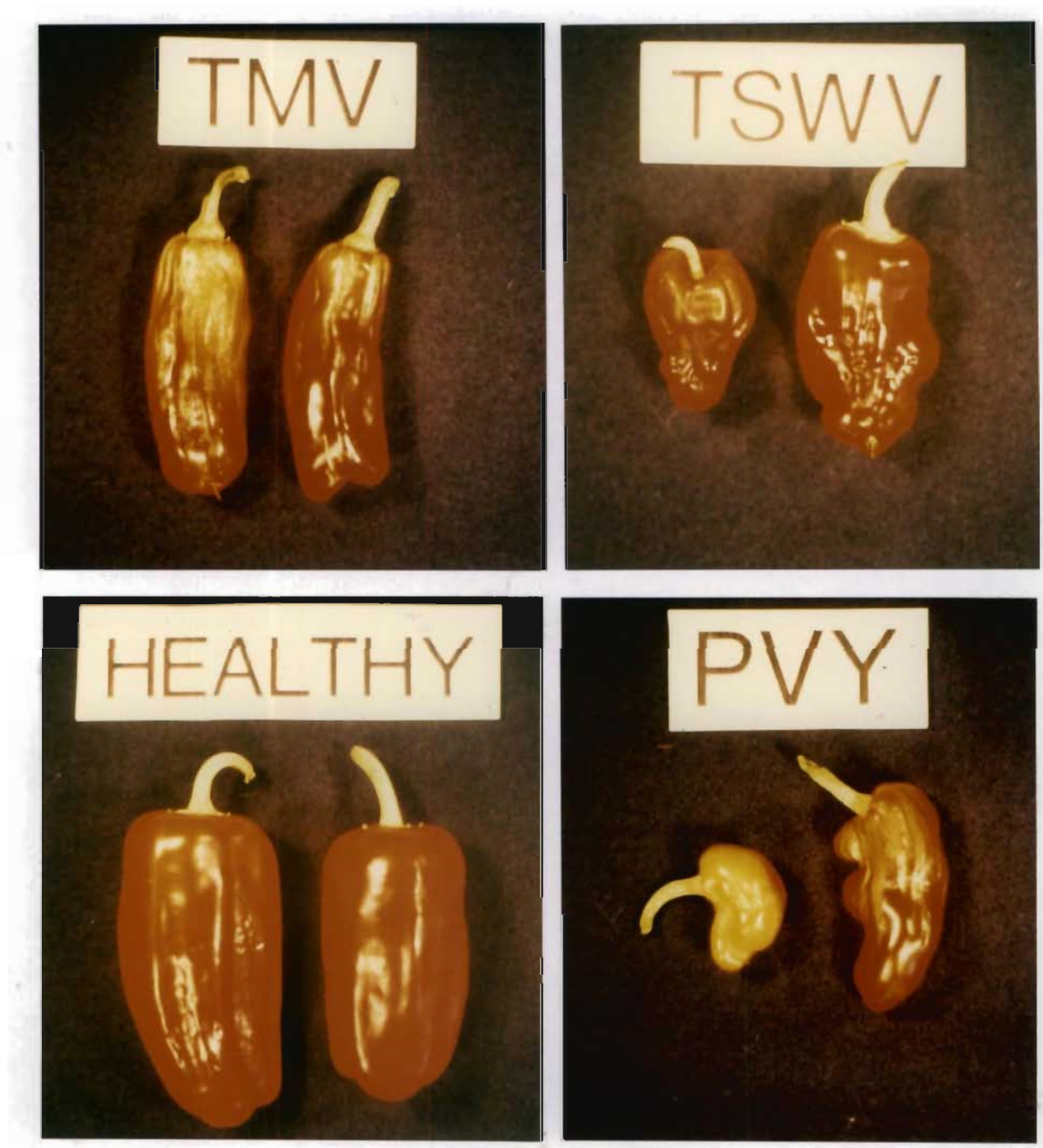

Figure 2.3.4 Virus symptoms on pepper fruit.

The damage that PVY causes depends upon the strains involved, the host cultivars grown, and whether it occurs singly or in mixed infections. Heavy losses occur in peppers when PVY is present together with other viruses (de Bokx and Huttinga, 1981). Insecticide application or roguing of infected plants is usually inadequate in reducing virus spread (Laird and Dickson, 1963).

2.3.3 Cucumber Mosaic Virus

Cucumber mosaic virus (CMV) is geographically one of the most widespread plant viruses. It is now considered that the host range of CMV is in excess of 800 species (Palukaitis *et al.*, 1992). Common symptoms caused by CMV are mottle, mosaic, yellow discoloration, veinclearing, leaf deformation and leaf narrowing (**Figures 2.3.2 and 2.3.3**) (Zitter *et al.*, 1984). Yield losses of more than 60% from CMV have been reported in peppers (Florini and Zitter, 1987).

Some seventy-five species of aphids are known to transmit CMV in a nonpersistent manner (Fritzsche *et al.*, 1972). For peppers, *M. persicae* seems to be the most efficient vector in cold climates, whereas *A. gossypii* is the major vector in warmer regions (Conti *et al.*, 1979). The virus can be acquired by aphids from infected plants in less than 1 minute of feeding, and can be instantly transmitted to a susceptible plant, with no latent period. CMV is stylet-borne by aphids and can be lost during probing directly after feeding. In the absence of feeding, the virus remains associated with the aphid for less then 4 hours (Palukaitis *et al.*, 1992). CMV does not seem to be seed-transmitted in pepper (Green and Kim, 1991) but it is in some weeds, which are important sources of the virus (Conti *et al.*, 1979). Recent evidence has shown that CMV infection can occur from infected soil debris via non-vectored soil transmission (Pares and Gunn, 1989).

2.3.4 Tomato Spotted Wilt Virus

Tomato spotted wilt virus (TSWV) is common on solanaceous crops in tropical and subtropical regions throughout the world. The virus has a large host range which includes more than 400 species belonging to the Monocotyledonae and Dicotyledonae (Green and Kim, 1991). In peppers, the virus causes chlorosis, bright, often sudden yellowing (**Figure 2.3.2**) and browning, and chlorotic rings on the leaves. Necrotic leaf spots, necrosis of

terminal shoots and general stunting may also be encountered (**Figure 2.3.3**). Fruit show chlorotic spots, green or red areas surrounded by yellow halos, large necrotic blotches and sometimes concentric rings. Fruit distortions have also been reported (Cho *et al.*, 1989) (**Figure 2.3.4**). Necrosis and abortion of developing flowers are common. Incidences of TSWV infection as high as 60% in pepper production areas (Greenough and Black, 1984) and considerable yield loss due to this virus have been reported (Burgmans *et al.*, 1986).

TSWV is known to be transmitted by six species of thrips: *Thrips tabaci* (Lind.), *T. setosus* (Lind.), *Frankliniella schultzei* (Trybom), *F. occidentalis* (Pergande), *F. fusca* (Hinds) and *Scirtothrips dorsalis* (Hood) in a persistent manner. Although only larvae can acquire the virus, transmission is both by infectious larvae and adults after an incubation period of 4 to 10 days (Cho *et al.*, 1989). Seed transmission has been reported in tomato and several weed species but apparently does not occur in air-dried seeds of peppers (Green and Kim, 1991).

Weeds, other vegetables (tomato [*Lycopersicum esculentum* Mill.], potato, eggplant [*Solanum melongana* L.], celery [*Apium graveolens* L.] and lettuce [*Lactuca sativa* L.]), fruit (papaya [*Carica* spp.] and pineapple [*Ananas comosus* (L.) Merr.]), and ornamentals (marigold [*Tagetes erecta* L.], *Zinnia* spp., *Chrysanthemum* spp., dahlia [*Dahlia rosea* Cav.] and *Gerbera* spp.) are important reservoirs of TSWV (Cho *et al*,. 1986, 1989). Besides vector control by insecticide or use of reflective mulches (Greenough *et al.*, 1985), the eradication of weeds is important to reduce virus incidence. Sequential plantings of susceptible crop species should also be avoided.

2.3.5 Tobacco Mosaic Virus

Tobacco mosaic virus (TMV) is known to occur on peppers world-wide (Green and Kim, 1991). Symptoms on peppers are mosaic, mottle, necrotic lesions and leaf drop (**Figures 2.3.2 and 2.3.3**). Reduction in flowering has also been noted (Tanzi *et al.*, 1986). A defoliating strain of TMV has been reported on peppers in Nigeria (Igwegbe and Ogungbade, 1985). Although the virus is generally not considered to be economically important in the pepper crop, crop losses may reach 100% because these viruses often induce changes to the fruit, such as mosaic, blistering, necrotic flecks and deformations (**Figure 2.3.4**) (Conti and Lavisolo, 1983).

TMV is transmitted by contact and also to a considerable degree by seed. Virus-carrying seeds and debris in the soil from a previous pepper crop often serve as primary sources of infection (Pares and Gunn, 1989). The virus is present mainly as an external contamination of the seed.

Skimmed milk, concentrated soap solutions or dilute sodium hypochlorite used as a dip for hands and tools while handling plants during pruning and harvesting can be effective in inhibiting TMV infection. TMV can be eliminated from seed coats by soaking the seed in 4.2% calcium hypochlorite or in 2.6% sodium hypochlorite for 15 minutes or in a 10% solution of trisodiumphosphate (Na_3PO_4) for two hours either immediately or during the first month after harvest (Demski, 1981). Immersing seed in 9% hydrochloric acid for 30 minutes has also resulted in elimination of TMV from seed (Demski, 1981). Next to preventative seed treatments, genetic resistance offers the best chances of control.

2.4 Identification and Prevalence of Pepper (*Capsicum annuum* L.) Viruses in Natal, South Africa.

2.4.1 Introduction

Virus diseases cause serious losses in the capsicum industry and can become the most limiting factor affecting pepper production (Makkouk and Gumpf, 1974). For this reason commercial pepper production in Natal has not been successful with plantings displaying a very high percentage of infection and low yields. Thompson (1980) identified a strain of PVY as the predominant cause of virus disease of Capsicums in Natal, limiting production. No other virus, including TMV, was isolated in host range studies from any plants infected in the field.

The present study was initiated to survey for the following three viruses: potato virus Y (PVY), tomato spotted wilt virus (TSWV) and cucumber mosaic virus (CMV). Since PVY, CMV and TSWV have become some of the most important virus diseases affecting peppers in other areas of the world (Agranovsky, 1993; Nono-Womdim *et al.*, 1993; Stobbs *et al.*, 1992; Abdalla *et al.*, 1991; Marchoux *et al.*, 1991; Cho *et al.*, 1986; Agrios

and Walker, 1985; Conti *et al.*, 1979) it was thought that a reinvestigation of these viruses would reveal their significance and relative importance in the pepper-growing regions of Natal. TMV was not included in the survey because it is not considered to be of economic importance as a disease of peppers, with resistant cultivars widely available and control not difficult to implement.

2.4.2 Materials and Methods

Survey Procedures

The following principal pepper growing areas of Natal were surveyed (**Figure 2.4.1**):

(1) Northern Natal / Zululand

Makhatini Flats (North of Richards Bay)

(2) Natal South Coast

Umkomaas (South of Durban) *and Port Shepstone areas*

(3) Natal Midlands

Pietermaritzburg and Eston District (Tala Valley)

In addition three nurseries producing Capsicum speedlings were surveyed:

(1) Scottburgh on the Natal South Coast;

(2) New Hanover, north of Pietermaritzburg;

(3) Ixopo, inland from Scottburgh.

Pepper crops or seedlings in privately owned fields and experimental plots were surveyed. Samples of pepper plants with symptoms of virus diseases (10-20 samples per field) were taken to the laboratory for identification. In the case of nursery seedlings random samples were collected as no virus symptoms were visible. Field virus incidence in each area was estimated by visual inspection.

Virus Identification

Viruses were identified by serological and electron microscopy tests. Commercial kits of double-sandwich ELISA to PVY were purchased from Phytodiagnostica Boehringer Mannheim and used in accordance with manufacturers directions. Indirect $F(ab')_2$ ELISA kits obtained from Dr. G. Pietersen of Plant Protection Research Institute in Pretoria were used to test for CMV and TSWV. The results of ELISA were evaluated using a Biotek Instruments EL-307 plate reader at A_{405nm}. Results were considered positive when ELISA

values of double or more than that of the negative control were obtained. All the results obtained from ELISA were the averages of three replications. For electron microscopy, leaf sap was stained with 2% phosphotungstate at pH 6-7 for 10-15 seconds. Examination of electron microscope preparations of selected samples with ELISA values double that of the control verified whether virus particles were present.

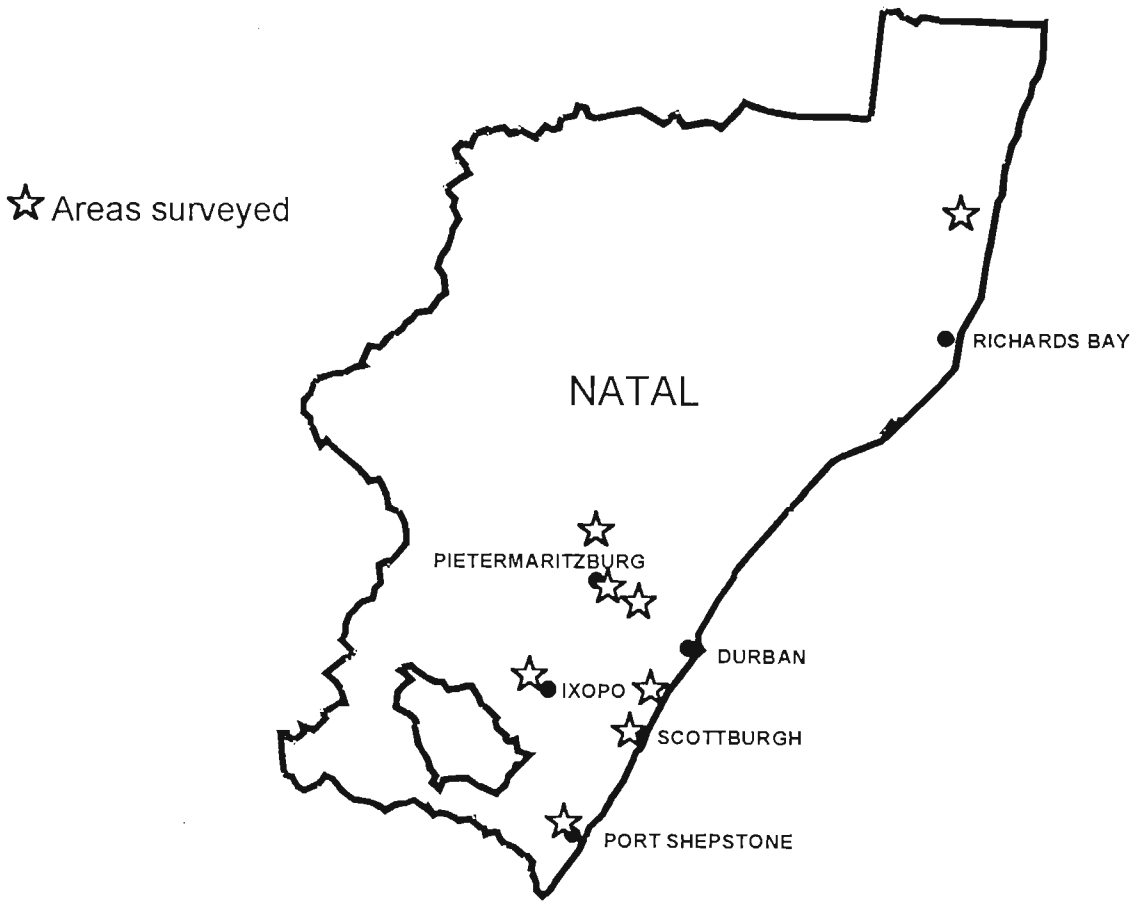

Figure 2.4.1 Map of Natal showing areas surveyed for capsicum viruses.

2.4.3 Results

Northern Natal / Zululand Survey

Samples from two chilli varieties (Britz and Spitfire) at the Makhatini Research Farm were collected twice in 1993, early and late during the season. The incidence of mosaic, leaf distortion and streaking of fruit was estimated at 60-80% (**Figure 2.4.2**) at the end of the season. PVY and CMV were identified in the samples (**Table 2.4.1**). The incidence of CMV was low and always occurred in mixed infections with PVY. Although PVY incidence in

both chilli varieties was high, lower incidence of CMV was observed in the Britz variety, which is a landrace originating from the Northern Transvaal.

Table 2.4.1 Virus incidence in two chilli varieties in Northern Natal, early and late in the season and percentage of samples collected which tested ELISA positive for PVY and CMV.

	% virus incidence		% ELISA positive samples	
	Britz	Spitfire	PVY	CMV
May 1993	20	40	30	0
September 1993	60	80	100	28

Natal South Coast Survey

The farmers' small irrigated fields were repeatedly surveyed in 1993-1994 in the Port Shepstone and Umkomaas areas. Pepper crops cv. California Wonder were severely affected by virus diseases, varying from 60-100% incidence. PVY was the only virus detected in pepper samples from the Port Shepstone area. In the Umkomaas region, however, low incidence of CMV was detected in pepper crops late in the season (**Table 2.4.2**).

Table 2.4.2 Field virus incidence and percentage ELISA positive samples collected in two regions of the Natal South Coast.

		% ELISA positive samples	
	% virus incidence	PVY	CMV
Umkomaas	100	100	1
Port Shepstone	60	50	0

Natal Midlands Survey

The survey of small privately owned fields in the Eston district and experimental plots at Ukulinga in Pietermaritzburg conducted in 1992 and 1993 revealed high incidence of the mosaic disease caused by PVY. The virus incidence in pepper crops in the Eston district was estimated at 50%. In Pietermaritzburg, however, pepper and chilli plants at Ukulinga

exhibited high levels of mosaic with up to 80% incidence. No other virus disease was detected (**Table 2.4.3**).

Table 2.4.3 Field virus incidence and percentage ELISA positive samples collected in two regions of the Natal Midlands.

	% virus incidence	% ELISA positive samples	
		PVY	CMV
Eston	80	50	0
Ukulinga	80	80	0

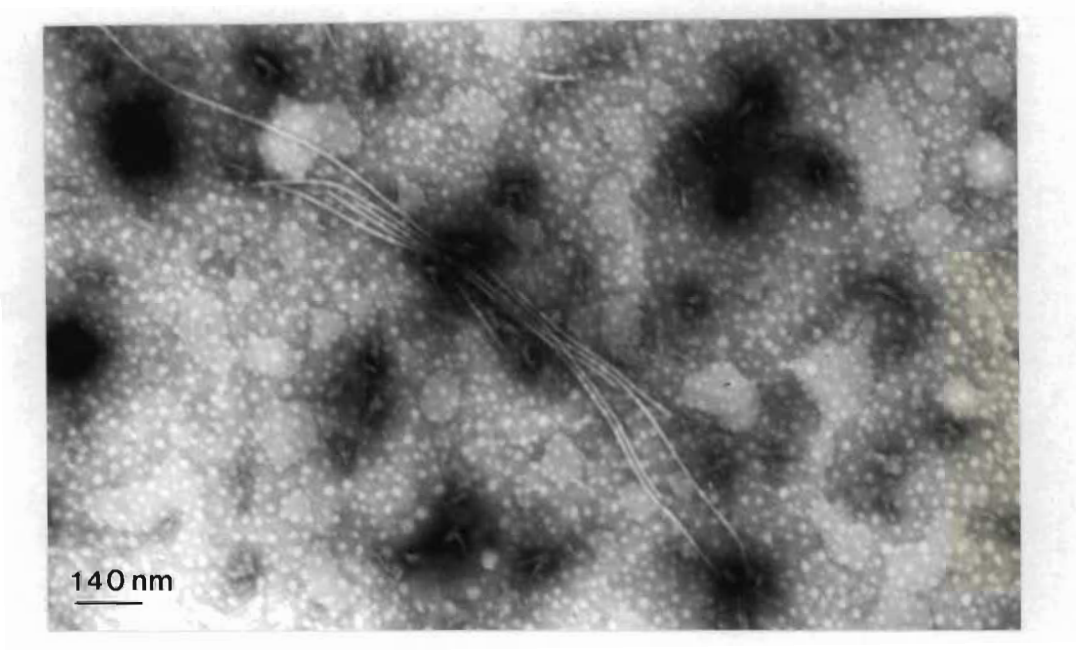

Figure 2.4.2 PVY virus particles from chilli plants collected on the Natal South Coast.

Nursery Survey

No virus was detected in any of the nurseries surveyed verifying natural field infections of most pepper crops surveyed.

Virus Identification

Filamentous particles ca. 700-750 nm in length, characteristic of PVY in leaf dip preparations (Delgado-Sanches and Grogan, 1970), were present in the sap from diseased pepper plants collected in all locations (**Figure 2.4.2**). No CMV-like virus particles were

observed from samples which reacted positively to ELISA. This may be due to the relatively low stability of the CMV virion, and possibly due to the mixed infection with PVY. No purification procedures were carried out due to time constraints.

2.4.4 Discussion

The results of the surveys conducted in 1992-1994 clearly show the prevalence of PVY in the principal Capsicum growing areas of Natal. Although CMV was detected in certain regions, its incidence was low and the virus was apparently of minor importance. No TSWV was detected in any of the areas surveyed. Since no virus was detected in any of the nursery seedlings, it is safe to assume that a widely spread alternate host or hosts exist for PVY and that weed control activities and care in selecting secondary crops may lower virus incidence. A survey and identification of possible alternate weed hosts to Capsicum viruses was carried out and is presented in Chapter 3. Other control practices, such as the use of reflective mulches, sticky yellow traps, oils and insect repellents may provide means to control the disease and are reviewed in Chapter 4.

The disease incidences of PVY were considerably higher on the Natal South Coast than in the other regions. This can probably be explained by the climate of the region which favours continuous capsicum production. The annual temperatures for this region average at 20°C with no frost in most areas (Schulze, 1982). In addition, the probability of maintaining high virus populations in weed and other crop hosts as well as high aphid populations is greater, facilitating higher inoculum pressures on the pepper crop. In contrast, the Natal Midlands experience low winter temperatures with frost occurring frequently, possibly interrupting the disease cycle sufficiently to reduce virus incidence during the early stages of the season. Similarly, Northern Natal/Zululand experiences extreme summer temperatures unfavourable for most vegetable production.

Although the incidence of CMV in peppers in Natal seems negligible at the moment, the possibility exists that it may in the future become a serious pathogen. It has a natural host range greater than any other aphid-borne virus, including perennial species and plants in which it is seed-borne (Quiot *et al.*, 1975). In addition, the most severe yield losses in pepper are due to mixed infections (Conti and Masenga, 1977). Mixed infections of two or

more viruses in a single pepper plant have been found frequently in California (Abdalla *et al.*, 1991), amounting to as much as 90% of diseased pepper plants. Thus, the potential for CMV infections of peppers to gain significance is great. Differential hosts may be useful in separating and further identifying viruses in mixed infections.

The pepper virus disease situation in Natal is unlikely to improve and will probably become even more serious unless disease control measures not currently practised are implemented widely. The pattern and spread of migrating aphids should be established with a view to minimising virus spread. Also, other viruses not included in this study, such as pepper veinal mottle virus (PVMV) reported in West Africa and Ethiopia (DeWijs, 1973; Agranovsky, 1993), should be investigated in future studies.

2.5 Literature Cited

Abdalla, O.A., Desjardins, P.R. and Dodds, J.A. 1991. Identification, disease incidence, and distribution of viruses infecting peppers in California. Plant Dis. 75 : 1019-1023.

Agranovsky, A.A. 1993. Virus diseases of peppers (*Capsicum annuum* L.) in Ethiopia. J. Phytopathol. 138 : 89-97.

Agrios, G.N. and Walker, M.E. 1985. Effect of cucumber mosaic virus inoculation at successive weekly intervals on growth and yield of pepper (*Capsicum annuum*) plants. Plant Dis. 69 : 52-55.

Anon., 1992. Hot stuff: The chilli pepper in Mexico. Courier Agrochem 2.92. pp. 21-22.

Burgmans, J.L., Fry, P.R. and Sunde, R.G. 1986. Peppers: Survey of virus diseases of *Capsicum annuum* in Hawke's Bay and Poverty Bay. N. Z. J. Expt. Agr. 14 : 459-463.

Cho, J., Mau, R., Gonsalves, D. and Mitchell, W.C. 1986. Reservoir weeds of tomato spotted wilt virus. Plant Dis. 70 : 1014-1017.

Cho, J.J., Mau, R.F.L., German, T.L., Hartmann, R.W., Yudin, L.S., Gonsalves, D. and Provvidenti, R. 1989. A multidisciplinary approach to management of tomato spotted wilt virus in Hawaii. Plant Dis. 73 : 375-383.

Conti, M. and Lovisolo, O. 1983. Virus problems in protected vegetable crops. Acta Hort. 127 : 83-99.

Conti, M. and Masenga, V. 1977. Identification and prevalence of pepper viruses in Northwest Italy. Phytopath. Z. 90 : 212-222.

Conti, M., Caciagli, P. and Casetta, A. 1979. Infection sources and aphid vector in relation to the spread of cucumber mosaic virus in pepper crops. Phytopath. Medit. 18 : 123-128.

Cook, A.A. 1959. Virus disease resistance in peppers. Proc. Florida State Hort. Soc. 72 : 110-112.

de Bokx, J.A. and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descrip. Plant Viruses No.242.

Delgado-Sanches, S. and Grogan, R.G. 1970. Potato virus Y. CMI/AAB Descrip. Plant Viruses No.37.

Demski, J.W. 1981. Tobacco mosaic virus is seedborne in pimento peppers. Plant Dis. 65 : 723-724.

DeWijs, J.J. 1973. Pepper veinal mottle virus in Ivory Coast. Neth. J. Pl. Path. 79 : 189-193.

Florini, D.A. and Zitter, T.A. 1987. Cucumber mosaic virus (CMV) in peppers (*Capsicum annuum* L.) in New York and associated yield losses. Phytopathology 77 : 652 (Abstr.).

Fritzsche, R., Lehemann, K.E. and Proesler, G. 1972. Tierische vectoren pflanzenpathogener viren. Fischer, Jena. (In: **Palukaitis *et al.*, 1992**)

Gorter, G.J.M.A. 1977. Index of Plant Pathogens. Dept of Agricultural Technical Services, South Africa, Science Bulletin No. 392, 177p.

Green, S.K. and Kim, J.S. 1991. Characteristics and control of viruses infecting peppers: A literature review. Technical Bulletin No.18. Asian Vegetable Research and Development Centre. 60p.

Greenleaf, W.H. 1986. Pepper breeding. In: Breeding Vegetable Crops. M.J. Bassett (Ed.). AVI Publishing Co. Inc., Westport, Connecticut. 548p.

Greenough, D.R. and Black, L.L. 1984. Occurrence of *Frankliniella occidentalis* in Louisiana. Phytopathology 75 : 1362.

Greenough, D.R., Black, L.L. and Bond, W.P. 1990. Aluminium-surfaced mulch: An approach to the control of tomato spotted wilt virus in Solanaceous crops. Plant Dis. 74 : 805-808.

Heiser Jr., C.B. and Smith, P.G. 1953. The cultivated *Capsicum* peppers. Econ. Bot. 7 : 214-226.

Hull. R. 1993. Nucleic acid hybridisation procedures. In: Diagnosis of plant virus diseases. R.E.F. Matthews (Ed.). CRC Press, London. pp. 253-272.

Igwegbe, E.K.C. and Ogungbade, O.K. 1985. Evaluation of pepper cultivars under greenhouse conditions for resistance to a defoliation strain of tobacco mosaic virus. Plant Dis. 67 : 317-320.

Laird, E.F. and Dickson, R.C. 1963. Tobacco etch virus and potato virus Y in pepper, their host plants and insect vectors in Southern California. Phytopathology 53 : 48-52.

Makkouk, K.M. and Gumpf, D.J. 1974. Further identification of naturally occurring virus diseases of pepper in California. Plant Dis. Reptr. 58 : 1002-1006.

Marchoux, G., Gébré-Selassie, K. and Villevieille, M. 1991. Detection of tomato spotted wilt virus and transmission by *Frankliniella occidentalis* in France. Plant Pathol. 40 : 347-351.

Marco, S. and Cohen, S. 1979. Rapid detection and titer evaluation of viruses in pepper by enzyme-linked immunosorbent assay. Phytopathology 69 : 1259-1262.

Marte, M. and Wetter, C. 1986. Occurrence of pepper mild mottle virus in pepper cultivars from Italy and Spain. J. Plant Dis. Prot. 93 : 37-43.

Martelli, G.P. and Quacquarelli, A. 1983. The present status of tomato and pepper viruses. Acta Hort. 127 : 39-64.

Mills, P.R. and Abdul-Magid, A.G.M. 1987. Infection of *Capsicum frutescens* with potato virus Y and tobacco etch virus in the Sudan. Plant Dis. 71 : 557 (Abstr.).

Nono-Womdim, R., Gébré-Selassie, K., Palloix, A., Pochard, E. and Marchoux, G. 1993. Study of multiplication of cucumber mosaic virus in susceptible and resistant *Capsicum annuum* lines. Ann. appl. Biol. 122 : 49-56.

Pares, R.D. and Gunn, L.V. 1989. The role of non-vector soil transmission as a primary source of infection by pepper mild mottle and cucumber mosaic viruses in glasshouse-grown *Capsicum* in Australia. J. Phytopathol. 126 : 353-360.

Palukaitis, P., Roossinck, M.J., Dietzgen, R.G. and Francki, I.B. 1992. Cucumber mosaic virus. Adv. Virus Res. 41 : 281-339.

Quiot, J.B., Douine, L., Marchoux, G. and Devergne, C. 1975. Ecologie du virus de la mosaique du cocombre dans le Sud-Est de la France. I. Recherches des plantes spontanees hotes du V.M.C. caracterisation des populations virales. 4th Congr. Medit. Phytopath. Union, Zadar, Oct. 5-11, pp. 159-160. (In **Conti and Masenga, 1977**).

Raccah, B., Gal-On, A. and Eastop, V.F. 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus Y to peppers in Israel. Ann. appl. Biol. 106 : 451-460.

Roodeplaat Bulletin. 1991. Paprika inisiërings-vergadering gehou op Roodeplaat. No. 29

Schulze, R.E. 1982. Agrohydrology and climatology of Natal. Agricultural Catchments Research Unit, Report no. 14. Dept. of Agricultural Engineering, Univ. of Natal.

Sharma, O.P., Sharma, P.P., and Chowfla, S.C. 1989. Inheritance of resistance to potato virus Y in garden pepper (*Capsicum annuum* L.). Euphytica 42 : 31-33.

Sherwood, J.L., Reddic, B.B., and Conway, K.E. 1986. Reaction of Bahamian hot pepper to single and double infection with tobacco mosaic virus (TMV) and potato virus Y (PVY). Phytopathology 76 : 1126 (Abstr.)

Statistics on Fresh Produce Markets. Report No. 29. Issued by Directorate Agricultural Economic Trends. Dept. of Agriculture, Pretoria.

Stobbs, L.W., Broadbent, A.B., Allen, W.R. and Stirling, A.L. 1992. Transmission of tomato spotted wilt virus by the Western Flower Thrips to weeds and native plants found in Southern Ontario. Plant Dis. 76 : 23-29.

Sunday Times, Business Times. 1992. Paprika booster. February 2, 1992

Tanzi, M., Betti, L. and Canova, A. 1986. Analysis of growth and yield in some commercial pepper varieties infected by different TMV pepper strains. In: EUCARPIA VI. Meeting on Genetics and Breeding on *Capsicum* and Eggplant. 204 p.

Thompson, G.J. 1980. Plant host virus interactions: Studies on a *Capsicum* mosaic and tomato spotted wilt. M.Sc Thesis. University of Natal, Pietermaritzburg. 174 pp.

Villalón, B., 1981. Breeding peppers to resist virus diseases. Plant Dis. 65 : 557- 562.

Zitter, T.A., Florini, D. and Provvidenti, R. 1984. Virus diseases of pepper. Vegetable Crops Fact Sheet. # 730.00 Cornell University, Washington.

CHAPTER 3

Weeds as Capsicum Virus Sources

3.1 Introduction

Weeds...."plants growing in ground that are or have been in cultivation, usually to the detriment of the crop or to the disfigurement of the place"

(Webster, 1976).

"plants with harmful or objectionable characteristics that grow where they are unwanted"

(Duffus, 1971).

Infections of uncultivated plants, including virus infections have long escaped attention. Virus infections were first detected in cultivated plants by clear symptoms, by often heavy (epidemic) incidence due to the uniformity of the crop, and by their impact on yield and quality (Bos, 1981). The last makes virus infections of crops economically important. So crops are closely watched by their growers down to the level of individual plants, and weights (yields) are determined. Thus, even "latent" viruses may attract attention if they only affect plant or crop weight. Plants that have been killed because of infection may still betray their earlier presence by gaps in otherwise even stands. In contrast, most virus infections in mixed wild vegetations escape attention.

Wild species, including weeds, are generally very variable (Bos, 1981). No two plants react identically to infection. High natural competition rapidly leads to the selection for resistance and latency (symptomless infection) and sensitive host plants and virulent virus strains disappear. For example, *Stellaria media* (L.) Cyr. plants showed more severe reactions when infected with alien strains of CMV than when infected with virus of the same origin (Tomlinson and Walker, 1973). Moreover, the spacial distribution of diverse plants in natural vegetation, prevents or reduces direct contact between susceptible plants and thus reduces the chance of rapid epidemic infection that would be noticed.

However, it was soon realised that the mere presence of viruses in wild plants may have a bearing on the health of nearby crops. Since the 1920s, when Doolittle and Walker (1925)

substantiated the overwintering of CMV in uncultivated species, the information on the role of virus infections of wild plants has expanded enormously. For crop management, one needs to decide whether, when and to what extent wild plants should be removed to avoid economic damage by viruses in crops. So quantitative data on their relative importance is useful in pathosystems management.

3.2 The Identification of Possible Capsicum Virus Alternate Hosts in Natal

3.2.1 Introduction

Potato virus Y (PVY) affects production of capsicums in Natal. The mosaic disease causes heavy commercial losses, frequently reaching 100% incidence in certain areas. Since PVY is not seed-borne in peppers (Raccah *et al.*, 1985), the primary infection occurs from external sources. In order to establish the potential host range of a Capsicum strain of PVY isolated from field infected peppers in Natal, a glasshouse study was initiated using several solanaceous species commonly found in vegetable producing areas of Natal.

In addition, although TSWV has not been found to infect peppers and only low levels of CMV were detected in certain areas of Natal (Section 2.4), a survey to determine the natural weed hosts of these viruses was carried out near Capsicum plantings. The indexing of naturally occurring weeds which could act as reservoirs for PVY was also carried out.

3.2.2 Materials and Methods

Glasshouse study

Datura stramonium L., *Nicandra physaloides* L., *Physalis viscosa* L., *Solanum elaegnifolium* Cav., *S. nigrum* L., *S. velosum* L., *S. aculeastrum* L., and *S. mauritianum* L. were grown from field-collected seed (**Figure 3.2.1**). Seeds from *S. mauritianum*, *S. nigrum* and *D. stramonium* were first shaken in a 10^{-4} M solution of GA_3 (gibberellic acid) overnight to overcome seed dormancy and alternating temperature and light requirement factors (M. Rijkenberg, personal communication). Thereafter, all seed was germinated in moist petri plates under natural light. Young seedlings were transplanted into pots (6 per

species) and maintained in a controlled temperature glasshouse between 18-35° C for approximately 3 weeks prior to inoculation.

Figure 3.2.1 Solanaceous weeds grown in the glasshouse. Left to right: ***P. viscosa, S. aculeastrum, S. elaegnifolium, S. velosum, D. stramonium, N. physaloides, S. mauritianum*** **and** ***S. nigrum.***

A mechanical inoculation technique was used to inoculate the test plants with a PVY isolate obtained from a field-infected pepper plant exhibiting severe mosaic symptoms. PVY was maintained in a pepper plant in the glasshouse. Infected leaf tissue was crushed in buffer (0.02 M potassium phosphate buffer pH 7.5 + 0.02 M 2-mercaptoethanol) with a sterilised mortar and pestle. Cotton wool was dipped into the crude sap and rubbed gently over the test plant leaves previously dusted with carborundum. Rubbed leaves were washed with a stream of water. Uninoculated controls consisted of one representative of each species used in the treatment. The plants were maintained in the glasshouse for about 4 weeks and observed for symptom development.

Test plants were assayed using a commercially available double-antibody sandwich ELISA for PVY from Boehringer Mannheim as per manufacturers instructions. The results of ELISA were evaluated using a Biotek Instruments EL-307 plate reader at A_{405nm}. Results were considered positive when ELISA values of double or more than that of the negative

control were obtained. All the results obtained from ELISA were the averages of two replications. *Nicotiana tabacum* L. "Samsun NN" were inoculated with crude sap from each test plant to confirm ELISA results. Leaf dip electron microscopy was carried out with a Jeol 100CX transmission electron microscope when a positive reaction was observed. Leaves from infected Samsun NN and test plants were dipped several times in a drop of sterile water placed onto a formvar coated grid. The water was removed after 30 seconds and the grid was stained with 2% phosphotungstic acid at pH 6-7 for 10-15 seconds.

Field survey

Major weeds growing in or adjacent to capsicum crops in the Pietermaritzburg and South Coast regions of Natal were collected for identification and sampled for virus infection by ELISA. Weed plants collected were pressed and stored for later identification. A book, Weeds of Crops and Gardens in Southern Africa (Grabandt, 1985) and the help of the staff at the Natal Herbarium were used in weed identifications. Concurrently, leaf samples of weeds were collected and brought into the laboratory for serological testing. Commercially available ELISA kit for PVY from Boehringer Mannheim, and TSWV and CMV indirect $F(ab')_2$ ELISA kits obtained from Dr. G. Pietersen of Plant Protection Research Institute in Pretoria were used for virus detection, as per directions specified in the kits. Three replicate wells were used for each test sample. Substrate absorbance was measured at A_{405nm} with a Biotek EL-307 microplate reader.

A *D. stramonium* L. plant sample showing unusual virus symptoms was rub-inoculated onto *N. tabacum* "Samsun NN" for purification. The purification method of Gooding and Herbert (1967) was used. Leaves were ground with a mortar and pestle in two volumes of 0.5M Na_2HPO_4-KH_2PO_4 buffer (pH 7.2) containing 1% 2-mercaptoethanol. The homogenate was then strained through cheesecloth. n-Butanol was then added to make up a final concentration of 8% by volume. The solution was stirred for 15 minutes and then centrifuged for 30 minutes at 10 000g. The supernatant was saved. Polyethylene glycol (PEG) at 4% w/v was added and stirred until dissolved. The solution was centrifuged for 15 minutes at 10 000g and the pellet resuspended in 0.01M phosphate buffer. The PEG step was then repeated adding 0.4g PEG and 0.4g NaCl per 10 ml suspension, stirring until dissolved. The solution was then centrifuged again for 15 minutes at 10 000g and the pellet

resuspended in 0.01M phosphate buffer, clarified in a microfuge and the supernatant stored. Electron microscopic examination of the supernatant was then carried out, with preparations negatively stained with 2% phosphotungstate. A back-inoculation technique was also carried out from virus infected Samsun NN into a healthy *D. stramonium* plant grown in the glasshouse.

3.2.3 Results

Glasshouse Study

The ELISA results and expression of PVY symptoms in Samsun NN obtained in the study are listed in **Table 3.2.1**. *D. stramonium*, *P. viscosa*, *S. velosum* and *S. mauritianum* were not infected with PVY. Only *S. aculeastrum* showed clear symptoms of virus infection. These were mosaic, vein clearing and slight blistering of the leaves (**Figure 3.2.2**). Leaf dip electron microscopy of PVY infected *S. aculeastrum* and of Samsun NN inoculated with crude sap from *S. aculeastrum* revealed typical PVY-like particles (**Figure 3.2.3**). Uninoculated controls gave negative ELISA results. Typical symptoms of vein clearing and mottling on Samsun NN were observed for all species which gave positive ELISA results except for *S. elaegnifolium* and *S. aculeastrum* which induced only slight mottling with no noticeable vein clearing. This may be due to the difficulty in obtaining crude sap from the thick and leathery leaves of these species.

Field Survey

Over 100 samples of commonly occurring weed species growing in or adjacent to Capsicum crops in the Pietermaritzburg and South Coast regions of Natal were collected. The results of ELISA assays for PVY, TSWV and CMV infection and any symptoms noted are listed in **Table 3.2.2**. *N. physaloides* and *S. nigrum* were the most common weeds growing in capsicum crops or in fallows directly adjacent to the crop. These tested positive for PVY in all samples collected (10/10 and 7/7 respectively). Only 1 of the 10 *N. physaloides* samples collected was infected with CMV. TSWV incidence in *S. nigrum* was low with 1 of 7 samples testing positive for the virus.

Figure 3.2.2 PVY symptoms on *S. aculeastrum*. (A) Plant symptoms, (B) Leaf symptoms.

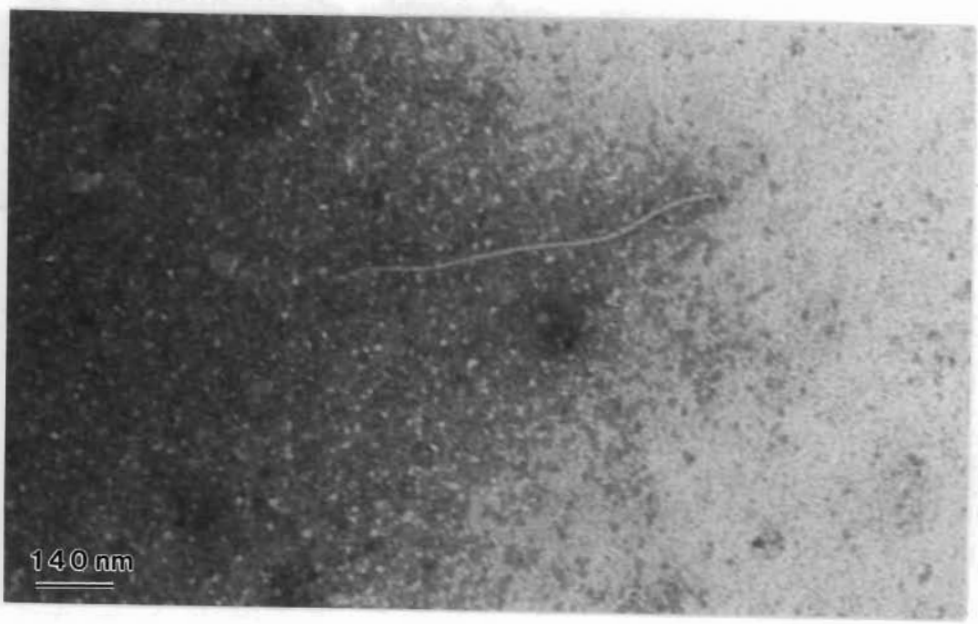

Figure 3.2.3 TEM micrograph of PVY-like virus particles from *S. aculeastrum*.

Table 3.2.1 Weed species mechanically inoculated with PVY, ELISA results and reactions in Samsun NN tobacco inoculated with sap extracted from the test plants.

Weed species	PVY ELISA Result[1]	Samsun NN Symptoms[2]
Datura stramonium	0/5	None
Nicandra physaloides	5/5	vc, m
Physalis viscosa	0/5	None
Solanum elaegnifolium	4/5	m
S. nigrum	5/5	vc, m
S. velosum	1/5	None
S. aculeastrum	5/5	m
S. mauritianum	0/5	None

[1]No. of plants tested positive/no. inoculated

[2]vc = veinclearing, m = mottle

Two *D. stramonium* plants exhibiting mosaic, veinclearing and leaf blistering symptoms were collected in the Pietermaritzburg area from within a tomato crop. These were ELISA positive to PVY. Samsun NN tobacco inoculated with sap from these plants, exhibited typical PVY symptoms (**Figure 3.2.4**). Virus purified from the tobacco showed typical potyvirus-like flexuous particles approximately 670nm long (**Figure 3.2.4**). However, attempts to reinoculate virus-free *D. stramonium* raised in the glasshouse failed.

Species commonly occurring in pepper growing regions which did not appear to be infected with PVY, CMV or TSWV were *Sida alba* L., *Corchorus* sp., *Lantana camara* L., *Amaranthus hybridus* L., *A. thumbergii* Moq., *Ageratum* sp., *A. houstonianum* Mill., *Chromolaena odorata* L., *Senecio brachypodus* DC., *Justicia flava* (Forssk.)Vahl, and *Senna occidentalis* (L.) Link. It is important to point out that *A. hybridus* was identified as a major weed host of TSWV and its thrips vector in Hawaii and Canada (Cho *et al.*, 1986; Stobbs *et al.*, 1992).

Table 3.2.2 Plant species from two pepper growing areas in Natal naturally infected with PVY, CMV and TSWV in 1993 and 1994.

Scientific and common name	ELISA Results[1] PVY	CMV	TSWV	Symptoms[2]
Chenopodiaceae				
Chenopodium album L.	0/1	1/1	0/1*	
White goosefoot				
C. carinatum L.	0/1	1/1	0/1	
Green goosefoot				
Compositae				
Acanthospermum hispidum DC.	2/2	0/2	0/2	
Upright starbur				
Bidens pilosa L.	1/3	2/3	1/3*	
Common blackjack				
Galinsoga parviflora Cav.	0/2	1/2*	0/2*	
Gallant soldier				
Tagetes minuta L.	0/1	1/1	0/1	
Mexican marigold				
Malvaceae				
Sida cordifolia L.	0/1	0/1	1/1	
Flannel weed				
Solanaceae				
Nicandra physaloides (L.)Gaertn.	10/10*	1/10	0/10*	nc
Apple of Peru				
Solanum nigrum L.	7/7*	0/7*	1/7*	
Black nightshade				
Datura stramonium L.	2/5	0/5*	0/5*	sm, ld,
Jimsonweed				

[1]No. of samples infected/Total no. indexed

[2]sm=systemic mosaic/mottling; ld=leaf deformation; nc=necrotic spots. Absence of description indicates no symptoms or latent infection.

*previously recorded virus hosts

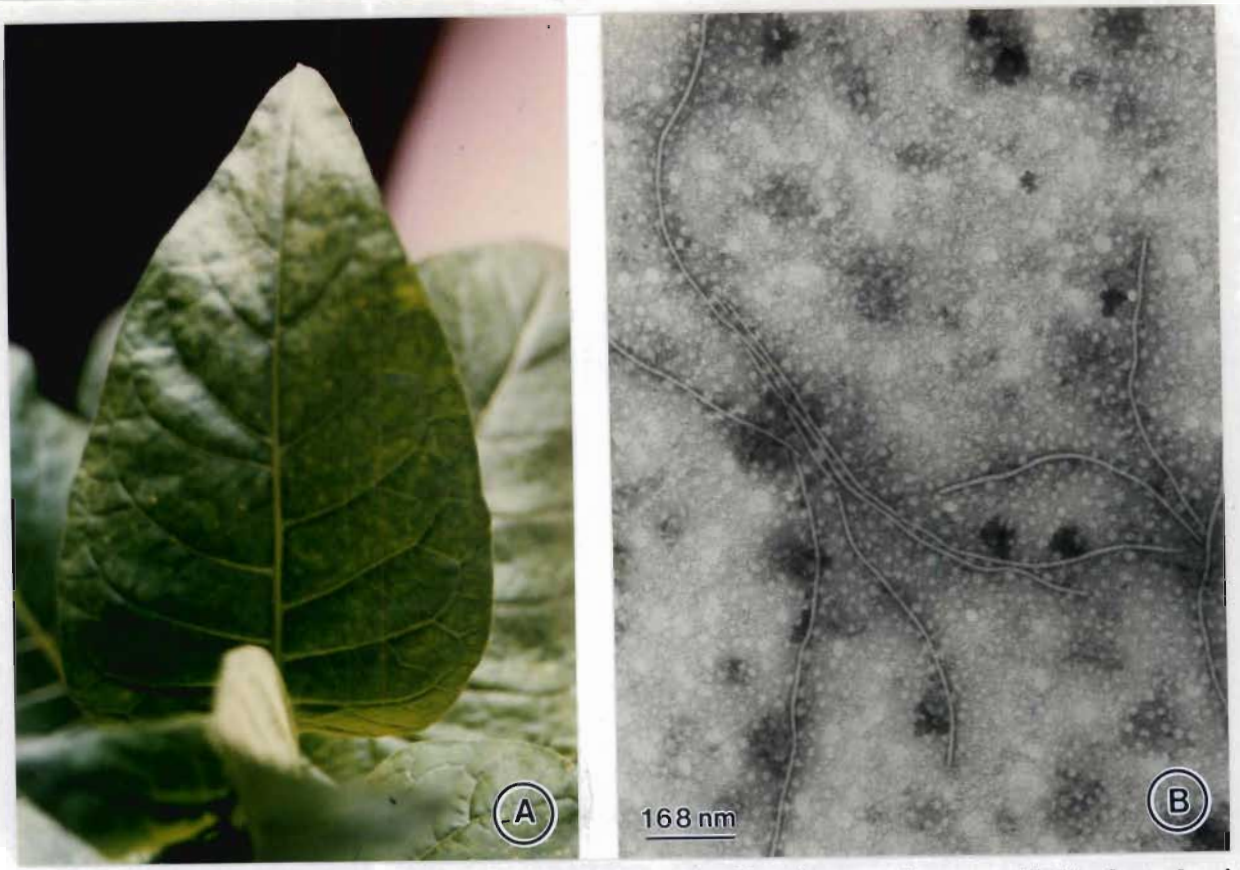

Figure 3.2.4 (A) PVY-like virus symptoms on *N. tabacum* Samsun NN infected with Datura virus. (B) TEM micrograph of PVY-like virus particles purified from Samsun NN infected with Datura virus.

3.2.4 Discussion

Few viruses cause conspicuous, recognisable symptoms in wild hosts (Tomlinson, 1987), and as a result, the importance of potential naturally occurring virus hosts is overlooked. Several solanaceous weeds collected in vegetable producing areas were found to be susceptible to PVY, with only one, *S. aculeastrum*, exhibiting easily recognisable virus symptoms. In addition, symptom expression is often variable, depending on the age of the plant, when it became infected, its nutritional level and environmental conditions (Stobbs *et al.*, 1992). Symptoms apparent in controlled environment conditions may not necessarily be the same as those seen under field conditions. The importance of wild plants as virus sources does not only depend on their susceptibility to a virus, however. It also depends on vector behaviour, efficiency and abundance, on other sources of infection and on the wild plants themselves, their abundance, time of availability and distance from the sensitive crop (Bos, 1981). Association between vector and virus source plants and subsequent movement to other susceptible plants are essential for transmission of plant virus diseases by insects (Carter, 1961).

PVY was detected in four species of wild plants growing either in the proximity of, or inside the pepper fields investigated. A fifth species, *D. stramonium*, was found exhibiting virus symptoms in the Pietermaritzburg district. It reacted positively to PVY ELISA, although it is reported to be immune to all tested strains of the virus (de Bokx and Huttinga, 1981). PVY-like virus particles were purified from rub-inoculated Samsun NN tobacco. Attempt to reinoculate *D. stramonium* with the virus originally isolated from it, however, failed. It is unlikely that a false ELISA positive was obtained since other *D. stramonium* samples collected in the same area, which did not exhibit symptoms, did not react positively in the same experiment. The inability to reinfect *D. stramonium* with the Datura virus isolate may be attributed to the possible loss of some unknown helper factor during the transfer of virus from the weed to tobacco. It has been observed that the infection of a plant by one virus may considerably enhance the accumulation of a co-infecting virus inoculated simultaneously or later (Valkonen, 1992). This phenomenon has often been interpreted as the ability of the "helper" virus to complement defective movement functions of the "helper-dependent" virus. The conclusion has been drawn from the observation that, although the cells of the host are susceptible to the helper-dependent virus following inoculation of isolated protoplasts, very low titres of the helper-dependent virus accumulate in whole plants when infected by this virus alone. The complementation may be in cell-to-cell and/or long distance movement and it has been shown to be non-specific amongst plant viruses (Taliansky *et al.*, 1982; Malyshenko *et al.*, 1989). For example, the accumulation of PVY in *Solanum brevidens* Phill., was enhanced over 1000-fold when doubly infected with TMV or PSTVd (potato spindle tuber viroid) (Valkonen, 1992). However, since the occurrence of PVY infected *D. stramonium* was limited to only two examples, and the characterisation of this virus was beyond the scope of this study, no further work in this regard was carried out.

Although sampling of peppers in Natal failed to reveal any evidence of TSWV in field populations and CMV incidence was insignificant, sampling of weeds in or near pepper crops yielded several species naturally infected with strains of these viruses. Thus, these may present a potential threat to Capsicum production. It is possible that virulent strains of these viruses may be introduced through the distribution of infected seedlings and become established in these hosts, causing a significant increase in virus incidence. Several species such as *A. hybridus*, *B. pilosa* and *N. physaloides* have been described as important weed

hosts of TSWV in vegetable producing areas of Hawaii (Cho *et al.*, 1986). Similarly, *S. nigrum* and *G. parviflora* are reported hosts of CMV in pepper fields in Italy (Conti *et al.*, 1979; Crescenzi *et al.*, 1993).

The majority of species naturally infected with PVY, CMV or TSWV were symptomless and therefore could only be detected by checking the natural vegetation at random. The question of whether and to what extent these weeds are major sources of inoculum for mosaic disease outbreaks in peppers depends on the fact that only the species found infected before peppers were transplanted to the field can be regarded as potential sources of virus infection for the local crops. Weeds found infected when virus was already widespread in pepper crops should therefore be regarded more as storage than donor hosts, although it cannot be excluded that they may occasionally act as secondary sources of infection. Further investigations into the associations between virus reservoir plants, vectors and crops are needed.

3.3 The Spread of PVY into Capsicums on the Natal South Coast from Two Solanaceous Weed Species

3.3.1 Introduction

Previous investigations on virus diseases of pepper (*Capsicum annuum* L.) in Natal have shown that these are severely affected by aphid transmitted viruses of the nonpersistent type (K.Budnik *et al.*, unpublished). Potato virus Y (PVY) was found to be the most important, responsible for severe losses, reaching as much as 100% incidence in areas of the Natal South Coast. The present study reports on the epidemiology of PVY on the Natal South Coast with particular regard to its spread in the local pepper crops.

3.3.2 Materials and Methods

Pepper Field

The spread of PVY into a pepper crop in the Umkomaas region of the Natal South Coast was monitored during May-September 1993 (**Figure 3.3.1**). Pepper seedlings cv. California Wonder were sampled before planting and thereafter in the field at 15-25 day intervals,

starting 1 week after planting. Samples from the field were collected on a random basis, however the position of each sample collected was mapped for its location within the field. Approximately 30 samples were collected each time. A commercially available antibody sandwich ELISA for PVY from Boehringer Mannheim was used for virus determination as per manufacturer's instructions.

Winged aphids were trapped using Bug Traps (Green Research, P.O. Box 541, Caledon 7230) (**Figure 3.3.2**). Ten yellow traps, each 11.5 x 14 cm coated with a clear adhesive, were placed in the pepper field at the level of the plant tops.

Virus Reservoirs

Surveys started before peppers were transplanted to the field and continued during the growing season. All the cultivated and wild plants growing adjacent to the pepper crop were surveyed. PVY infection was determined using ELISA. Serological tests to determine PVY field hosts were made on 30 individuals of 7 species. These were crops of peppers, chillies and brinjals cultivated adjacent to the pepper crop studied as well several weed species, *Datura stramonium* L., *Nicandra physaloides* (L.)Geartn., *Solanum nigrum* L., *Bidens pilosa* L., and volunteer tomatoes from the previous season, growing in and around the crop (**Figure 3.3.3**).

3.3.3 Results

Virus Reservoirs

The species infected with PVY before the peppers were transplanted to the field were: other crops of chillies and peppers, old *S. nigrum* plants and dense populations of young *N. physaloides* growing in a bank adjacent to the field (**Figure 3.3.4**). Later in the season, from August to September, PVY was isolated from peppers and young *S. nigrum* plants appearing in the same area where dense populations of *N. physaloides* previously occurred (**Figure 3.3.5**).

Figure 3.3.1 Pepper crop studied in the Umkomaas region of the Natal South Coast.

Figure 3.3.2 Bug traps used in aphid trapping on the Natal South Coast.

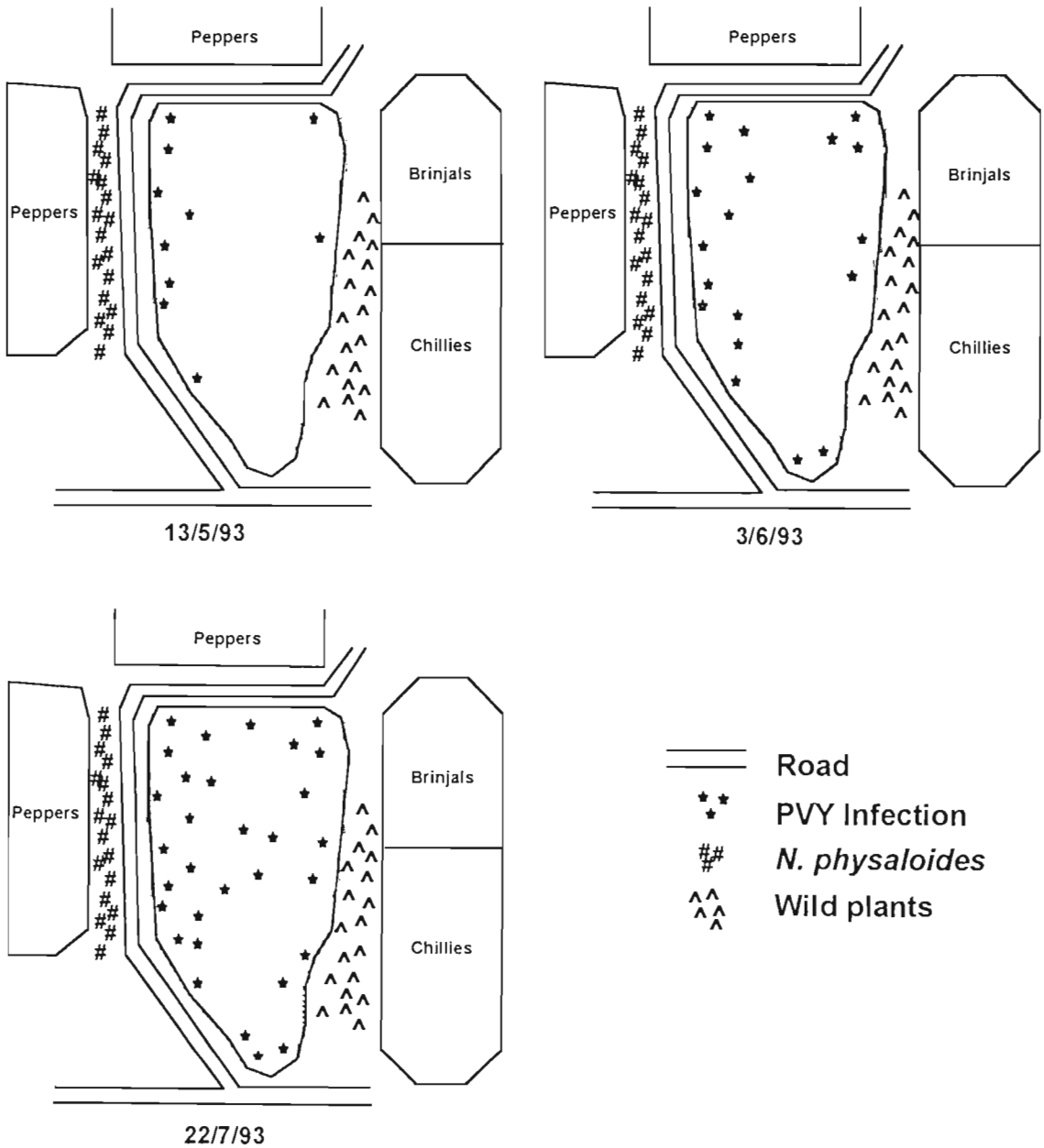

Figure 3.3.3 PVY spread in a pepper field near Umkomaas on the Natal South Coast from *N. physaloides*.

Figure 3.3.4 Bank of *N. physaloides* growing adjacent to a pepper crop on the Natal South Coast.

Figure 3.3.5 (A) Young *Nicandra physaloides* plants. (B) Young *Solanum nigrum* plants observed around and within pepper crops.

Pepper Field

Healthy peppers were planted on May 6th, and PVY was first detected on May 13th in about 15% of samples collected (see **Figure 3.3.3**). Most of the infected plants were positioned on the edges of the field, in particular closest to the bank of *N. physaloides*. By June 3rd virus incidence increased sharply to about 55% of samples collected. On the 22nd of July over 70% of samples collected was ELISA positive and by August 30th, 100% infection was detected, confirmed by visual inspection of the field with all pepper plants exhibiting virus symptoms, ranging from severe mosaic and stunting to mild vein clearing and leaf deformation. The peak number of aphids trapped occurred between May-June; 650 aphids were trapped during the period May 24th and June 8th (**Figure 3.3.5**). Although no aphid colonies were observed on peppers, several colonies were noticed on nearby *N. physaloides* plants and later on *S. nigrum* (**Figure 3.3.6**).

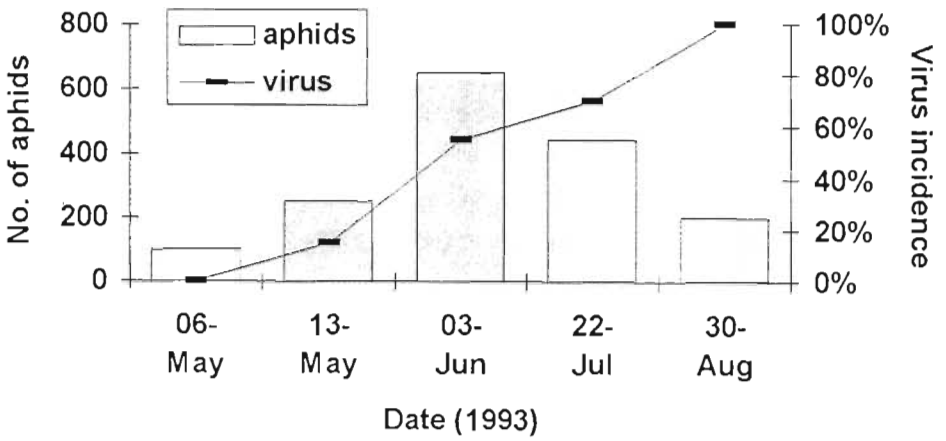

Figure 3.3.5 Progress of PVY infection in peppers, and winged aphid frequency, in a crop on the Natal South Coast.

Figure 3.3.6 Aphid colonies on *Nicandra physaloides* (A) and *Solanum nigrum* (B) growing adjacent to a pepper crop on the Natal South Coast.

3.3.4 Discussion

N. physaloides populations adjacent to the crop appear to be the primary source of PVY infecting peppers on the Natal South Coast. Virus spread into the pepper crop was very rapid with first signs of infection occurring only 1 week after planting. The incidence of PVY in the pepper crop was so high and developed over such a short time that only movement from local source plants can account for it. In addition, the bulk of early infected plants was regularly dispersed on the edge of the field (see **Figure 3.3.4**) suggestive of aphid transmission from a source short distance away. The progress of PVY infection in peppers appears to be closely related with the density of winged aphids trapped. Virus incidence increased most rapidly during May-June, when aphid density was at its peak. Conti *et al.* (1979) suggest that where infection sources are present close to crops, heavy aphid influx causes rapid spread of virus in the vicinity of such sources. The presence of aphid colonies on *N. physaloides* supports this evidence.

PVY infected *S. nigrum* plants appeared later in the season when most *N. physaloides* plants reached the end of their life cycle. *S. nigrum*, therefore does not appear to play a role in the primary spread of PVY into pepper crops, but may aid in the maintenance of high

levels of PVY during the summer, when high temperatures prohibit cultivation of plants which may act as virus hosts (**Figure 3.3.7**).

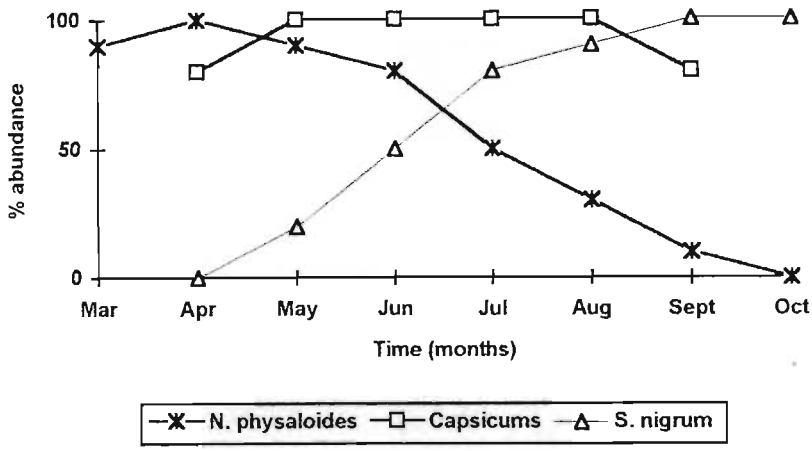

Figure 3.3.7 Relative abundance of hosts to PVY during the year, on the Natal South Coast.

Laird and Dickson (1963) found that the rapidity of virus spread appears to be more important than the final disease incidence, from the standpoint of fruit yield and quality in peppers. Pepper plants infected at any time up to the appearance of newly set fruit yielded little or no fruit,or distorted fruit. Diseased plant roguing and insecticide treatment have not been successful in the past (Raccah, 1986; Loebenstein and Raccah, 1980). Primary spread control by eliminating virus reservoir plants may be a practical solution to slowing virus spread for long enough to obtain satisfactory yields.

3.4 Literature Cited

Bos, L. 1981. Wild plants in the ecology of virus diseases. In: Plant diseases and vectors: Ecology and epidemiology. K. Maramorosch and K.F. Harris (Eds.). Academic Press, New York. pp. 1-33.

Carter, W. 1961. Ecological aspects of plant virus transmission. Annu. Rev. Entomol. 6 : 347-370.

Cho, J.J., Mau, R.F.L., Gonsalves, D. and Mitchell, W.O. 1986. Reservoir weed hosts of tomato spotted wilt virus. Plant Dis. 70 : 1014-1017.

Conti, M., Caciagli, P. and Casetta, A. 1979. Infection sources and aphid vectors in relation to the spread of cucumber mosaic virus in pepper crops. Phytopath. Medit. 18 : 123-128.

Crescenzi, A., Barbadossa, L., Gallitelli, D. and Martelli, G.P. 1993. Cucumber mosaic cucomovirus populations under natural epidemic conditions and after a satellite-mediated protection test. Plant Dis. 77 : 28-33.

de Bokx, J.A. and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descr. Plant Viruses. No. 242.

Doolittle, S.P. and Walker, M.N. 1925. Further studies on the overwintering and dissemination of cucurbit mosaic. J. Agric. Res. 31 : 1-58.

Duffus, J.E. 1971. Role of weeds in the incidence of virus diseases. Ann. Rev. Phytopathol. 9 : 319-340.

Gooding, G.V. and Herbert, T.T. 1967. A simple technique for purification of tobacco mosaic virus in large quantities. Phytopathology 57 : 1285.

Grabandt, K. 1985. Weeds of crops and gardens in Southern Africa. Seal Publishing, Johannesburg, 134 pp.

Laird, E.F. and Dickson, R.C. 1963. Tobacco etch virus and potato virus Y in pepper, their host plants and insect vectors in Southern California. Phytopathology 53 : 48-52.

Loebenstein, G. and Raccah, B. 1980. Control of non-persistently transmitted aphid-borne viruses. Phytoparasitica 8 : 221-235.

Malyshenko, S.I., Kondakova, O.A., Taliansky, M.E. and Atabekov, J.G. 1989. Plant virus transport function: complementation by helper viruses is non-specific. J. Gen. Virol. 70 : 2751-2757.

Raccah, B. 1986. Nonpersistent viruses: Epidemiology and control. Adv. Virus Res. 31 : 387-429.

Raccah, B., Gal-On, A. and Eastop, V.F. 1985. The role of flying aphid vectors in the transmission of cucumber mosaic virus and potato virus Y to peppers in Israel. Ann. appl. Biol. 106 : 451-460.

Stobbs, L.W., Broadbent, A.B., Allen, W.R. and Stirling, A.L. 1992. Transmission of tomato spotted wilt virus by the Western Flower thrips to weeds and native plants in Southern Ontario. Plant Dis. 76 : 23-29.

Taliansky, M.E., Malyshenko, S.I., Pshennikova, E.S., Kaplan, I.B., Ulanova, I.B. and Atabekov, J.G. 1982. Plant virus-specific transport function. I. Virus genetic control required for systemic spread. Virology 122 : 318-326.

Tomlinson, J.A. 1987. Epidemiology and control of virus diseases of vegetables. Ann. appl. Biol. 110 : 661-681.

Tomlinson, J.A. and Walker, V.M. 1973. Further studies on seed transmission in the ecology of some aphid-transmitted viruses. Ann. appl. Biol. 73 : 293-298.

Valkonen, J.P.T. 1992. Accumulation of potato virus Y is enhanced in *Solanum brevidens* also infected with tobacco mosaic virus or potato spindle tuber viroid. Ann. appl. Biol. 121 : 321-327.

Webster, N. 1976. Webster's Third New Dictionary of the English Language (unabridged). Merriam Co., Springfield, Mass., USA. 2662 p.

CHAPTER 4

The Control of Capsicum Viruses

4.1 Introduction

"Indeed, a rather impressive amount of advanced research has already been reported on the occurrence, identity, and mode of action of a variety of antiviral agents in plants. However, not one of these agents, whether of natural or synthetic origin, has yet been developed to a stage whereby it can be applied in the field for the control of infection or spread of a virus disease in a crop. Hence, with plant virus chemotherapy being still in its infancy, the only available means of controlling plant viruses are, at least for the time being, in the area of indirect control approaches."

Isaak Harpaz, 1982

Control of aphid-borne viruses, particularly those transmitted in a nonpersistent manner, has proven very difficult to accomplish (Simons, 1982). Such obvious approaches as vector control, have largely failed to effect acceptable levels of control. Thus researchers are looking towards procedures which are involved with the interference in the transmission process. Quiot *et al.* (1982) suggest that the first aim in controlling virus disease is to reduce or eliminate virus spread. The second is to avoid the appearance of virulent strains that are able to produce unexpected epidemics that disrupt harvesting forecasts. Because the transmission cycles of vectors are complex, many opportunities can be found by virologists to break the "virus-vector-plant" cycle and stabilise virus populations.

Cultural and technical measures can be used to interfere with the transmission process at any of its phases, thereby arresting the spread of virus in the field (Harpaz, 1982). In particular,

(1) genetic manipulation aimed at producing plant varieties that are resistant to virus infection and/or to the pathogen's vectors,

(2) elimination of inoculum sources, by actual eradication of infected and suspected wild plant material,

(3) cultivation practices aimed at breaking the infection cycle by introducing sufficiently wide gaps in the availability of susceptible host plants to the virus, its vector, or both, e.g. rotation of crops,

(4) evasive measures based on taking advantage of the epidemiological pattern of a virus disease in order to adjust planting and harvesting dates to evade infection, and

(5) measures devised to reduce, to a maximum possible extent, the number of inoculative vector individuals that are active in the field, can be used.

For obvious reasons, the measure devised to reduce vectors, most commonly applied in the control of virus diseases, is still conventional treatment with pesticides in a variety of formulations. However, in cases where nonpersistent viruses are involved, spraying with aphicides is sometimes likely to increase rather then decrease the spread of disease in the crop (Loebenstien and Raccah, 1980). The reduction of inoculative vectors active in a field should rather be achieved by luring them away from crop plants, or by repelling them from and thus barring access to crop plants (Harpaz, 1982).

With increasing concern throughout the world over the environmental effects of continued reliance on toxic chemicals for pest control, let alone the rising costs of these products, far greater attention has been devoted to the development of alternative, less toxic, and less disruptive methods for controlling pests in general and vector-borne viruses in particular. This chapter reviews some research results in the area of less conventional control of vector transmission of plant viruses, and compares the efficacy of five management practices in the control of potato virus Y (PVY) in capsicums on the Natal South Coast.

4.2 Management of Vector-borne Viruses - Several Case Studies

4.2.1 Use of Insecticides

The control of air-borne insect vectors with insecticides has been more effective against persistently than nonpersistently transmitted viruses (Tomlinson, 1987). This is because with the former, aphid vectors require several hours to acquire and transmit the virus. In these situations systemic insecticides can provide effective control (Hull and Heathcote, 1967), especially when applied in granular form at planting so that the active ingredient is slowly released to maximise the period during which the plant is protected. In contrast, however, aphid transmission of nonpersistent viruses is almost immediate (Loebenstein and Raccah, 1980) and the action of the insecticide is not fast enough to kill the vector before

acquisition and inoculation (**Table 4.2.1**). In certain cases, organophosphate (OP) insecticides even caused an increase in infection with treatment, in comparison with untreated plants (Raccah, 1986). This is commonly explained by an increased excitation of sprayed aphids, which probes and inoculates more. Rice *et al.* (1983) found that an OP toxicant, Demethon-*S*-methyl, caused an increase in corticle secretion, and following that also the dispersion of the aphid colony. An exception to this is sometimes encountered, when spread is contributed by one colonising aphid species and there is little if any spread which might be attributed to incoming vectors (Raccah, 1986). In such a case, application of insecticides which reduce colonisation and the number of aphids which are likely to move between plants in the field results in a reduced virus infection.

Table 4.2.1 Effect of vector control with insecticides on dissemination of nonpersistent viruses.

Crop	Virus	Insecticide	% Infection	
			Treatment	Control
Potato	Potato virus Y	Thimet	19.5	15.7
		Rogor	18.0	15.8
		Temik	25.9	27.6
		Disyston	31.3	27.6
Sweet peas	Common pea mosaic	Menazon	54	68
		Disyston	56	68
Iris	Iris severe mosaic	Phosphamidon	13	15

(from Loebenstein and Raccah, 1980)

The failure of conventional insecticides led researchers to look for alternative insecticides. The major reason for the failure of most insecticides in reducing spread of nonpersistent viruses is their relatively slow action (Raccah, 1986). However, if the vector aphid recognises the crop as unsuitable before probing, or if the aphid can be killed before transmitting the virus, then the level of virus within the crop would be lower (Kennedy, 1976). More than 30 years ago pyrethrum was found to have a fast knock-down action. It was of little economic use at that time, since pyrethrum was photosensitive and expensive. Nevertheless, photostable synthetic pyrethroids were recently developed some of which

were found to be fast-acting insecticides. Deltamethrin and permethrin, have been reported to bring about an effective reduction in the transmission of some nonpersistent viruses (Rice *et al.*, 1983). Their efficacy appears to be due to a particularly fast intoxication of the vectors, preliminary symptoms of which may be an inhibition of feeding (Sassen, 1983). Aphid intoxication is vital to ensure that hyperactivity does not result in increased dispersal of viruliferous aphids. A recent report was on the effectiveness of the synthetic pyrethroids deltamethrin (Decis) and lamda-cyhalothrin (Karate) on the spread of CAMV (cowpea aphid-borne mosaic virus) and CMV (cucumber mosaic virus) in cowpea (Roberts *et al.*, 1993). However, it showed that neither of the two pyrethroids studied prevented the initial introduction of virus into the crop and, when incoming aphid incidence was high, virus incidence was higher in the sprayed than in the unsprayed plots. Similarly, Sassen (1983) observed that transmission of the nonpersistent bean yellow mosaic virus (BYMV) could not be effectively reduced by deltamethrin and permethrin as their activity did not prevent the short 5-10 second probes which were needed to transmit the virus. Marco (1993) found that pyrethroids that reduced PVY infection in potatoes did not protect peppers in the field to a significant extent. Thus, although pyrethroids were found to reduce virus incidence in the laboratory (Gibson and Rice, 1986), they seldom give adequate results in the field, and their combination with oil is preferred (Raccah, 1986).

4.2.2 Use of Oils

Since Bradley *et al.* (1962) first reported that mineral oil interfered with aphid transmission of PVY, many researchers have investigated the possibility of using oils for control of certain aphid-borne viruses. Research effort has been significant in Israel and Europe (Simons and Zitter, 1980), where oils are used commercially on such crops as seed potatoes, peppers, and lettuce (**Table 4.2.2**).

The mechanism by which oil prevents aphids from transmitting viruses is not understood. Both acquisition and inoculation of virus are affected by oil on the leaf (Bradley, 1963), with activity apparently greater against acquisition than against inoculation. Oil appears to have little effect on the probing and feeding behaviour of aphids (Simons *et al.*, 1977).

Table 4.2.2 Field experiments for control of nonpersistent viruses in field and vegetable crops by oil sprays.

Crop	Virus	% Control
Potato	PVA	30-50
	PVY	56-88
Bell pepper	PVY + CMV	40-90
Lettuce	Lettuce mosaic virus	43
Celery	Celery mosaic virus	34-40
Pea	Pea mosaic virus	66

(From Loebenstein and Raccah, 1980)

Oil does not appear to denature virus particles (Simons and Zitter, 1980). Transmission of particles of differing morphologies seems equally suppressed by oil. The oil's effect could involve adherence of the virus to the aphid's stylets (Bradley, 1963) or interference with sap ingestion in the food canal or possibly sap egestion, if this occurs. Efforts to elucidate the exact mechanism of action have not been successful. Vanderveken's (1977) review covers the subject in considerable detail (**Table 4.2.3**).

Oil sprays have been reported to significantly reduce primary virus spread. Small plot field trials have consistently shown 3 to 8 fold reductions in virus spread (Zitter and Ozaki, 1978), whereas under large scale field situations it has been common to observe almost total suppression of virus spread (Simons, 1982). However, oil sprays loose effectiveness as inoculum potential increases (Simons and Zitter, 1980). Factors such as aphid density, titer of transmissible virus in infected plants and plant density are all important. In addition, oil viscosity and type of spray nozzle and pressure (Tomlinson, 1987) influence the effectiveness of oil sprays, necessitating careful selection of oils and emulsifiers as well as costly spraying equipment. These factors and the fact that some oils are phytotoxic (Simons and Zitter, 1980) limit their use.

A much more pronounced effect than that of oil or pyrethroid alone has been obtained by combining the two (Raccah, 1983). In this case a fast acting pyrethroid was used in combination with Virol for the control of CMV in cucumbers. The work, however is still in its early phase, and requires clarification of the preferred oil and pyrethroid, as well as the mode of application which will yield the best results.

Table 4.2.3 Effect of oil sprays at different stages of the transmission sequence of beet mosaic virus (BMV), beet yellows virus (BYV), and pea enation mosaic virus (PEMV) by *Myzus persicae* (M.p.) or *Acyrtosiphon pisi* (A.p.).

Virus/ Vector	Stages of Aphid Transmission Sequence				
	Before acquisition	At acquisition	Between acquisition and inoculation	At inoculation	After inoculation
BMV/ M.p.	+	+	+	+	-
BYV/ M.p.	+	+	+	+	-
PEMV/ A. p.	-	-	-	-	-

+ = inhibition; - = no inhibition

(after Vanderveken, 1977)

4.2.3 Use of Plastic Mulches and other Reflective Surfaces

Aphids, like most insects, respond preferentially to certain wavelengths of light (Simons, 1982). Shortly after development of wings, aphids become strongly attracted to short wave light and fly towards the sky in a migratory or dispersal flight. After flying for several hours their response to short wave light is reversed and they are repelled by it (Kennedy 1986). Using knowledge based on insect visual stimuli, reflective mulches have been used to repel insect vectors. The first experiments were done with aluminium foil or black or transparent polyethylene sheets. Results, reviewed by Smith and Webb (1969), showed that maximum repellency was obtained when at least 50% of the crop was covered. Since then the technique has been investigated by many others and various improved types of mulches have been tested. The data on the degree of aphid repellence and the control of virus spread in several crops is summarised in **Table 4.2.4.** In Israel, mulches of grey

plastic sheeting decreased numbers of aphids in peppers by more than 80% and the spread of PVY and CMV by more than 90% (Loebenstein *et al.*, 1975). More recently, Greenough *et al.* (1990), found that an aluminium-surfaced mulch is an effective method of reducing losses from the thrips-borne tomato spotted wilt virus (TSWV) in tomato and bell pepper.

Table 4.2.4 Field experiments for control of nonpersistent viruses with reflective surfaces.

Crop or plant	Virus	Reflective material	Aphids in treated plots	% Control
Gladiolus	CMV	Aluminium foil	7-9	73-80
		White plastic	12	70
		Aluminium powder	19	55
Squash	WMV	Aluminium foil	7-10	72-94
Lettuce	CMV	Aluminium foil	25	94
Bell peppers	PVY + CMV	Aluminium foil	2-18	86
		White on black plastic	9-35	88
		Grey plastic	6-18	90

CMV = Cucumber mosaic virus; WMV = Watermelon mosaic virus;
PVY = Potato virus Y.

(after Loebenstein and Raccah, 1980)

Despite its effectiveness, this technique has not achieved widespread commercial acceptance. Three principal problems which have limited the use of reflective surfaces (Harpaz, 1982). Firstly, the cost of reflective materials is high. It can only be economical for high value crops, taking into consideration the additional beneficial effects of mulching on yields; e.g., soil temperature adjustment, weed control and water conservation. In addition, soil temperature adjustment is not always beneficial, as illustrated by Loebenstein *et al.* (1975), who observed an occasional drop in pepper yields, presumably due to a too low a temperature underneath the aluminium mulch (this will not be a factor where average temperatures are constantly high). Secondly, with the growth of the crop plants, their developing foliage progressively shades the repellent reflection of the mulch surface.

Lastly, in the case of aluminium foil, disposal after the growing season is difficult. However, some recent advancements have been made to address these problems. Plastic mulches are now available in a rainbow of colours, depending on factors such as crop type, season of the year, and whether insect management is desired (Lamont, 1993). New trends have been identified in plastic mulches, as presented at the recent 24th Congress of the American Society for Plasticulture (1993), including more recycling, reformulations and down-gauging (creating a stronger but thinner film, at a lower price), new colours, photoselectivity and most importantly, controlled degradation aimed at eliminating disposal problems. The use of silver spray paint specially formulated for application on planting beds has been the most recent advancement in the use of reflective surfaces for the control of virus spread (Summers, 1994). The treatment is reported to repel aphids and delay the onset of virus infection by 10 days to 2 weeks. At the end of the season, 90% of the zucchini squash fruit on the untreated control was unmarketable in contrast to more than 50% which were still marketable on the crop treated with the silver spray-on mulch. In addition, the plants needed less water, because the mulch reduced evaporation and the crust formed on the spray-painted soil surface suppressed weeds. The task of gathering and disposing of plastic mulches at the end of the season was also eliminated; the water based paint can simply be disced down and incorporated into the soil with no harmful effects. Similarly, applications of reflective whitewash to plants as a foliar spray reduced the incidence of PVY and CMV in peppers (Marco, 1993). However, plant damage occurred and increased as the concentration of whitewash increased above 10%. Thus, although this could be a promising alternative to non-pesticidal virus control, the possible physiological effects of whitewashes on plants needs to be further investigated.

4.2.4 Use of Yellow Polyethylene Traps

The fact that aphids are attracted to yellow has been recognised for decades (Moericke, 1950). An early utilisation of this phenomenon in the practice of controlling aphid-borne virus diseases was the construction and operation of the yellow water pan trap (Harpaz, 1982). This was used widely as an important instrument in the sampling and monitoring of aphid vector populations in the field (Gonzalez and Rawlins, 1968).

A significant step forward in the direction of utilising yellow traps not merely as an ancillary, indicatory tool, but rather as a primary device for direct vector control, was made by Cohen and Marco (1973) in Israel. They used yellow polyethylene sheets covered with a layer of transparent glue that remains sticky for about three weeks, after which it has to be removed. The sheets were erected 70 centimetres above ground at a distance of 6 meters from the windward border of a pepper field, four days before germination. At the end of a 114 day period, the cumulative percentage of infection of PVY and CMV in the protected plots reached only 26.2%, compared to 52% in the unprotected plots. Zimmerman-Gries (1979) achieved even better results in protecting seed potato from potato leafroll virus (PLRV). Here the percentage infection was 17.2% in the untreated control in contrast to only 2% in the protected crop.

Based upon the work of Cohen and Marco (1973), sticky yellow plastic sheets have since become a standard, widely applied practice in Israel for the control of PVY and CMV in peppers (Harpaz, 1982). However, in an experiment carried out by Weiss *et al.* (1977) to control the spread of vector-borne viruses in a squash field by the above-described method, the percentage of plants that became infected in plots surrounded by yellow polyethylene sheets was consistently higher than in unprotected ones. He postulated that different aphid species may be sensitive to different wavelengths of light and thus some may be attracted to the yellow traps while others will not be. In this case, the aphid responsible for the spread of virus into the crop was less attracted to the trap than other species commonly found in the area. Thus the sticky trap control measure which is highly effective for one crop-virus-vector combination can be equally ineffective, or even harmful, for another combination, and care should be taken not to extrapolate successful examples to other situations comprising different combinations of crops, viruses and vectors.

4.2.5 Use of Insect Repellents

Aphids are known to release an alarm pheromone, which apparently serves as a defence mechanism (Nault and Montgomary, 1977), causing aphids to disperse rapidly. The alarm pheromone for most aphids is *trans*-ß-farnesene. However, it is not sufficiently persistent for long term protection against aphid colonisation and has not prevented aphid virus

transmission in laboratory tests (Yang and Zettler, 1975) or in tests in field crops (Hille Ris-Lambers and Schepers, 1978).

More recently, plant derived, insect behaviour-controlling chemicals (anti-feedants) have been re-evaluated in their use for vector control (Isman *et al.*, 1990). Hunter and Ullman (1992), found that foliar applications of azadirachtin, present in the oil of the Neem tree, delayed symptoms of zucchini yellow mosaic virus (ZYMV) in 81% of zucchini squash plants treated with a 1% concentration of the formulation. They observed a significant difference in the number of aphids colonising treated and untreated zucchini squash (**Figure 4.2.1**). Due to its preprobing repellent property, azadirachtin may be useful in reducing virus spread in the field. However, increased aphid activity and wandering behaviour was also observed, which may increase vector intensity and cause virus spread more rapidly than in untreated fields.

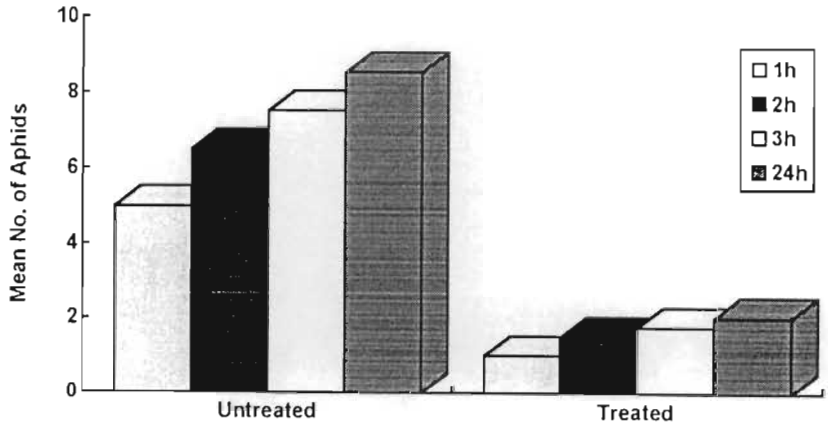

Figure 4.2.1 Number of aphids for each observation period (1, 2, 3, and 24hrs) on untreated zucchini and zucchini treated with 1% concentration of RD-Repelin (azadirachtin) (from Hunter and Ullman, 1992).

4.3 Evaluation of the Efficacy of Five Virus Management Practices on Capsicums on the Natal South Coast

4.3.1 Introduction

Pepper (*Capsicum annuum* L.) is an important crop world-wide. Virus diseases cause serious losses and can be the most important limiting factor for production (Marco, 1993). In Natal, the crop is severely affected by potato virus Y, a nonpersistently aphid-transmitted virus (K. Budnik *et al.*, unpublished). Marketable yields are dramatically decreased because of reductions in fruit set, quality, and fruit size. The high levels of virus in certain areas of Natal currently make the commercial production of peppers unfeasible.

Epidemics of nonpersistently transmitted viruses are among the most difficult to control due to extremely short aphid acquisition and inoculation periods. Dispersing widely, alate aphids land on plants in response to visual and olfactory stimuli (Miller and Strickler, 1984). Upon landing on plant, host choice is based entirely on gustatory stimuli received during short-duration sample probes. During sampling, an aphid inserts its stylets into the sub-epidermal leaf tissues and draws plant sap into the stylet food canal and precibarium where internal chemoreceptors are located (McLean and Kinsey, 1984). If the plant is perceived as palatable (i.e. a host) by the aphid, stylet insertion continues and sustained ingestion from the phloem eventually takes place. If the plant is perceived as unpalatable (i.e. a non-host), the aphid will withdraw its stylets, make several additional sample probes and then move to a different plant (Irwin and Ruesink, 1986). Short duration sample probes have been shown to be the most efficient in acquiring and transmitting nonpersistent viruses (Sylvester, 1962). Therefore, the most important and efficient vectors are frequently transient, non-colonising aphid species because these make the greatest number of sample probes and move most frequently between plants (Schultz *et al.*, 1989). Since insecticide applications have generally proved to be ineffective in nonpersistent virus control (Loebenstein and Raccah, 1980), increased efforts to find alternative pest control measures have been made.

The primary objectives of this investigation was to evaluate the effect of several control measures on the spread of PVY into pepper crops on the Natal South Coast, with a view to slowing the virus epidemic sufficiently to enable viable commercial pepper production in this region.

4.3.2 Materials and Methods

Experiments

During the summer of 1993 a pilot study was carried out at the University of Natal Research farm, Ukulinga. The following measures were investigated: use of plastic mulch, use of yellow sticky traps, use of whitewash, use of reflective foil suspended above the crop, use of insect repellents (Azatin™ and Virol) and use of insecticides. Although the trial did not yield significant results due to technical problems, information gathered in that trial was used in the present work. In May 1994, seedlings of the pepper cultivar California Wonder were obtained from a local nursery and tested for PVY infection using a commercially available PVY ELISA test kit from Boehringer Mannheim as per manufacturer's instructions. Healthy seedlings were then transplanted into the experimental field on a farm on the Natal South Coast in the Umkomaas region, where virus incidence in pepper was previously established (**Chapter 2**).

Six treatments were carried out, with plots arranged in a randomized complete blocks with four replicates. Sprinkler irrigation was provided once a week. The plots were 5 x 3 meters, 1.5 meters apart, and consisting of 5 rows, for a total of 35 plants per plot.

Treatments

The following six treatments were carried out (**Figure 4.3.1**):

(1) Insecticide applications - Mercaptothion, an insecticide registered for the control of aphids on capsicums;

(2) Virol applications - a mineral oil kindly donated by Dr. G.J. Thompson of the Vegetable and Ornamental Plant Research Institute in Pretoria;

(3) Plastic mulch - a commercially available white-on-black strawberry mulch was used;

(4) Yellow sticky traps - 300 mm x 50 m polyethylene strips precoated with a transparent adhesive which remained sticky for approximately 2 weeks, supplied by AgriBiol cc;

(5) Azatin™ applications - an emulsifiable concentrate containing the phytochemical azadirachtin, present in neem oil; and

(6) Control - untreated plot.

Sprays were carried out once a week, 1 day after irrigation, with a hand held-type sprayer (Killasprayer) with an adjustable nozzle. 5% insecticide, 1% Virol and 1.5% Azatin™ concentrations were applied. Spraying commenced 24 hours after planting due to the rapid virus spread previously experienced in the area. Plastic mulch and yellow sticky traps were installed 24 hours prior to planting. The yellow plastic was cut in half (150 mm) to ease in installation and placed around the plot 1m from the plants 0.5m above the plant tops. The height of the traps was adjusted for the growth of the pepper plants when required.

Figure 4.3.1 Experimental plots in the Umkomaas region on the Natal South coast, 24 hours before planting.

Incidence of virus

Incidence of virus-diseased plants was visually assessed according to symptoms, on a weekly basis until the termination of the trial. Visual assessments were validated using PVY ELISA assays of 20 individual plants with and without symptoms. These correlated with visual evaluations. Virus spread was determined by calculating the number of diseased plants over the total number of plants in each plot.

Effect of treatments on fruit yield

All the fruit from each treatment were harvested on a regular basis and total fresh weight was determined that same day. In addition, fruit diameter was measured in order to quantify fruit quality and to supplement yield data. Fruit with a diameter between 60-70 mm was considered marketable.

Aphid traps

Aphid catches within each plot were monitored over a four week period, starting during the fourth week of the experiment and continuing until termination. 11.5 x 14 cm yellow Bug Traps (Green Research, P.O. Box 541, Caledon 7230) coated with a transparent adhesive were used.

Statistics

AUDPC (area under the disease progress curve) was calculated from weekly virus incidence data from each plot. AUDPC values and yield and fruit diameter data was analysed by one-way analysis of variance (ANOVA). Means of AUDPC, yields and fruit diameter were separated by their LSD at a 95% level of confidence.

4.3.3 Results

Effect of treatments on pepper plants

Virol and Azatin™ exhibited levels of phytotoxicity to young pepper seedlings when applied at 1% and 1.5% concentrations respectively. Damage appeared as slight leaf deformation and discoloration, especially on margins, followed by some plant stunting, but symptoms did not resemble those of typical virus infection. Azatin™ caused more damage than Virol. The other treatments did not visibly damage the plants.

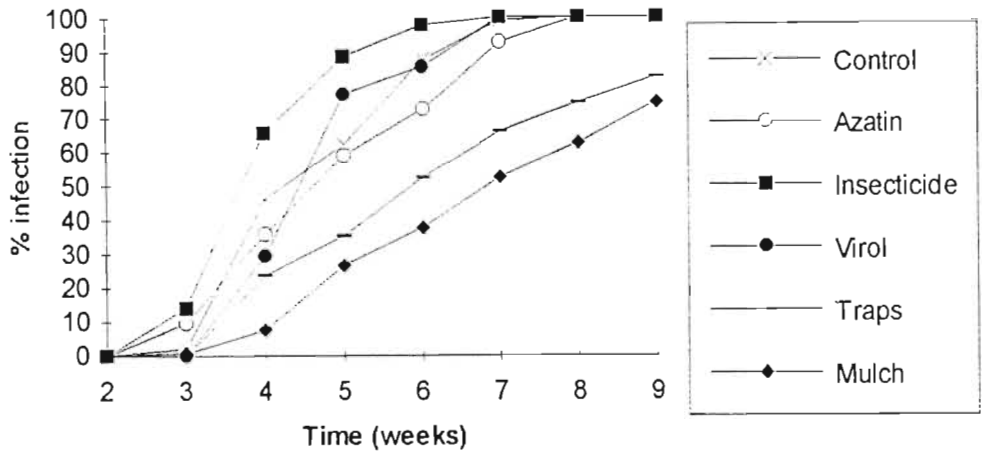

Figure 4.3.2 Virus spread into pepper plots treated with Virol, Azatin™, insecticide, yellow sticky traps and plastic mulch.

Effect of treatments on virus spread

Figure 4.3.2 presents the disease progress curves of virus spread into the pepper plots. The effect of the six treatments on disease progress is summarised in **Table 4.3.1.** Neither Virol nor Azatin™ differed significantly from the untreated control. Both mulch and sticky traps reduced disease progress significantly, by 50% and 35% respectively. Mulching provided significantly higher reduction in disease progress than sticky traps. Insecticide applications increased virus spread, with disease progress in treated plots significantly higher (15%) than in the control.

Effect of treatments on yield and fruit diameter

Mulching resulted in significantly higher yields than all other treatments, with a 62% yield increase when compared to the untreated control (**Table 4.3.2**). Due to high intraplot variability in yield, none of the other treatments differed significantly from the control. However, trends are clear and reflect the AUDPC values for virus spread. The insecticide treatment gave the worst yield, less than 50% of the control. Azatin™ and Virol resulted in yields similar to that of the control, while yellow sticky traps provided for a 25% increase in yield on the untreated control.

The effect of the different treatments on fruit quality (as determined by the average fruit diameter) was marked (**Table 4.3.3**). Mulching and the use of sticky traps resulted in a significant increase in fruit diameter when compared to the control. In addition, mulching resulted in a significant increase in fruit quality when compared to the use of yellow sticky traps. No other treatment differed significantly from the control. **Figure 3.3.3** illustrates the effect of the different treatments on fruit quality.

Table 4.3.1 Disease progress in peppers treated with mulch, yellow sticky traps, Virol, Azatin™, and insecticide and in untreated controls.

Treatment	AUDPC mean values[1]	Means Separation[2]	% of untreated control
Plastic Mulch	1574	a	50
Yellow sticky traps	2053	b	65
Virol	2937	c	94
Azatin™	3095	c	99
Control	3129	c	100
Insecticide	3613	d	115

[1]F-test significant @ 0.000 level

[2]Treatments with different letters differ at the 95% level of confidence

Table 4.3.2 The effect of six treatments on the yield of pepper plants.

Treatment[1]	Mean yield (g/plant)	Means separation[2]	% untreated control
Insecticide	21.49	a	49
Control	44.05	ab	100
Azatin™	45.96	b	104
Virol	46.86	b	106
Sticky traps	54.89	bc	125
Plastic mulch	71.38	c	132

[1]F-test significant @ 0.000 level

[2]Treatments with different letters differ at the 95% level of confidence

Table 4.3.3 The effect of six treatments of fruit quality (expressed as average fruit diameter).

Treatment[1]	Mean fruit diameter (mm)	Means separation[2]	% of untreated control
Insecticide	48.13	a	97
Control	49.60	ab	100
Azatin™	51.22	ab	103
Virol	54.06	b	109
Sticky traps	61.61	c	124
Plastic mulch	69.58	d	140

[1]F-test significant @ 0.000 level

[2]Treatments with different letters differ at the 95% level of confidence

Effect of treatments on aphid catches

The effect of the different treatments on the number of aphids trapped within pepper plots and virus incidence have been presented graphically below (**Figure 4.3.4**). Virus incidence within pepper plots correlates with the number of aphids trapped. Mulched plots and plots surrounded by yellow sticky traps had the greatest effect on the number of aphids trapped. The full data set is presented in **Table 4.3.4**.

Table 4.3.4 Weekly number of aphids trapped within each experimental plot over a four week period[1].

Treatment	Week 4	Week 5	Week 6	Week 7
Mulch	3	4	6	8
Yellow Traps	3	6	9	11
Azatin™	11	14	27	33
Virol	13	22	25	30
Control	12	15	26	27
Insecticide	11	18	30	23

[1]Trapping begun during the 4th week of pepper trial.

...continued on next page

Figure 4.3.3 Effect of mulching, use of yellow sticky traps, Virol, Azatin™(Neem), and insecticide on the quality of green peppers produced, in comparison to untreated control.

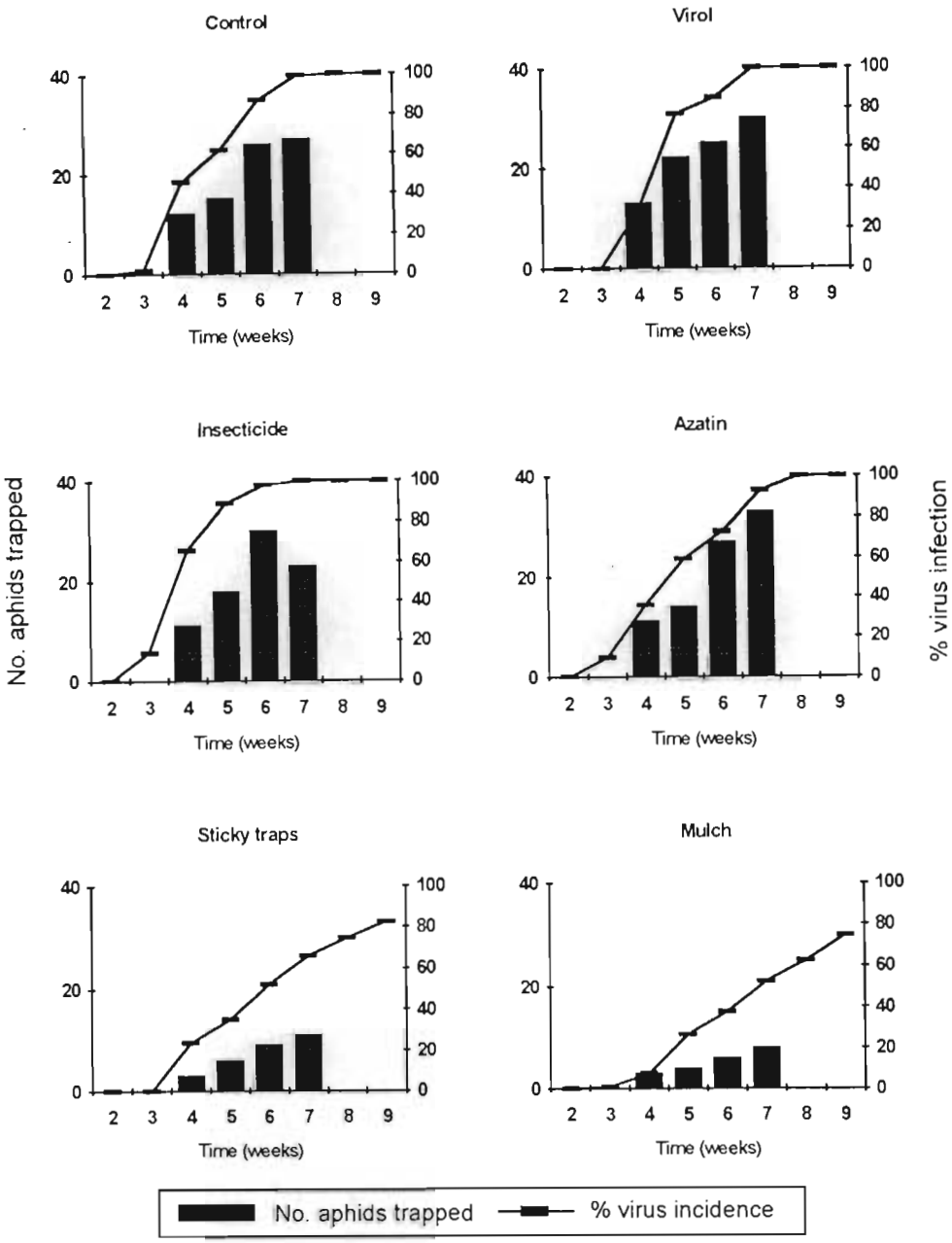

Figure 4.3.4 The effect of different treatments on virus spread and the number of aphid vectors trapped within each test plot on the Natal South Coast.

4.3.4 Discussion

Mulching and the use of yellow sticky traps reduced the incidence of PVY in peppers significantly when compared to an untreated control. Similar effects on the incidence of CMV and PVY as well as TSWV in peppers and other solanaceous crops have been reported elsewhere (Cohen and Marco, 1973; Greenough *et al.*, 1990; Brown *et al.*, 1993). These control methods utilise the knowledge based on insect visual stimuli to either attract or repel aphid vectors (Smith and Webb, 1969). Hence the low aphid numbers trapped

within the treated plots. Mulching reduced virus spread more than the use of sticky yellow traps, and resulted in improved fruit quality when compared to the use of sticky yellow traps.

The results obtained in this experiment (**Table 4.3.1**) confirm that insecticides provide no protection against nonpersistently transmitted viruses (Loebenstein and Raccah, 1980), even though they may be effective aphicides (Lowery and Boiteau, 1988). In fact, the application of insecticide resulted in increased virus spread when compared to an untreated control. A similar effect was observed by Marco (1993) on pepper crops treated with pirimicarb (Pirimor). This is commonly explained by an increased excitation of sprayed aphids, which probe and inoculate more (Rice *et al.*, 1983). Despite the increase in disease progress as a result of insecticide application, fruit quality and yield were not significantly different from that of the control.

Field experiments carried out in Cyprus for the prevention of the spread of PVY in seed potatoes by oil sprays yielded 90-95% control (Ionnou and Iordanou, 1987). Similarly, Hunter and Ullman (1992) found that applications of a neem product, RD-Repelin®, significantly reduced the spread of zucchini yellow mosaic virus in 81% of plants treated. However, disappointing results were obtained in plots treated with Virol and Azatin™. Neither Virol nor Azatin™ reduced PVY spread in pepper plots when compared to the control. Similar results were obtained by Marco (1993), who obtained inconsistent results for the use of mineral oils. The effectiveness of oil sprays is dependent on many factors, such as spray pressure and volume, which is expensive to achieve, and climate which is impossible to control (Vanderveken, 1977). In addition, the efficacy of oils is reported to diminish as the inoculum potential increases (Simons and Zitter, 1980). Factors such as aphid density, titer of transmissible virus in infected plants and plant density are all important. Similarly, Hunter and Ullman (1992) observed increased aphid activity and wandering behaviour on leaves treated with RD-Repelin® and the possibility exists that in the present case aphids alighting on plants treated with Azatin™ may have been initially repelled but eventually probed the plants, which would have been sufficient for virus transmission. As a consequence neither Virol nor Azatin™ had a positive effect on fruit quality or yield when compared to the control.

Table 4.3.5 Composite results of the six treatments, their rank, and effectiveness expressed as a percentage of untreated control, for each parameter measured.

Treatment	Virus spread	Rank	Yield	Rank	Quality	Rank
Mulch	50	1	162	1	140	1
Yellow Traps	65	2	125	2	124	2
Virol	94	3	106	3	108	3
Azatin™	99	4	104	4	103	4
Control	100	5	100	5	100	5
Insecticide	115	6	49	6	97	6

Thus, the use of yellow sticky traps or mulching are identified as the best means of controlling PVY spread in peppers. Both treatments rank consistently well for each parameter measured (**Table 4.3.5**). Although neither treatment prevents virus infection, virus spread is slowed sufficiently (50% and 35% respectively) to allow for adequate yields and fruit quality. Mulching is the preferred treatment, not only because of its higher levels of virus control, but also because the installation of yellow sticky traps was found to be difficult and time-consuming and traps tended to be destroyed by high winds. In addition, mulching has the added benefits of weed control and water conservation. The negative aspects of mulching are the cost and difficulty of collection and disposal of the plastic at the end of the season, and its high cost of implementation. However, the use of specially formulated silver paint as a mulch, recently described by Summers (1994), largely solves the problems of cost, application and disposal. Production of a similar product for this purpose is under investigation by a local paint company (Natal Associated Agencies) on our instigation.

4.4 Literature Cited

Brown, J.E., Dangler, J.M., Woods, F.M., Tilt, K.M., Henshaw, M.D., Griffey, W.A. and West, M.S. 1993. Delay in mosaic virus onset and aphid vector reduction in summer squash grown on reflective mulches. HortScience 28 : 895-896.

Bradley, R.H.E. 1963. Some ways in which a paraffin oil impedes aphid transmission of potato virus Y. Can. J. Microbiol. 9 : 369-380.

Bradley, R.H.E., Wade, C.V. and Wood, F.A. 1962. Aphid transmission of potato virus Y inhibited by oils. Virology 18 : 327-328.

Cohen, S. and Marco, S. 1973. Reducing the spread of aphid-transmitted viruses in peppers by trapping the aphids on sticky yellow polyethylene sheets. Phytopathology 63 : 1207-1209.

Gibson, R.W. and Rice, A.D. 1986. The combined use of mineral oils and pyrethroids to control plant viruses transmitted non- and semi-persistently by *Myzus persicae*. Ann. appl. Biol. 109 : 465-472.

Gonzalez, D. and Rawlins, W.A. 1968. Aphid sampling efficiency of Moericke traps affected by height and background. J. Econ. Entomol. 61 : 109-114.

Greenough, D.R., Black, L.L. and Bond, W.P. 1990. Aluminium-surfaced mulch: an approach to the control of tomato spotted wilt virus in solanaceous crops. Plant Dis. 74 : 805-808.

Harpaz, I. 1982. Nonpesticidal control of vector-borne viruses. In: Pathogens, vectors and plant diseases: approaches to control. Harris, K.F. and Maramorosch, K. (Eds.). Academic Press, New York. pp. 1-21.

Hille Ris-Lambers, D. and Schepers, A. 1978. The effect of trans-ß-farnesene, used as a repellent against landing aphid alatae in seed potato growing. Potato Res. 21 : 23-26.

Hull, R. and Heathcote, G. 1967. Experiments on the time of application of insecticide to decrease the spread of yellowing viruses of sugar beet 1954-1966. Ann. appl. Biol. 60 : 469-478.

Hunter, W.B. and Ullman, D.E. 1992. Effects of the neem product, RD-Repelin, on settling behaviour and transmission of zucchini yellow mosaic virus by the pea aphid, *Acrythosiphon pisum* (Harris) (Homoptera: Aphididae). Ann. appl. Biol. 120 : 9-15.

Ioannou, N. and Iordanou, N. 1987. Prevention of the spread of potato virus Y in seed potatoes by mineral oil sprays. Technical Bulletin, Agricultural Research Institute, Cyprus. Vol. 98. (Abstr.)

Irwin, M.E. and Ruesink, W.G. 1986. Vector intensity. In: Plant virus epidemics: Monitoring, modelling and predicting outbreaks. G.D. Mclean, R.G. Garrett and W.G. Ruesink, (Eds.). Academic Press, London. pp. 13-34.

Isman, M.B., Koul, P., Luczynski, A, and Kaminski, J. 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to Azadirachtin contact. J. Agric. Food Chem. 38 : 1406-1411.

Kennedy, G.G. 1976. Host plant resistance and spread of plant viruses. Environ. Entomol. 5 : 827-832.

Kennedy, G.G. 1986. Plant-plant-pathogen-insect interactions. In: Ecological theory and integrated pest management practice. M. Kogan (Ed.). John Wiley & Sons, New York. pp. 203-216.

Lamont Jr., W.J. 1993. Colour your decision. Am. Veg. Grower. 41 (11) : 22-24.

Loebenstein, G. and Raccah, B. 1980. Control of non-persistently transmitted aphid-borne viruses. Phytoparasitica 8 : 221-235.

Loebenstein, G., Alper, M., Levy, S., Palevitch, D. and Menagem, E. 1975. Protecting peppers from aphid-borne viruses with aluminium or plastic mulch. Phytoparasitica 3 : 43-53.

Lowery, D.T. and Boiteau, G. 1988. Effects of five insecticides on the probing, walking, and settling behaviour of the green peach aphid and the buckhorn aphid (Homoptera: Aphididae) on potato. J. Econ. Entomol. 81 : 208-214.

Marco, S. 1993. Incidence of nonpersistently transmitted viruses in pepper sprayed with whitewash, oil, and insecticide, alone or combined. Plant Dis. 77 : 1119-1122.

McLean, D.L. and Kinsey, M.G. 1984. The precibarial valve and its role in the feeding behaviour of the pea aphid, *Acrythosiphon pisum*. Bull. Entomol. Soc. Am. 30 : 26-33.

Miller, J.R. and Strickler, K.L. 1984. Finding and accepting host plants. In: Chemical ecology of insects. W.J. Bell and R.T. Carde, (Eds.). Sinauer Associates, Inc., Massachusetts. pp. 127-157.

Moericke, V. 1950. Über das farbsehen der pfirsichblattlaus (*Myzodes persicae*. Sulz.) Z. Tierpsychol. 7 : 265-274.

Nault, L.R. and Montgomery, M.E. 1977. Aphid pheromones. In: Aphids as virus vectors. K.F. Harris and K. Maramorosch, (Eds.). Academic Press, New York. pp. 527-545.

Quiot, J.B., Labonne, G., and Marrou, J. 1982. Controlling seed and insect-borne viruses. In: Pathogens, vectors and plant diseases: approaches to control. Harris, K.F. and Maramorosch, K. (Eds.). Academic Press, New York. pp. 95-122.

Raccah, B. 1983. Monitoring insect vector populations and the detection of viruses in vectors. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T. Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, London. pp 147-159.

Raccah, B. 1986. Nonpersistent viruses: Epidemiology and control. Adv. Virus Res. 31 : 387-429.

Rice, A.D., Gibson, R.W. and Stribley, M.F. 1983. Effect of deltamethrin on walking, flight and potato virus-Y transmission by pyrethroid resistant *Myzus persicae*. Ann. appl. Biol. 102 : 229-236.

Roberts, J.M.F., Jackai, L.E.N., Thottappilly, G. and Singh, S.R. 1993. Interaction between two synthetic pyrethroids and the spread of two non-persistent viruses in cowpea. Ann. appl. Biol. 122 : 57-67.

Sassen, B. 1983. The effect of two pyrethroids on the feeding behaviour of three aphid species and transmission of two different viruses. Z. Pflanzenkrankh. 90 : 119-126.

Shultz, G.A., Irwin, M.E. and Goodman, R.M. 1985. Relationship of aphid (Homoptera: Aphididae) landing rates to the field spread of soybean mosaic virus. J. Econ. Entomol. 78 : 143-147.

Simons, J.N. 1982. Use of oil sprays and reflective surfaces for control of insect-transmitted plant viruses. In: Pathogens, vectors and plant diseases: approaches to control. Harris, K.F. and Maramorosch, K. (Eds.). Academic Press, New York. pp. 71-93.

Simons, J.N. and Zitter, T.A. 1980. Use of oils to control aphid-borne viruses. Plant Dis. 64 : 542-546.

Simons, J.N., McLean, D.L. and Kinsey, M.G. 1977. Effects of mineral oil on probing behaviour and transmission of stylet-borne viruses by *Myzus persicae*. J. Econ. Entomol. 70 : 309-315.

Smith,F.F. and Webb, R.E. 1969. Repelling aphids by reflective surfaces, a new approach to the control of insect-transmitted viruses. In: Viruses, vectors and vegetation. K. Maramorosch (Ed.). Wiley & Sons, New York. pp. 631-640.

Summers, C. 1994. Sky's reflection repels aphids. Calif. Agric. 48(4) : 4.

Sylvester, E.S. 1962. Mechanisms of plant virus transmission by aphids. In: Biological transmission of disease agents. K. Maramorosch, (Ed.). Academic Press, London. pp. 11-32.

Tomlinson, J.A. 1987. Epidemiology and control of virus diseases of vegetables. Ann. appl. Biol. 110 : 661-681.

Vanderveken, J.J. 1977. Oils and other inhibitors of virus transmission. In: Aphids as virus vectors. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp 435-454.

Weiss, M., Cohen S., Marco, S. and Harpaz, I. 1977. Failure of sticky yellow traps to reduce aphid-borne virus incidence in squash. J. Econ. Entomol. 72 : 139-143.

Yang, S.L. and Zettler, W.F. 1975. Effects of alarm pheromone on aphid probing behaviour and virus transmission efficiency. Plant Dis. Reptr. 59 : 902-905.

Zimmerman-Gries, S. 1979. Reducing the spread of potato leafroll virus, alfalfa mosaic virus and potato virus Y in seed potatoes by trapping aphids on sticky yellow polyethylene sheets. Potato Res. 22 : 123-131.

Zitter, T.A. and Ozaki, H.Y. 1978. Aphid-borne vegetable viruses controlled with oil sprays. Proc. Fla. State Hort. Soc. 91 : 287-289.

CHAPTER 5

Breeding Capsicums to Resist Virus Diseases

5.1 Introduction

Disease patterns are to a great extent a product of our plant-breeding and agricultural practices.

N.W. Simmonds (1962)

Virus diseases are an international problem of pepper production. Mosaic or virus-like symptoms on peppers have been reported world-wide (see **Appendix 1**). There are approximately 45 different names for pepper virus diseases, but not all have been fully characterised (Green and Kim, 1991). Some are responsible for moderate to heavy losses annually in most pepper-growing areas of the world; these include cucumber mosaic virus (CMV), potato virus Y (PVY), tobacco etch virus (TEV), tobacco mosaic virus (TMV), and tomato spotted wilt virus (TSWV). Occasionally, pepper crops are abandoned without harvesting, as a result of these virus diseases (Greenleaf, 1986).

Peppers, through the process of natural selection, probably originated thousands of years ago, but the greatest selection pressure by man originated over 485 years ago when Columbus took the small, hot red peppers back to Europe with him (Villalón, 1981). Through the process of selection for fruit uniformity and other desirable characteristics, man has limited the genetic base in peppers to them more susceptible than wild types to insects and pathogenic organisms. For example, the virus disease leaf curl of peppers in Sri Lanka is prevalent mostly in modern high-yielding varieties but not among the traditional varieties that are highly resistant (Shivanathan, 1983). Most cultivated pepper varieties were developed under ideal conditions and lack the genetic diversity to withstand stresses imposed by pests or unusual environmental conditions.

Some pepper diseases caused by fungal or bacterial pathogens can be often controlled or prevented by use of chemicals, but no similar direct method for controlling pepper virus diseases is available. Other than avoiding infection by methods previously described,

breeding for resistance is one of the most efficient ways to control viruses, provided that an adequate genetic base is employed (Loebenstein and Raccah, 1980).

In general, the methods used in breeding for resistance to vegetable viruses, consist of the development of screening methods, a search for resistance (in numerous new and older cultivars and often extending outside the species being studied), and its transfer to commercially-desirable breeding lines, often involving a backcrossing programme (Tomlinson, 1987). Several type of resistance are known, but those most commonly encountered can be broadly categorised into:

(1) immunity; no reaction when challenged,

(2) resistance to infection; tendency to escape infection or resistance to transmission,

(3) resistance to establishment; includes hypersensitivity,

(4) resistance to virus multiplication; reduced or negligible virus multiplication in the plant, and

(5) tolerance; systemic infection without symptom development (Quiot *et al.*, 1982; Tomlinson, 1987). Progress in breeding for resistance to vegetable viruses has been recorded in several vegetable crops (Walkey *et al.*, 1983). However some important vegetable virus diseases are known for which little or no resistance has been found. Some of these are of world-wide importance and are listed in **Appendix 2** (from Tomlinson, 1987).

So where can the desired sources of resistance be found ? The identification of genes for resistance can be achieved by different methods (Nelson, 1973; Russel, 1978). One commonly used method is to observe infections within experimental plots under field conditions, the diseased plants being detected by indexing them on a range of differential host plants. Then, the accuracy of this search for genes for resistance can be enhanced by repeating the assays under varied geographic and climatic conditions, and by manipulating the sources of virus or the vector populations to standardise the inoculum pressure. Another method often practised, consists of artificially inoculating seedlings grown under controlled conditions. In this way, a collection of strains or isolates covering the variability of the pathogen can be defined very accurately. When the search is oriented towards the detection of partial resistance, it may be necessary to design elaborate tests to clearly pull out the sources of such resistance (Quiot *et al.*, 1982). In some cases, several distinct partial

resistances may appear in a mix; then a study on the mechanism of resistance may be the only way of differentiating them.

Many "new genes" have been uncovered through screening procedures, but even in the popular species, the surface has just been scratched. The inventory of *Capsicum* spp. germ plasm, maintained at the Southern Regional Plant Introduction Station at Experiment, Georgia, contains over 2,000 accessions representing most pepper species (Villalón, 1981). This genetic material was obtained from over 50 countries. This collection of germ plasm provides a broad base of genetic material and is available to all pepper breeders.

Several difficulties must be overcome, when introducing virus resistance into a given variety. One is to design a reliable and easy method of testing and recognising resistant plants in the progeny of a hybridisation (Quiot *et al.*, 1982). Such tests may be easily planned and carried out when the selection concerns major genes; generally, an inoculation of the seedlings with a given virus strain is convenient. Villalón (1981) used a simple artist airbrush method, to inoculate 30 to 40-day old pepper seedlings with TEV isolates to select virus resistant plants. These tests are less easy when several partial resistances must be introduced, in which case an understanding of resistance mechanisms that are involved becomes necessary. Another sometimes encountered difficulty is a narrow linkage between the resistance to be introduced into the commercial variety and undesirable genes affecting the growth of the plant, or the quality of the crop. Villalón (1981) used 13 different exotic pepper germ plasm stocks for crossing to commercial bell pepper cultivars. When the F_2 populations were screened for virus resistance, no large, four-lobed sweet bell lines were recovered, and only the primitive pepper types exhibited resistance to TEV. However, virus resistant sweet bell lines were developed by the backcross method. More recently, Villalón (1990) announced the release of new bell pepper varieties with resistance to TEV, CMV, PVY, TMV, pepper mottle virus (PeMV), and tobacco ringspot virus (TRV).

It is important at this point to make a cautionary note. Using the traditional pedigree methods to breed for resistance, as described above, relies on a good source of resistance genes, which if not locally available, has to be imported. Such genes, however, are invariably part of a gene-for-gene relationship, and the resistance would be vertical

(Robinson, 1987), and might fail. Although such resistance has been successfully implemented in many vegetable crops (Tomlinson, 1987), attributed mainly to virus' incapacity for change (Buddenhagen, 1983), new virus strains of PVY have been encountered which could overcome single gene resistance in bell peppers (Loebenstein and Raccah, 1980). Thus, when breeding for horizontal resistance, the traditional good source of resistance should be replaced with a good gene base of susceptible parents (Robinson, 1987). The inheritance of horizontal resistance is usually controlled by polygenes. At the beginning of a breeding program, the polygenes controlling horizontal resistance may be rare but they do occur. Every polygene is still present in the host population, but each of them occurs so infrequently that the resistance resulting from their combined effects has largely been lost (Robinson, 1987). Consequently, it is not usually necessary to introduce them, and it is not essential to have a good source of resistance, when breeding for horizontal resistance (Robinson, 1987). By applying positive selection pressures, it is possible to increase the frequency of such polygenes in the breeding population sufficiently to accumulate good levels of resistance.

What are the factors which determine the efficiency of a resistant variety at the field level? This efficiency seems to be dependent on the interaction between intrinsic characteristics of the type of resistance being used, other specific characteristics of the virus which is to be controlled, such as its capacity for variation and adaptability, and the type of agricultural management, e.g., crop rotations or monocultures (Quiot *et al.*, 1982). The efficiency of a given resistance, at the field level, may be altered through the action of several factors. The best known factors are high temperatures which allow a virus to overcome resistance of the hypersensitivity type (for example, peppers which are resistant to tobacco mosaic virus), large numbers of viruliferous vectors which may break down the type of resistance described as a "tendency to escape virus infection", and growing conditions (e.g., the availability of water, nutrients, and nitrogen) which might alter some types of resistance (Buddenhagen, 1983). Nono-Womdim *et al.* (1991) found that the resistance of peppers cvs. Milford and Vania is dependent on temperature and the plants' developmental stage. The boundaries of a new resistant variety's efficiency have to be defined by preliminary experiments performed under controlled conditions, for all these factors.

Table 5.1.1 Principal classes of chemical plant factors (allelochemics) and the corresponding behavioural and physiological effects on insects.

Allelochemical Factors	Behavioural or Physiological Factors
Allomones	**Give adaptive advantage to the producing organism**
Antixenotics	Disrupt normal host selection behaviour
Repellents	Orient insects away from plant
Locomotary excitants	Start or speed up movement
Suppressants	Inhibit biting or piercing
Deterrents	Prevent maintenance of feeding or oviposition
Antibiotics	Disrupt normal growth and development of larvae; reduce longevity and fecundity of adults
Toxins	Produce chronic or acute intoxication syndromes
Digestibility reducing factors	Interfere with normal processes of food utilisation
Kairomones	**Give adaptive advantage to the receiving organism**
Attractants	Orient insects toward host plant
Arrestants	Slow down or stop movement
Feeding or oviposition excitants	Elicit biting, piercing, or oviposition; promote continuation of feeding

(From Kogan, 1986)

Resistance to the vector offers promise of limiting the spread of plant viruses. It is based on the premise that apart from primary metabolites, as essential nutrients, (Kennedy, 1965), secondary substances (defence chemicals) exist which play a part in insect host selection and confer resistance of some crops to some insect species respectively. Secondary plant compounds perform allelochemical functions (Whittaker and Feeny, 1971) either as allomones or as kairomones. Allelochemicals exist primarily to perform allomonal functions in a defensive mode (Pasteels, 1976). The reciprocal adaptation of insects has resulted not only in the evolution of mechanisms to render allomones ineffectual (e.g. detoxification, behavioural avoidance), but also in converting them into kairomones, or cues for host-finding and feeding or oviposition extraction (**Table 5.1.1**) (Kogan, 1986). Thus for example, the allelochemical cucurbitacin, is an effective feeding deterrent or even toxin for

most plant-eating insects, but is a powerful feeding excitant for many diabroticine beetles (Norris and Kogan, 1980). However, many such compounds exist and could play a role in reducing virus spread by reducing vector populations or by reducing the frequency of vector-plant contacts (Kennedy, 1986). But, the situation is complex, and depends on the type of resistance, the type of transmission, and the relative importance of resident and transient vectors. For a comprehensive review on plant resistance to insects see Kogan (1986).

5.2 A Screening Method to Test Susceptibility Levels in Capsicum Breeding Lines

5.2.1 Introduction

Diseases can be reduced in severity by the development of less susceptible cultivars. It is possible that not only immunity but all aspects of resistance to vectors, including non-preference, as well as resistance to virus infection, decreased susceptibility to virus invasion and increased tolerance, can be useful (Buddenhagen, 1983).

Testing breeding lines for monogenically controlled resistance is simple. It becomes a matter of detecting viruses within such lines and discarding those that are hosts to the disease being bred against (Villalón, 1981; Quiot *et al.*, 1982). Such resistance, however, is not desirable from an epidemiological point of view, due to its lack of stability (Robinson, 1987). In bell peppers, resistance to potato virus Y (PVY) depends on only one gene and new virus strains can overcome this resistance (Loebenstein and Raccah, 1980).

Selecting for horizontal resistance to virus infection, on the other hand, may necessitate the design of specific tests to detect small incremental increases in quantitative resistance (Quiot *et al.*, 1982), and demands several breeding cycles to establish acceptable levels (Robinson, 1987). A general observation based on a comprehensive review of the literature on recurrent selection is that as little as four selection cycles will result in a totally susceptible population moving to a highly resistant level (M.D. Laing, personal communication). Similarly, selecting for tolerance requires some form of quantification, usually by comparing the interactions of virus/ontogeny/host genotype/environment in infected plants to

uninfected plants, involving only ontogeny/host genotype/environment. (Buddenhagen, 1983).

When faced with a large population of breeding lines it may be of benefit to establish a breeding "stock" of suitable genes for resistance or tolerance. Selection based on methods described above may not be practical at such an early stage, requiring a lot of time and manpower. On the other hand, one may wish to eliminate from this population, undesirably susceptible individuals. A quick and effective method of monitoring susceptibility to virus infection within a large population of capsicum breeding lines is presented.

5.2.2 Materials and Methods

Breeding lines

125 early generation chilli breeding lines were provided by ProSeed cc. (Pietermaritzburg). These were bred for their horticultural characteristics only and no virus resistance or tolerance was taken into consideration. For comparison, a landrace, Britz, from Thabazimbi in the Transvaal was used, which is known from previous experience to be highly tolerant to virus infection. A popular commercial chilli variety, Long Slim Cayenne, was used as a susceptible control. Seedlings were planted on the 22 October 1993 at Ukulinga, the University of Natal research farm. Plots were arranged in a randomized complete blocks design and treatments replicated three times. Sprinkler irrigation was provided once a week. For practical reasons and because only 16 seeds of each breeding line were provided, the plots consisted of one row of 4 plants each, 1m apart. The remaining four seedlings were kept for replacement in case of early plant death. Borders of Britz selection were established around each block. Since virus incidence in the area was found to be consistently high, the plants were not inoculated, and natural virus infection was relied upon. Virus infection was confirmed by evaluating several randomly selected plants by ELISA for PVY, CMV and TSWV.

Evaluation

The evaluation of virus tolerance was carried out at the end of the growing season, early in 1994. Plants were rated on a scale of 1 to 9 based on the severity of virus symptoms (**Figure 5.2.1**). In addition, fruit development was evaluated based on a similar scale

(**Figure 5.2.2**). Plants and pods were rated highly susceptible (1), exhibiting severe reactions to virus infection, up to the point of death, and resistant/tolerant (9), showing no virus symptoms. Plots were coded to eliminate bias during the rating procedure.

Statistical analyses

One-way analysis of variance (ANOVA) was carried out on plant and pod ratings. Plant and pod ratings were compared by linear regression. A frequency distribution histogram was constructed from pod rating means, to facilitate interpretation.

5.2.3 Results

As determined by ELISA, PVY was the only virus prevalent in samples collected. No healthy plants were observed during the rating procedures, confirming high incidence of PVY in the area, as well as the lack of any breeding lines totally resistant to the virus.

Significant differences between breeding lines were observed (**Table 5.2.1**). Multiple range analysis (see **Appendix 3** for details) showed the Britz line to be superior in both plant and pod ratings. Conversely, Long Slim Cayenne chilli cultivar was highly susceptible to virus infection. The frequency-of-occurrence histogram (**Figure 5.2.3**) follows a normal distribution for a population, with the two controls at each end of the scale.

Table 5.2.1 Analysis of variance of pod (A) and plant (B) ratings on breeding lines.

(A)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	237.7427	125	1.901941	1.596349	0.000979	1.283389
Within Groups	296.6667	249	1.191432			
Total	534.4093	374				

(B)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	228.1427	125	1.825141	1.585326	0.001144	1.283389
Within Groups	286.6667	249	1.151272			
Total	514.8093	374				

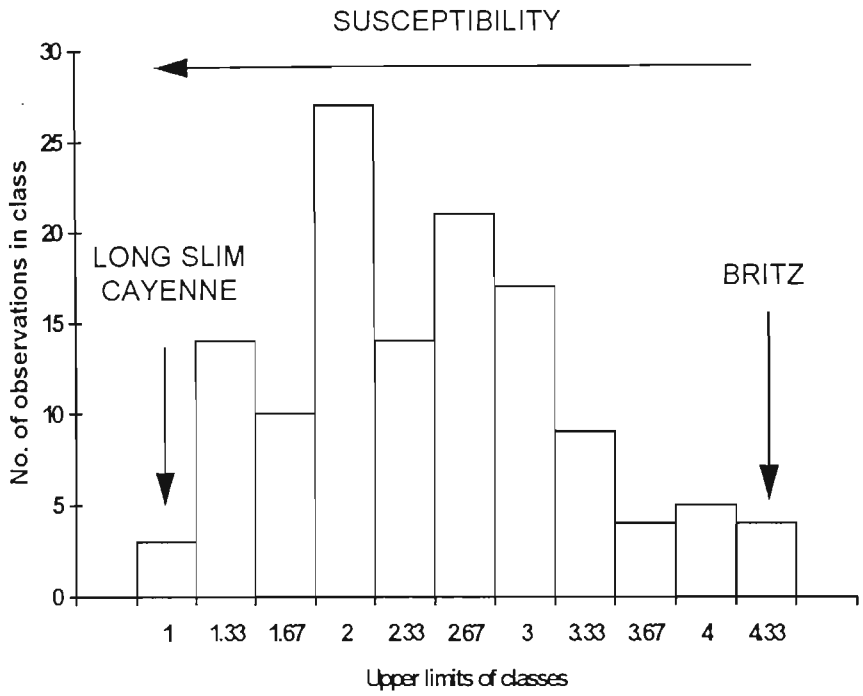

Figure 5.2.3 Histogram showing the frequency of distribution of pod ratings.

A 70% correlation was observed between plant and pod ratings (**Table 5.2.2**), suggesting a significant association between pod and plant susceptibility, and that either parameter can be used as an estimate of the other.

7 = Low susceptibility

Plant does not appear stunted, leaves show mild mosaic and veinclearing

5 = Medium susceptibility

Slight stunting, leaf mosaic evident, leaves show some blistering and underdevelopment

3 = Moderate susceptibility

Plant stunted, leaves show severe mosaic and blistering. Leaf area reduced.

1 = High Susceptibility

Plant severely stunted. Leaves small and deformed showing clear mosaic and deformations.

Figure 5.2.1 Plant rating scale used for evaluation of susceptibility to virus infection in breeding lines.

7 = Low susceptibility
Pods appear healthy; slight yellowing

5 = Medium susceptibility
Pod malformation becoming evident; slight yellowing

3 = Moderate susceptibility
Pods severely affected. Blistering and deformation clearly visible, marked yellowing.

1 = High susceptibility
No pod production or pods severely stunted and malformed with no economic value.

Figure 5.2.2 Pod rating scale to evaluate susceptibility to virus infection in breeding lines.

Table 5.2.2 Regression analysis between pod and plant ratings.

Dependent variable: Pod rating			**Independent variable: Plant rating**	
Parameter	Estimate	Standard Error	T Value	Prob. Level
Intercept	0.914522	0.0818523	11.1728	-0.00000
Slope	0.716598	0.0376535	19.0314	-0.00000

Analysis of variance

Source	Sum of Squares	Df	Mean Square	F-Ratio	Prob. Level
Model	269.54261	1	269.54261	362.1929	-0.00000
Residual	279.81784	376	0.74420		
Lack-of-fit	17.130933	8	2.141367	2.99986	0.00285
Pure error	262.68691	368	0.71382		
Total (Corr)	549.36045	377			

Correlation Coefficient = 0.700463 **R-squared = 49.06 percent**
Stnd. Error of Est. = 0.862668

5.2.4 Discussion

The results obtained indicate that it may be possible to differentiate between the levels of susceptibility to virus infection among breeding lines using a method of disease rating, thus aiding in the selection processes for tolerance or horizontal resistance. Although a large area of overlap exists, significant differences (**Appendix** 3) between highly susceptible lines and breeding lines with low susceptibility can be observed. It may thus be possible, for instance, to discard highly susceptible breeding lines and concentrate breeding efforts on the portion of the test population which is less susceptible. Lines which occur in the area of overlap may either be discarded as unacceptable or subjected to further examination. It is impossible at this stage to discern between tolerance and partial resistance as these would require more quantitative data. However, it is safe to assume that low susceptibility to virus infection can be accounted for by some level of tolerance or resistance within the individual. By selecting those breeding lines which exhibit some natural tolerance/resistance to disease, a sound genetic base can be acquired on which further selection procedures can be established (Robinson, 1987).

Two rating methods were used: plant and pod rating. From production point of view, the quality of fruit is important and seems a natural choice. However, using plant rating would have a time scale advantage, as it would not be necessary to maintain plants in the field until

fruit set. Since some degree of correlation is evident between the plant and pod ratings, it may be possible to rely on plant rating only. However, there are several kinds of tolerance/partial resistance (Quiot *et al.*, 1982). Most commonly, there is either mild or no apparent symptom expression on the plant, or no yield and fruit quality decrease. Therefore it may be necessary to carry out both rating methods to distinguish between the different kinds of tolerance/partial resistance being expressed.

It is important that breeders be aware of the necessity of high virus incidence within their plots when selecting for tolerance/resistance. The selection for tolerance/resistance to virus diseases in breeders' plots is dependent on the pathogen/host/environment systems and in most instances, a conscious effort has to be made to ensure that virus infection is a factor in cultivar selection.

A natural accumulation of resistance or tolerance will result if a positive selection pressure is applied (Robinson, 1987). If virus incidence in an area is consistently high, a breeder's plots will automatically select tolerant/resistant plants (Buddenhagen, 1983). This must have happened naturally in the selection of the landrace, Britz, judging by its high tolerance to virus infection. Conversely, if local viruses were patchy or sporadic in incidence or low in severity, breeders could at first ignore them and develop new, higher yielding cultivars by concentrating on plant type. Such cultivars could then turn out to be vulnerable to virus diseases. This is probably the case with the high susceptibility of Long Slim Cayenne to virus disease, where negative selection pressures eliminated gene frequencies for resistance or tolerance (Robinson, 1987).

Ensuring that virus infection is a factor in selection of new capsicum cultivars may be as simple as developing them in an area where high virus incidence is encountered. This, however, may not always be possible. In such cases, breeding lines will have to be artificially inoculated. Yeh *et al.* (1988) used virus inoculum, bulked up in suitably susceptible plants, blended with potassium phosphate buffer and an abrasive (carborundum), in a metal tank, connected to a spraying gun. They achieved 100% infection rates, and one person could inoculate 10,000 seedlings in 2 hours.

Monitoring yields, colour, texture, flavour, pungency, vitamins A and C, aroma, capsaicin level and distribution, extractability of red pigment, etc. (Villalón, 1981), may not be practical when faced with several hundred early generation genotypes. The rating technique described above may provide some solutions. It is quick and easily carried out and intended to narrow the range of the breeding stock to those which exhibit the least susceptibility to virus infection. From this point, more quantitative analyses and selection procedures may be applied.

5.4 Literature Cited

Buddenhagen, I.W. 1983. Crop improvement in relation to virus diseases. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T. Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 25-37.

Green, S.K. and Kim, J.S. 1991. Characteristics and control of viruses infecting peppers: A literature review. Technical Bulletin No.18. Asian Vegetable Research and Development Centre. 60p.

Greenleaf, W.H. 1986. Pepper breeding. In: Breeding vegetable crops. M.J. Bassett (Ed.). AVI Publishing Co. Inc., Westport, Connecticut. 548 p.

Kennedy, G.G. 1986. Plant-plant-pathogen-insect interactions. In: Ecological theory and integrated pest management practice. M. Kogan (Ed.). Wiley & Sons, New York. pp. 203-216.

Kennedy, J.S. 1965. Mechanisms of host plant selection. Ann. appl. Biol. 56 : 317-322.

Kogan, M. 1986. Plant defence strategies and host-plant resistance. In: Ecological theory and integrated pest management practice. M. Kogan (Ed.). Wiley & sons, New York. pp. 83-134.

Loebenstein, G. and Raccah, B. 1980. Control of non-persistently transmitted aphid-borne viruses. Phytoparasitica 8 : 221-235.

Nelson, R.R. 1973. Breeding plants for disease resistance. Pennsylvania State Univ. Press, Philadelphia. (in **Quiot *et al.*, 1982**)

Nono-Womdim, R., Marchoux, G., Pochard, E., Palloix, A. and Gébre-Slassié, K. 1991. Resistance of pepper lines to the movement of cucumber mosaic virus. J. Phytopath. 132 : 21-32.

Norris, D.M. and Kogan, M. 1980. Biochemical and morphological bases of resistance. In: Breeding plants resistant to insects. F.G. Maxwell and P.R. Jennings (Eds.). Wiley & Sons, New York. pp. 23-61.

Pasteels, J.M. 1976. Evolutionary aspects in chemical ecology and chemical communication, Proc. 15th Int. Congr. Entomol. (Washington, D.C.). pp 281-293. (in **Kogan, 1986**)

Quiot, J.B., Labonne, G., and Marrou, J. 1982. Controlling seed and insect-borne viruses. In: Pathogens, vectors and plant diseases: approaches to control. K.F. Harris and K. Maramorosch (Eds.). Academic Press, New York. pp. 95-122.

Robinson, R.A. 1987. Host management in crop pathosystems. MacMillan Publishing Co., New York. 263pp.

Russel, G.E. 1978. Plant breeding for pest and disease resistance. Butterworths, London.

Shivanathan, P. 1983. The epidemiology of three diseases caused by whitefly-borne pathogens. In: Plant virus epidemiology: The spread and control of insect-borne viruses. R.T Plumb and J.M. Thresh (Eds.). Blackwell Scientific Publications, Oxford. pp. 323-328.

Simmonds, N.W. 1962. Variability in crop plants, its use and conservation. Biol. Rev. 37 : 442-465.

Tomlinson, J.A. 1987. Epidemiology and control of virus diseases of vegetables. Ann. appl. Biol. 110 : 661-681.

Villalón, B. 1981. Breeding peppers to resist virus diseases. Plant Dis. 65 : 557-562.

Villalón, B. 1990. Genetic multiple virus resistant bell peppers. Phytopathology 80 : 981 (Abstr.).

Walkey, D.G.A., Tomlinson, J.A., Innes, N.L. and Pink, D.A.C. 1983. Breeding for virus resistance in British vegetable crops. Acta Hort. 127 : 125-135.

Whittaker, R.H. and Feeny, P.P. 1971. Allelochemicals: Chemical interactions between species. Science 171 : 757-770.

Yeh, S.-D., Gonsalves, D., Wang, H.-L., Namba, R., and Chiu, R.-J. 1988. Control of papaya ringspot virus by cross protection. Plant Dis. 72 : 375-380.

CHAPTER 6

General Discussion

6.1 The Epidemiology and Control of Capsicum Viruses in Natal

The ultimate objective of epidemiological studies is to reduce infection spread in crops. Knowledge about factors involved in spread aids in selecting a control measure, and in applying it at the proper time.

(Raccah, 1986).

Studies in Natal have identified potato virus Y (PVY) as the most common virus of capsicums that leads to the majority of crop loss (**Section 2.4**). The virus is a member of the potyvirus group and is transmitted from plant to plant by aphid vectors. It is transmitted in a nonpersistent fashion, which has the following implications:

(1) only a short feeding time is necessary for an aphid to acquire the virus from an infected plant; an acquisition time of as little as 15 seconds has been reported (de Bokx and Huttinga, 1981),

(2) there is no time period required between acquisition and transmission; an aphid can transmit virus immediately to a healthy plant,

(3) the aphid can infect a healthy plant with a short inoculation probe, once again in as little as 15 seconds. Such brief acquisition and inoculation probes limit the usefulness of insecticides to reduce the spread of PVY (**Section 4.3**), because it generally requires longer than 15 seconds for aphids to obtain a lethal dose of the insecticide. In fact, in an experiment carried out by the author (**Section 4.3**), insecticide use resulted in an increase in disease spread, probably due to a hyperactivity response of the aphid to sub-lethal doses of the chemical (Rice *et al.*, 1983), and finally,

(4) aphids retain the virus for a short time. Aphids which have acquired PVY lose their ability to transmit virus in about 1 hour (de Bokx and Huttinga, 1981), although longer retention times have been reported (Raccah, 1986).

PVY infections cause mosaic symptoms on capsicums (see **Figure 2.3.2**) and generally result in more yield loss when plants are inoculated early in the growth cycle (Marco, 1993).

1993). The present studies indicate that PVY spread in the field occurs rapidly; virus was first detected in young peppers only one week after planting (**Section 3.3**). When infection occurs early, the plant may abort its existing fruit or fruit may become deformed (see **Figure 2.3.4**). If infection is delayed for long enough, however, less severe fruit damage will occur, even if the final disease incidence is the same in both early, and late infected plants (Laird and Dickson, 1963).

PVY has the potential to infect numerous wild and cultivated plants (**Section 3.2**). Several commonly occurring weed species have been found to be hosts to PVY and may play a part in its survival during periods when no susceptible crops are cultivated. In particular, two weed species have been identified which play an intricate role in the ecology of this virus (**Section 3.3**). *Nicandra physaloides* (L.) (Geartn.) (commonly known as Apple of Peru), was identified as the main source of PVY spreading into pepper crops on the Natal South Coast. In addition, another annual weed, *Solanum nigrum* L. (commonly known as Black Nightshade), was found to host PVY during periods when *N. physaloides* has died off. The occurrence of these two weed species overlaps the growing season of peppers, thus maintaining a high virus incidence throughout the year, in crops and in surrounding vegetation (**Figure 3.3.7**).

For any plant to become infected, a defined pattern of events must take place sequentially (**Figure 6.1**). First, an aphid of a species that is capable of transmitting virus must be present; not all aphid species will vector viruses. A winged form must receive stimuli causing it to leave the plant on which it was born, which may or may not be the plant from which it acquires virus. Examples of stimuli that might cause aphid migration include declining host conditions (caused by drought, freezing or natural plant senescence), crowding or escape from predators (Kring, 1972; Van Harten, 1983).

If the aphid is not viruliferous (carrying the virus), it must fly to and land on an infected plant. The aphid must probe the virus source plant, and if its stylets contact a cell with transmissible virus, it will become viruliferous (Marte *et al.*, 1990). At this time another set of stimuli is required to cause the aphid to leave the virus source plant. If the aphid

colonises the plant, then the epidemiological cycle will be interrupted until the aphid, or its offspring, receives stimuli to leave the source plant (Kring, 1972).

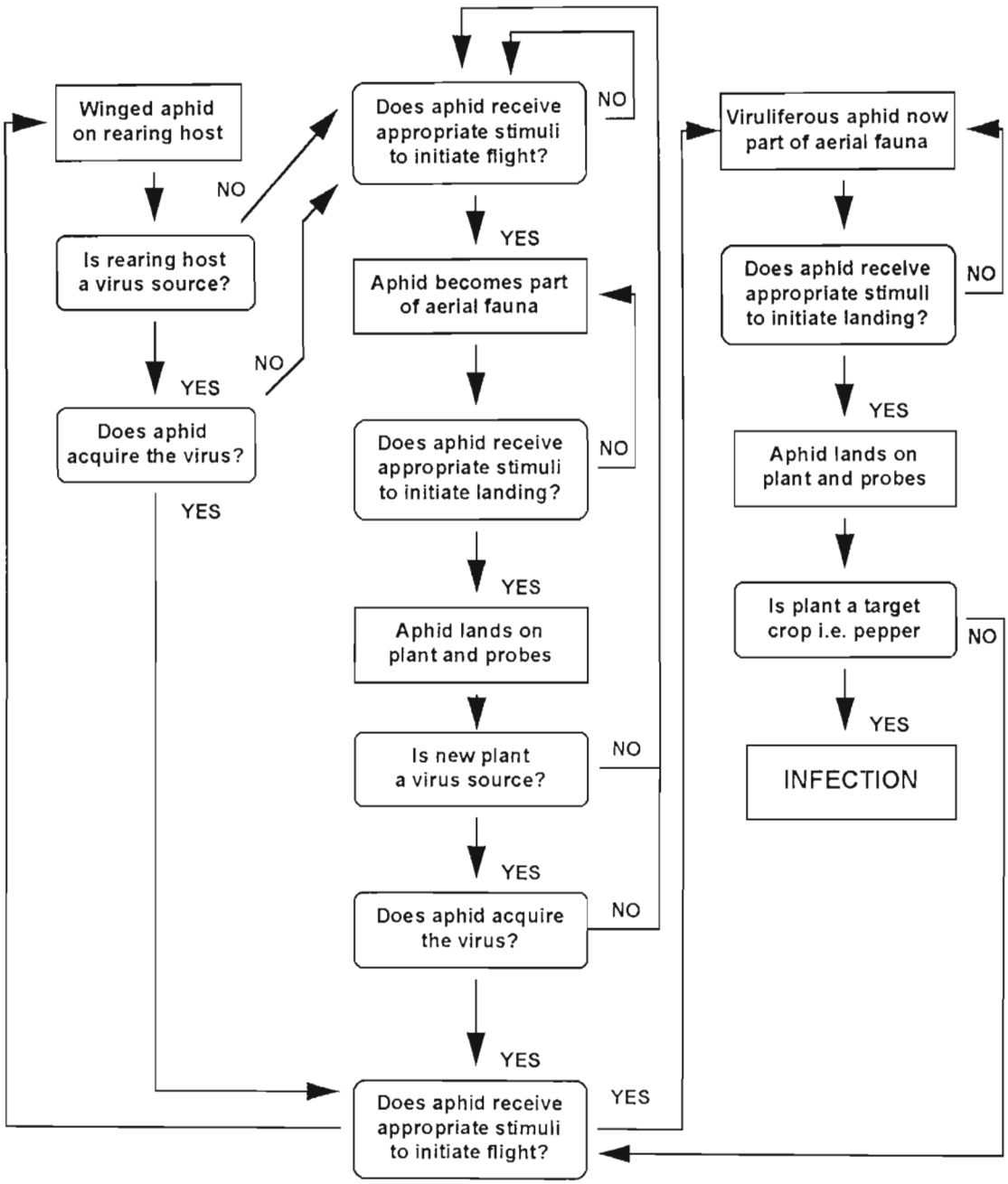

Figure 6.1.1 Model of steps involved in the epidemiology of aphid-transmitted viruses; aphid acquisition of virus and transmission to a healthy host. (Adapted from Perring *et al.*, 1992)

After leaving the source plant, the aphid must find a healthy pepper plant, alight on this plant and probe. Given the manner by which aphids select their host plants, brief probes following random landing on hosts and nonhosts alike, nonpersistent viruses are not restricted in their host range to plants colonised by their vectors (Irwin and Goodman, 1981). Hence the rapid infection of peppers. It is likely that the rapidity in which PVY is spread through a pepper field is because peppers are not preferred hosts to aphids. In three years of field work, aphids were observed to colonise peppers only on one occasion; a single refugee plant left in fallow ground from a previous season.

Knowing this series of defined events and knowing that each has to happen in the proper order, one would think that the probability of a plant becoming infected is low. However, 100% virus incidence in peppers was recorded on the Natal South Coast (**Section 3.3**). Epidemics of such proportions can occur only when the number of available virus source plants or the density of winged aphids is high. With this as a working model, research directed towards interrupting or slowing down of the epidemiological cycle was initiated in 1994, which could be used in an integrated virus management program.

Eliminating virus sources is the obvious first step in reducing virus spread. Growers should be keenly aware of surrounding crops and weeds that may serve as virus sources. Nearby weeds should be removed, and planting of crops equally susceptible to the virus should be avoided. Particular attention should be paid to early susceptible crops abandoned after harvest. Equally important are crops and wild plants which may act as aphid sources. Aphids were found colonising *N. physaloides* and *S. nigrum* (see **Figure 3.3.6**). If removing these weeds is not practical, then the application of aphicides may be helpful in reducing the aphid populations prior to planting the crop, although it is important to stress once again, that such a tactic has not been shown to produce a profitable return when applied to field crops (**Section 4.3**) because of the rapidity with which virus transmission occurs. Not only should the grower develop an awareness of the sources of virus inoculum around his field, but also of other hosts which may act as virus refuges during the off-season. Once these are determined, the producer can then concentrate on reducing these parameters so that the probability for growing healthy capsicums is increased.

Being aware of virus vector behaviour is the second step in the control of virus diseases. The flight behaviour of aphids and their attraction to certain wavelengths of light were discussed in Chapter 1. Repelling aphids with a reflective surface covering the ground between plants (such as aluminium or plastic mulches) was found to be effective in reducing PVY spread into peppers (**Section 4.3**). The effect of mulching resulted in a higher quantity and quality of pepper plants. A different approach based on attracting and immobilising aphids on sticky yellow sheets positioned vertically around a pepper plot, resulted in a similar reduction of infection (see **Figure 4.3.3** and **Tables 4.3.2** and **4.3.3**). Other methods based on reducing the apparency of the pepper crop to viruses were tested but have met with little success, not because they did not have the potential to reduce virus infection but because of practical limitations. Whitewash as a foliar application is reported to be effective (Marco, 1993). However, the local product appeared to be highly contaminated with coarse matter which blocked the spray nozzle of the applicator used. Use of reflective foil suspended above the pepper crop was suggested (F.W. Nutter, personal communication) to interfere with aphid landing behaviour. The method is not practical, however, because of the frequently high winds that Natal experiences. For the same reason, the use of yellow traps is not recommended. Thus, mulching is by far the best method available to reduce virus spread. The only negative factor is its high cost of implementation. This, however, can be offset by the gain in yields. A farmer in the Northern Transvaal, recovered these costs from his first chilli crop produced with a mulch, and almost tripled his yields (D. Marshall, personal communication).

Responsibility for the third step in bringing PVY infections of peppers under control, lies with the breeders producing new capsicum cultivars. Genetically based resistance and tolerance to plant viruses have been used to reduce losses to disease in a number of crops (Kennedy, 1986). This approach seeks to use directly the plant's natural defences. To develop tolerant or resistant capsicum cultivars, breeders will have to be aware of the necessity of a constant positive selection pressure, for resistance or tolerance to become apparent (Robinson, 1987). In areas where virus incidence is consistently high, this will happen naturally, but where patchy or sporadic virus incidence occurs, the levels of virus will have to be artificially elevated. A simple and effective method for selection of

genetically desirable breeding "stock" is described in **Section 5.2**. This can serve as the first level of selection procedures to ensure that the least susceptible individuals from a large breeding population become the parents of the next generation. From here, selection for tolerance or resistance can proceed. An important point to remember is that resistance to one type of virus does not necessarily operate against another type. Thus, when applying selection pressures it is imperative that all locally important viruses are defined.

Therefore, in order to minimize capsicum virus diseases in Natal, the following management practices should be implemented (see also **Figure 6.2**):

Precrop phase,

1) Crop rotation with nonsusceptible crops to reduce build-up of inoculum source.

2) Crop placement to avoid planting PVY susceptible crops adjacent to each other.

3) Control of PVY refuge hosts (e.g. *S. nigrum*).

Crop phase,

1) Use of virus free seedlings.

2) Use of plastic or spray-on mulch, to repel aphid vectors.

3) Eliminating virus sources (e.g. *N. physaloides*).

Postharvest phase,

1) Removal of crop plants as soon as possible to avoid maintaining high virus levels.

2) Control of PVY refuge hosts (including weeds and volunteers).

There are various other procedures for reducing the spread of nonpersistent viruses. These include several cultural practices which involve a reduction in crop apparency in time and space, for example, implementing a crop-free period, thus disrupting the synchrony between the crop and the pathogen and its vector; or changing plant spacing, thus changing the way plants are perceived by the vector (Kennedy, 1986). These have been developed largely on the basis of an understanding of the virus/vector/plant relationship, the virus transmission process, and the epidemiology of nonpersistent plant viruses. It did not emerge from any particular body of theory. Rather, those studies have contributed to the development of a sound conceptual basis for approaching the management of nonpersistent viruses.

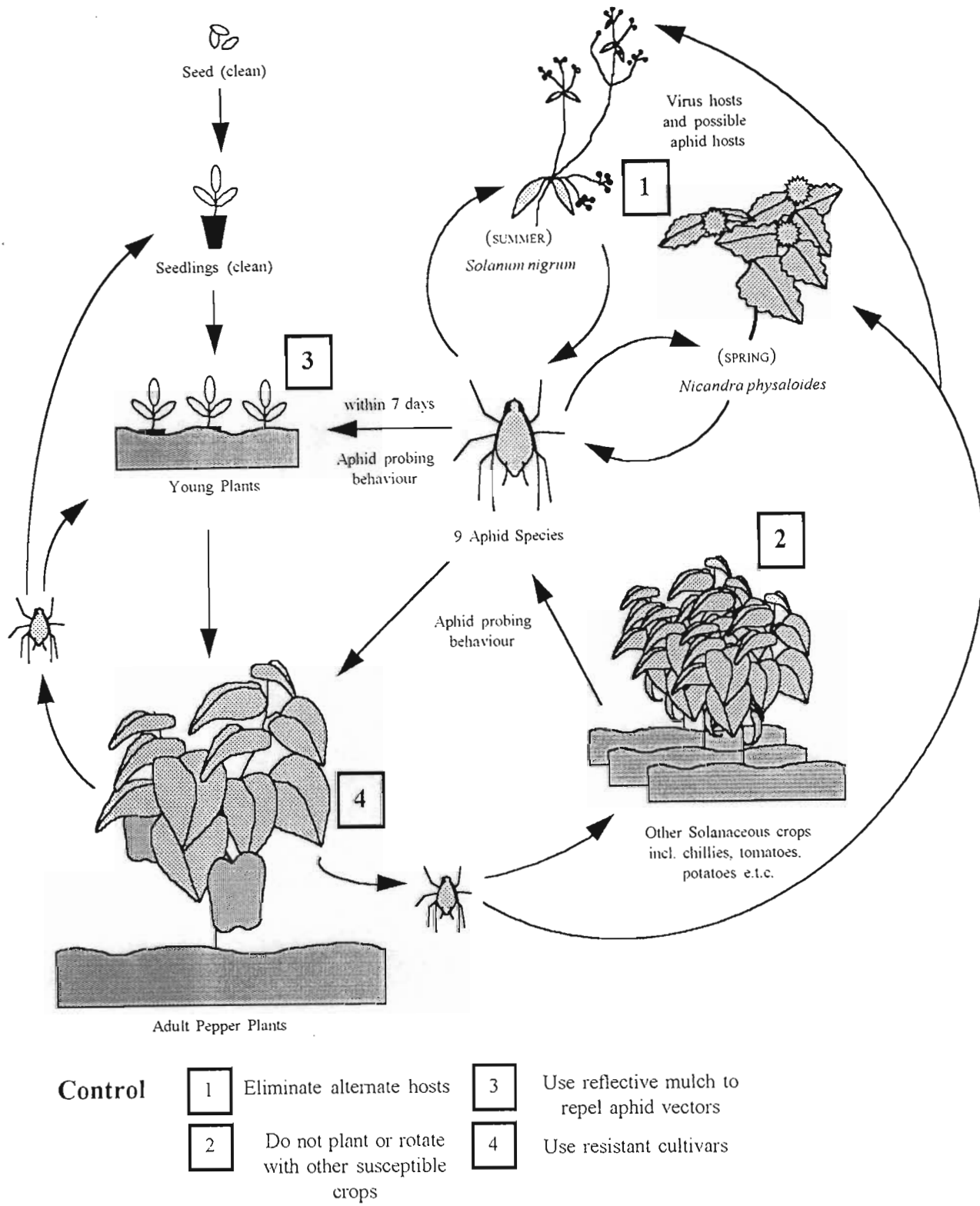

Figure 6.2 An ethograph of potato virus Y (PVY) on capsicums in Natal, indicating possible measures to disrupt the disease cycle.

6.2 Literature Cited

de Bokx, J.A. and Huttinga, H. 1981. Potato virus Y. CMI/AAB Descrip. Plant Viruses No. 242.

Irwin, M.E. and Goodman, R.M. 1981. Ecology and control of soybean mosaic virus. In: Plant diseases and vectors: Ecology and epidemiology. K. Maramorosch and K.F. Harris (Eds.). Academic Press, New York. pp. 182-220.

Kennedy, G.G. 1986 . Plant-plant-pathogen-insect interactions. In: Ecological theory and integrated pest management practice. Kogan, M. (Ed.). John Wiley & sons, New York. pp. 203-216.

Kring, J.B. 1972. Flight behaviour of aphids. Ann. Rev. Entomol. 17 : 461-492.

Laird, E.F. and Dickson, R.C. 1963. Tobacco etch virus and potato virus Y in pepper, their host plants and insect vectors in Southern California. Phytopathology 53 : 48-52.

Marco, S. 1993. Incidence of nonpersistently transmitted viruses in pepper sprayed with whitewash, oil, and insecticide, alone or combined. Plant Dis. 77 : 1119-1122.

Marte, M., Bellezza, G. and Polverari, A. 1991. Infective behaviour and aphid-transmissibility of Italian isolates of potato virus Y in tobacco and peppers. Ann. appl. Biol. 118 : 309-317.

Perring, T.M., Farrar, C.A., Mayberry, K. and Blua, M.J. 1992. Research reveals pattern in cucurbit virus spread. Calif. Agric. 46(2) : 35-40.

Raccah, B. 1986. Nonpersistent viruses: epidemiology and control. Adv. Virus Res. 31 : 387-429.

Rice, A.D., Gibson, R.W. and Stribley, M.F. 1983. Effect of deltamethrin on walking, flight and potato virus-Y transmission by pyrethroid resistant *Myzus persicae*. Ann. appl. Biol. 102 : 229-236.

Robinson, R.A. 1987. Host management in crop pathosystems. Macmillan Publishing Company, New York. 263pp.

Van Harten, A. 1983. The relation between aphid flights and the spread of potato virus Y^N (PVY^N) in the Netherlands. Potato Res. 26 : 1-15.

APPENDICES

Appendix 1

Viruses reported to infect peppers. (From Green and Kim, 1991)

Taxonomic Group	Virus	Particle Size (nm)	Vector	Geographical Distribution	Host Range
Filamentous Viruses					
Potyviruses	Chili veinal mottle (CVMV)	750x12	A	Asia	*Capsicum* spp.
	Pepper mild mosaic	714	A	Venezuela	Solanaceae
	Pepper mottle (PeMV)	737	A	El Salvador, USA, Thailand	Solanaceae
	Pepper severe mosaic (PeSMV)	761x13	A	Argentina	Solanaceae
	Pepper veinal mottle (PVMV)	770x12 850x12	A	West Africa	Solanaceae
	Peru tomato virus (PTV)	750x12	A	Peru	Solanaceae
	Potato virus Y (PVY)	730x11	A	Worlwide	Solanaceae
	Tobacco etch (TEV)	730x12-13	A	USA, Mexico, Sudan, Nigeria, Venezuela	Dicotyledonae (mainly Solanaceae)
Carlaviruses	Potato virus M (PVM)	650x12	A	USSR, India	Solanaceae
	Potato virus S (PVS)	650x12	A	USSR, India	Solanaceae
Potexviruses	Potato aucuba mosaic (PAMV)	580x11-13	C	Worldwide	Solanaceae
	Potato virus X (PVX)	515x11-13	C, S	Worldwide	Solanaceae
Rod-Shaped Viruses					
Tobamoviruses	Pepper mild mottle (PMMV)	312x18	C	North America, Australia, Japan, Europe	*Capsicum* spp.
	Tobacco Mosaic (TMV)	300x18	C, S	Worldwide	Wide
	Tomato mosaic (ToMV)	300x18	C	Worldwide	Wide

	Bell pepper mottle virus (BPeMV)	300x18	C	Argentina	Solanaceae
	Tobacco mild green mosaic virus (TMGMV)	310x18	C	Worlwide	Solanaceae, Umbelliferae, Commelinaceae Gesneriaceae
	Dulcamara yellow fleck virus (DYFV)	300x19	C	Hungary	Solanaceae
Tobraviruses	Tobacco rattle (TRV)	2 components: 21-23x46-117 21-23x185-197	N	USA, Europe, Brazil, Japan	Wide
Isometric Viruses					
Luteoviruses	Beet western yellows (BWYV)	26	A	Europe, USA, Japan	Wide
Tobacco necrosis and satelite viruses	Tobacco necrosis (TNV)	26-28	F	Worldwide	Wide
Tombusviruses	Tomato bushy stunt (TBSV)	30	S	USA, Europe, North Africa	Wide
	Morroccan pepper virus	30	S	Europe, North Africa	Solanaceae
Fabaviruses	Broad bean wilt (BBWV)	25	A	Argentina, Egypt, Europe, Japan, Morrocco	Wide, mainly Dicotyledonae
Nepoviruses	Tobacco ringspot (TRSV)	28	N	North America	Wide, woody, herbaceous, ornamentals
	Tomato ringspot (TomRV)	28	N	USA	Wide, ornamentals, woody, semi-woody plants
	Tomato black ring (TBRV)	30	N	Europe	Wide
Tymoviruses	Belladonna mottle (BMV)	27	B	Europe, USA	Solanaceae
Alfalfa mosaic virus group	Alfalfa mosaic (AMV)	5 components: 18x18 18x29 18x38 18x49 18x58	A	Worldwide	Wide (Dicotyledonae)
Cucomoviruses	Cucumber mosaic (CMV)	28	A	Worlwide	Wide
	Tomato aspermy (TAV)	30	A	USA, Europe, New Zealand	Wide
Harviruses	Tobacco streak (TSV)	27-35	T	USA, New Zealand, Argentina, Europe, Japan	Wide
Cryptic viruses	Red pepper cryptic virus	30	-	Japan	*Capsicum annuum*

	(RPCV)				
Geminiviruses	Curly top (CTV)	18-20x32	L	North America, Turkey	Wide (Dicotyledonae)
	Tobacco leaf curl (TLCV)	15-20x25-30	W	Japan, India	Solanaceae Compositae Caprifoliae
	Pepper mild tigré (PMTV)	20x30	W	Mexico	*N. tabacum* *Datura stramonium*
	Chino del tomate (CdTV)	18x20	W	Mexico	Leguminosae Solanaceae *Cynanchum acutum* *Malva* sp.
	Serrano golden mosaic	20x30	W	Mexico, USA	*L. esculentum* *D. stramonium* *Capsicum* spp.
Enveloped Viruses					
Tomato spotted wilt virus	Tomato spotted wilt (TSWV)	70-90	T	Possibly Worldwide	Wide
Other Not Well Characterised Viruses					
	Bell pepper dwarf mosaic	?	?	India	Solanaceae
	Brinjal mosaic	?	A	India	*Capsicum* spp.
	Chili leaf curl	?	W	Sri Lanka, India	Solanaceae
	Green veinbanding	?	A	Cuba	*Capsicum* spp.
	Launaea mosaic	?	A	India	*Capsicum* spp *L. esculentum*
	Marigold mottle	?	N	India	*Capsicum* spp.
	Pepper veinbanding	17x679	A	India	*Capsicum* spp.
	Pepper yellow vein mosaic	?	F	England, Holland, Hungary	*Capsicum* spp *Lactuca sativa* *Solanum villosum*

*A= aphid; B= beetle; C= contact; F= fungus; L= leafhopper; N= nematode; T= thrips; W= whitefly; S= soil, no insect vector found; - = no vector
? = Particle size or vector not identified

Appendix 2

Vegetables requiring cultivars bred with improved resistance to viruses. (Tomlinson, 1987)

Crop (Latin name)	**Virus**
Beet (*Beta vulgaris*)	beet yellows beet western yellows
Bean (*Vicia faba*)	broad bean wilt beet wester yellows
Bean (*Phaseolus vulgaris*)	cucumber mosaic bean common mosaic bean yellow mosaic
Brassicas: including Brussels sprout (*B. oleracea* var. *gemmifera*) Cabbage (*B. oleracea* var. *capitata* and *B. pekinensis*) Cauliflower (*B. oleracea* var. *botrytis*)	cauliflower mosaic turnip mosaic
Cassava (*Manihot esculenta*)	cassava mosaic
Carrot (*Daucus carota*)	carrot motley dwarf
Celery (*Apium graveolens*)	cucumber mosaic celery mosaic
Cowpea (*Vigna unguiculata*)	cucumber mosaic cowpea mild mottle
Cucurbits: including Cucumber (*C. sativus*) Courgette (*C. pepo*) Melon (*C. melo*) Watermelon (*Citrullus lanata*)	cucumber mosaic cucumber green mottle mosaic melon nectrotic spot watermelon mosaic (I & II) zucchini yellow mosaic
Groundnut (*Arachis hypogaea*)	groundnut rosette tomato spotted wilt virus
Leek (*Allium porrum*)	leek yellow stripe
Lettuce (*Lectuca sativa*)	beet western yellows cucumber mosaic lettuce big vein
Onion (*Allium cepa*)	onion yellow dwarf
Parsnip (*Pastinaca sativus*)	parsnip yellow fleck
Pea (*Pisum sativum*)	alfalfa mosaic
Pepper (*Capsicum annuum*)	**alfalfa mosaic** **cucumber mosaic**

Pepper (*Capsicum annuum*)	**capsicum mosaic** **potato virus Y** **pepper veinal mottle** **tomato spotted wilt**
Potato (*Solanum tuberosum*)	potato leaf roll potato virus Y
Spinach (*Spinacea oleracea*)	broad bean wilt
Tomato (*Lycopersicon esculentum*)	cucumber mosaic potato virus Y tomato spotted wilt tomato yellow leaf curl

Appendix 3

Multiple range analysis for Pod results by Cultivar No.

Method: 95 % LSD		
Line/Cultivar	Average	Homogeneous Groups
1.7-1-4-1	0.67	a
8.7-1-2-2	0.67	a
L/S CAYENNE	0.67	a
1.11-1-2-2	1.00	ab
19.1-2-5-1	1.00	ab
18.1-1-4-1	1.00	ab
18.11-2-1-1	1.00	ab
1.9-3-4-2	1.00	ab
T29	1.00	ab
OFFTYPE	1.00	ab
17.1-1-2-2	1.17	abc
17.1-1-3-2	1.17	abc
1.11-1-3-5	1.17	abc
15.1-1-3-2	1.17	abc
6.12-1-1-1	1.17	abc
19.1-3-5-4	1.17	abc
8.8-1-1-1	1.17	abc
1.5-2-2-2	1.33	abcd
3.10-2-1-2	1.33	abcd
16.4-1-1-4	1.33	abcd
PSR1891	1.33	abcd
Britz selection	1.50	abcde
6.8-1-1-1	1.50	abcde
18.1-2-3-1	1.50	abcde
19.1-3-5-1	1.50	abcde
14.7-1-4-2	1.50	abcde
22.4-03	1.50	abcde
1.5-2-7-1	1.67	abcdef
1.11-1-3-4	1.67	abcdef
6.9-1-1-1	1.67	abcdef
16.4-1-1-3	1.67	abcdef
19.1-3-3-3	1.67	abcdef
MFPP1693	1.67	abcdef
MFPP2293	1.67	abcdef
MFPP2193	1.67	abcdef
9.2-3-1-1	1.83	abcdefg
6.17-2-2-1	1.83	abcdefg
18.1-2-1-1	1.83	abcdefg
10.20-2-1-1	1.83	abcdefg
1.5-2-5-2	1.83	abcdefg
15.1-1-3-2	1.83	abcdefg
16.7-1-2-1	1.83	abcdefg
17.3-2-5-1	1.83	abcdefg
9.5-3-1-1	1.83	abcdefg
1.9-2-1-1	1.83	abcdefg

16.4-1-2-1	1.83	abcdefg
1.11-1-3-2	1.83	abcdefg
10.20-3-2-2	1.83	abcdefg
6.17-2-2-4	1.83	abcdefg
P/MATE	1.83	abcdefg
15.2-02	1.83	abcdefg
MFPP2093	1.83	abcdefg
PSR20490	1.83	abcdefg
17.1-1-3-3	2.00	abcdefgh
6.11-1-2-2	2.00	abcdefgh
18.1-2-2-1	2.00	abcdefgh
19.4-1-1-2	2.00	abcdefgh
1.7-1-4-2	2.00	abcdefgh
1.5-1-1-2	2.17	abcdefghi
10.20-2-2-2	2.17	abcdefghi
1.7-1-1-1	2.17	abcdefghi
9.5-3-5-2	2.17	abcdefghi
8.8-1-2-2	2.17	abcdefghi
6.15-2-2-1	2.17	abcdefghi
T2	2.17	abcdefghi
SPITFIRE	2.17	abcdefghi
1.5-2-2-1	2.33	abcdefghi
18.4-1-1-1	2.33	abcdefghi
14.8-1-2-1	2.33	abcdefghi
18.1-2-3-3	2.33	abcdefghi
19.1-3-3-2	2.33	abcdefghi
10.16-1-1-2	2.33	abcdefghi
8.8-1-2-3	2.33	abcdefghi
1.5-2-3-1	2.50	bcdefghij
1.5-2-5-1	2.50	bcdefghij
1.5-2-6-2	2.50	bcdefghij
6.11-1-4-1	2.50	bcdefghij
9.5-2-3-4	2.50	bcdefghij
14.7-1-5-1	2.50	bcdefghij
19.3-1-1-1	2.50	bcdefghij
10.20-2-2-2	2.50	bcdefghij
9.5-2-3-2	2.50	bcdefghij
6.17-2-2-3	2.50	bcdefghij
2.5-1-1-3	2.50	bcdefghij
6.16-1-1-1	2.50	bcdefghij
6.8-1-1-1	2.50	bcdefghij
PSR2291	2.50	bcdefghij
9.5-2-3-1	2.67	bcdefghijk
18.1-1-3-1	2.67	bcdefghijk
10.20-4-2-1	2.67	bcdefghijk
10.20-3-1-2	2.67	bcdefghijk
12.17-1-1-1	2.67	bcdefghijk
13.3-2-1-1	2.67	bcdefghijk
14.7-1-4-1	2.67	bcdefghijk
PSR1991	2.67	bcdefghijk
14.7-1-1-2	2.83	cdefghijk
1.9-2-1-3	2.83	cdefghijk
3.7-2-1-2	2.83	cdefghijk
3.13-1-1-2	2.83	cdefghijk

9.16-1-1-2	2.83	cdefghijk
1.5-2-2-3	2.83	cdefghijk
1.2-1-1-1	2.83	cdefghijk
17.4-2-1-2	2.83	cdefghijk
18.11-1-1-3	2.83	cdefghijk
3.14-1-1-1	3.00	defghijk
10.20-3-2-3	3.00	defghijk
1.9-1-1-1	3.00	defghijk
1.10-1-2-1	3.00	defghijk
18.1-2-3-2	3.00	defghijk
18.12-1-1-1	3.00	defghijk
9.5-3-4-1	3.00	defghijk
9.5-1-1-1	3.17	efghijk
14.7-1-2-3	3.17	efghijk
19.1-3-6-1	3.33	fghijk
MFPP2893	3.33	fghijk
16.4-1-3-2	3.50	ghijk
9.5-2-4-1	3.50	ghijk
18.1-1-1-1	3.67	hijk
3.10-2-1-4	3.67	hijk
10.11-1-1-3	3.83	ijk
9.5-3-4-2	3.83	ijk
PENDANT	3.83	ijk
17.1-1-2-1	4.16	jk
BRITZ	4.16	jk
1.10-1-1-1	4.33	k
19.4-2-2-1	4.33	k

Multiple range analysis of plant results by cultivar/line.

Method: 95 % LSD		
Line/Cultivar	Average	Homogeneous Groups
1.7-1-4-1	0.67	a
8.7-1-2-2	0.67	a
L/S CAYENNE	0.67	a
9.2-3-1-1	1.00	ab
17.1-1-3-2	1.00	ab
1.11-1-2-2	1.00	ab
1.11-1-3-5	1.00	ab
18.1-2-1-1	1.00	ab
1.5-2-5-2	1.00	ab
19.1-2-5-1	1.00	ab
15.1-1-3-2	1.00	ab
3.10-2-1-2	1.00	ab
6.8-1-1-1	1.00	ab
18.1-2-2-1	1.00	ab
6.12-1-1-1	1.00	ab
18.1-1-4-1	1.00	ab
16.7-1-2-1	1.00	ab
9.5-3-1-1	1.00	ab
18.11-2-1-1	1.00	ab
8.8-1-1-1	1.00	ab
1.9-3-4-2	1.00	ab
16.4-1-2-1	1.00	ab
16.4-1-1-3	1.00	ab
1.7-1-4-2	1.00	ab
T29	1.00	ab
15.2-02	1.00	ab
PSR2291	1.00	ab
PSR1891	1.00	ab
MFPP2293	1.00	ab
PSR20490	1.00	ab
OFFTYPE	1.00	ab
17.1-1-2-2	1.17	abc
19.1-3-5-4	1.17	abc
9.5-2-3-2	1.17	abc
22.4-03	1.17	abc
MFPP1693	1.17	abc
1.5-2-2-2	1.33	abcd
1.5-2-5-1	1.33	abcd
1.9-2-1-1	1.33	abcd
16.4-1-3-2	1.33	abcd
17.4-2-1-2	1.33	abcd
10.20-2-2-2	1.50	abcde
1.5-2-3-1	1.50	abcde
9.16-1-1-2	1.50	abcde
17.3-2-5-1	1.50	abcde
18.12-1-1-1	1.50	abcde
14.7-1-4-2	1.50	abcde
10.16-1-1-2	1.50	abcde

13.3-2-1-1	1.50	abcde
8.8-1-2-3	1.50	abcde
6.15-2-2-1	1.50	abcde
T2	1.50	abcde
MFPP2193	1.50	abcde
Britz selection	1.67	abcdef
9.5-1-1-1	1.67	abcdef
17.1-1-2-1	1.67	abcdef
1.5-2-7-1	1.67	abcdef
1.9-1-1-1	1.67	abcdef
15.1-1-3-2	1.67	abcdef
6.11-1-2-2	1.67	abcdef
8.8-1-2-2	1.67	abcdef
1.11-1-3-2	1.67	abcdef
16.4-1-1-4	1.67	abcdef
10.11-1-1-3	1.67	abcdef
19.1-3-3-3	1.67	abcdef
P/MATE	1.67	abcdef
1.5-1-1-2	1.83	abcdef
10.20-2-1-1	1.83	abcdef
14.7-1-1-2	1.83	abcdef
18.4-1-1-1	1.83	abcdef
1.7-1-1-1	1.83	abcdef
9.5-2-3-1	1.83	abcdef
3.7-2-1-2	1.83	abcdef
1.11-1-3-4	1.83	abcdef
14.7-1-5-1	1.83	abcdef
6.9-1-1-1	1.83	abcdef
6.16-1-1-1	1.83	abcdef
10.20-3-1-2	1.83	abcdef
18.11-1-1-3	1.83	abcdef
9.5-3-4-1	1.83	abcdef
PSR1991	1.83	abcdef
1.5-2-2-1	2.00	abcdefg
14.7-1-2-3	2.00	abcdefg
14.8-1-2-1	2.00	abcdefg
19.1-3-5-1	2.00	abcdefg
10.20-3-2-2	2.00	abcdefg
3.13-1-1-2	2.17	abcdefgh
6.17-2-2-1	2.17	abcdefgh
1.10-1-2-1	2.17	abcdefgh
18.1-2-3-1	2.17	abcdefgh
19.1-3-3-2	2.17	abcdefgh
2.5-1-1-3	2.17	abcdefgh
6.17-2-2-4	2.17	abcdefgh
6.8-1-1-1	2.17	abcdefgh
14.7-1-4-1	2.17	abcdefgh
SPITFIRE	2.17	abcdefgh
MFPP2893	2.17	abcdefgh
3.14-1-1-1	2.33	abcdefghi
6.11-1-4-1	2.33	abcdefghi
18.1-1-3-1	2.33	abcdefghi
10.20-2-2-2	2.33	abcdefghi
6.17-2-2-3	2.33	abcdefghi

19.3-1-1-1	2.50	bcdefghi
10.20-4-2-1	2.50	bcdefghi
19.4-1-1-2	2.50	bcdefghi
9.5-3-5-2	2.50	bcdefghi
MFPP2093	2.50	bcdefghi
12.17-1-1-1	2.67	bcdefghi
19.1-3-6-1	2.83	cdefghij
1.5-2-6-2	2.83	cdefghij
1.9-2-1-3	2.83	cdefghij
9.5-2-3-4	2.83	cdefghij
17.1-1-3-3	2.83	cdefghij
1.5-2-2-3	2.83	cdefghij
18.1-2-3-2	2.83	cdefghij
3.10-2-1-4	3.00	defghij
PENDANT	3.17	efghij
18.1-1-1-1	3.33	fghij
19.4-2-2-1	3.33	fghij
1.10-1-1-1	3.67	ghij
9.5-2-4-1	3.67	ghij
BRITZ	3.67	ghij
1.2-1-1-1	3.83	hij
9.5-3-4-2	3.83	hij
10.20-3-2-3	4.00	ij
18.1-2-3-3	4.50	j