Control of Insect-Transmitted Viruses in Cucurbit Crops in KwaZulu-Natal

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Declaration

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

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ABSTRACT

The production of cucurbits (Cucurbitaceae) in KwaZulu-Natal faces the constant threat of viral diseases. These can be so severe as to severely limit or prevent production in the latter part of the growing season (December-April). The important viruses in this regard are zucchini yellow mosaic potyvirus (ZYMV), watermelon mosaic 2 potyvirus (WMV2), watermelon mosaic potyvirus - Morocco strain (WMV-M), papaya ringspot potyvirus - type W (PRSV-W), cucumber mosaic cucumovirus (CMV), and squash mosaic comovirus (SqMV). The potyviruses and CMV are vectored by aphids (Homoptera: Aphididae) and SqMV is vectored by cucumber beetles (Coleoptera: Coccinelidae). PRSV and SqMV were found to be absent from the region, while CMV was found not to be a serious threat to cucurbit production. ZYMV, WMV2 (now confirmed to occur in South Africa) and WMV-M are the major viral pathogens of cucurbits in KwaZulu-Natal. The distribution of these viruses and methods for their control were investigated.

Investigations of aphid morphology using the scanning electron microscope were undertaken to determine if taxonomic studies could be conducted using this form of microscopy. The best form of specimen preparation was the cryo-fixation technique, which resulted in less collapse of the body wall and general damage to the specimen when compared to the critical point drying technique. Due to the lack of mobility of the specimen while viewing, this form of microscopy is rejected as a means of identifying aphids to the species level.

ZYMV was found to occur in a number of weed species (*Galinsoga parviflora*, *Malva parviflora*, *Amaranthus* sp., *Solanum* spp.), which could serve as reservoirs of virus. WMV-M and CMV were also found in some weed species. All tests for the potyviruses and SqMV were done using the double-antibody sandwich (DAS) ELISA technique. CMV was tested for using indirect ELISA tests. A third of the plants tested were found to be infected with more than one virus which could have implications for disease severity.

Disease severity was found to increase at about midway through the growing season (December-January). This was concurrent with a massive increase in the general aphid population in the experimental area. As no aphids were seen on the cucurbits in the fields, these vectors are believed to be transient inhabitants of the crop at first testing and then rejecting the plants as a food source. All control measures applied in the trial were aimed at reducing the numbers of aphids in the plots. Aphids were trapped using yellow sticky traps. *Cucurbita pepo* (zucchini)

was used in the trials due to its bush growth habit and good virus symptom expression. The success of the treatments was determined by monitoring the numbers of aphids present in the plots, and the use of a rating scale which assessed the severity of virus disease in the plots. The two best treatments were the white reflective mulch and the straw mulch. In the cultivar trial which assessed ten different cultivars for their virus resistance/tolerance. The best three cultivars were 'SQ 229', 'Puma', and 'SQ 228'. 'SQ 229' and 'SQ 228' were withdrawn from the market by the seed company for unknown reasons.

From the results obtained from these investigations, a disease management programme can be suggested. All cucurbit crops should be grown over a white reflective mulch, drip irrigation should be used to reduce agitation of the plants which could unnecessarily disturb feeding aphids, and a resistant or tolerant cultivar should be used in the latter half of the growing season. The effectiveness of any treatment can be assessed by comparing the number of aphids caught with the number caught in the control plots.

CHAPTER 1: LITERATURE REVIEW

1.1 THE CUCURBITACEAE

Members of the Cucurbitaceae are predominantly found in the subtropical and tropical regions of the world. In prehistoric times, many members of the Cucurbitaceae were domesticated in both the New and the Old Worlds, with archaeological evidence dating back to 5 000B.C. This makes cucurbits one of the oldest cultivated plants. In many instances these crops were a major part of the staple diet of these people as well as food for livestock (Kochhar, 1986; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

Cucurbits have extensive, shallow root systems. The angled stems are hollow and have bicollated bundles. The large, palmately lobed leaves are simple, alternate and have a long petiole. The flowers are often yellow in colour and tendrils are borne in the leaf axil. The flowers tend to be large, and the plants monoecious, however dioecious and andromonoecious forms are known. Bees are the most important pollinators of cucurbit flowers. Environmental conditions are known to affect the sex expression in plants. Long days with high temperatures tend to promote staminate flowers, whilst short days with low temperatures favour pistillate flowers. Also, growth regulators can be used to modify sex expression. Ethylene, for example, promotes femaleness. When fertilized and developing fruit are present on a plant, they can have an inhibitory effect on the frequency and number of subsequent flowers. If a flower is inadequately pollinated, either the flower will abort or the resulting fruit is underdeveloped and misshapen. The mature fruits are large and normally indehiscent with a hard skin (Kochhar, 1986; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

There are two groups within the family: aggressive climbers, which are generally found in southeastern Asia and the neotropics; and xerophytes from arid areas in Africa, Madagascar and North America. The majority of crop species from the family are mesophytic annuals from the tribes Cucurbiteae and Sicyoideae, however some of the xerophytes are being studied as potential fuel and food sources. There are about 118 genera and 825 species in the Cucurbitaceae, which is not closely related to any other plant family. The genera *Cucurbita*, *Cyclanthera* and *Sechium* originated in the New World. The others are from the African or Asian tropics. As many of the

'wild' taxa are potentially useful as economically valuable crops, the cucurbit family is an important one (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

Cucurbits are warm season crops and very susceptible to the cold. They require warm, dry weather, sunshine, low humidity, and a frost-free growing period. The average temperature should range from 20°C and 27°C. Rubatzky & Yamaguchi (1997) stated that optimum temperatures are between 25° and 30°C, with 10°C being the lower limit for growth. Ideally the soil should be a well-drained sandy loam, a silt loam, or a clay loam. A pH range from slightly acid to slightly alkaline is preferred. When the plant is growing vigorously, the plants should be irrigated at frequent intervals (Kochhar, 1986; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The seeds are sown directly into the cultivated fields and then left. Weed control is necessary until the plants start vining (Kochhar, 1986; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

1.1.1 The Crops

***Cucumis metuliferus* E. Mey ex Schrad** (African horned cucumber)

Despite its common name, this plant is more closely related to melon than cucumber. The fruits measure 10-15 cm in length, are oblong in shape, and covered with spines about one centimetre long. In order to attain their maximum weight the fruits require a month on the vine. A further two weeks are required for maturation. During this latter period they become sweeter and orange in colour. Opinions on flavour vary from one combining the flavours of bananas, lemons and passion fruit to insipid and astringent. The fruit produced by wild plants is bitter, but in Africa the leaves are occasionally cooked and eaten (Robinson & Decker-Walters, 1997).

New Zealand growers are marketing this fruit under the name 'kiwano'. As studies to boost seed germination, stabilize fruit characteristics (size ; uniform colouration), and enhance flavour, the potential for commercial exploitation of this plant continues to grow. However, it does have the potential to become a serious problem plant should it 'escape' from cultivation (Robinson & Decker-Walters, 1997).

***Cucumis sativus* L.** (Cucumber)

Although determinate cultivars have been developed for the home grower and mechanical harvesting, most cucumber plants have indeterminate growth. The vines measure 1-3 m in length, with single, unbranched tendrils at each leaf axil. The triangularly ovate, 3-5 lobed leaves and angular stems have trichomes. Typically, male and female flowers occur at different nodes (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

At the edible stage the immature fruits are usually green, although in some cultivars they may be white or yellow. The shape of the fruit may be round to oblong or cylindrical. The rind has small tubercles and spines of trichome origin on it (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The cucumber is of Asiatic origin. In eastern Iran, the remains of cucumbers have been dated back to the third millennium B.C. In India cucumbers have been cultivated for at least 3000 years, while in China this practise has been conducted for about 2000 years. After having been introduced to the Mediterranean region 3000-4000 years ago by early travellers, it spread through Europe to the United Kingdom where the fruit was referred to as 'cowcumbers'. The Portuguese introduced it to West Africa, and Columbus introduced cucumbers to the New World (Haiti) in 1494. The cucumber is now cultivated throughout the world either in glasshouses, on commercial farms, or by home gardeners (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The most common use of cucumbers is as food, either fresh in salads or pickled. In Asia the seeds are eaten, and in southeastern Asia the young leaves and stems are cooked. Non-food related uses include use in lotions, perfumes, shampoos and soaps. The leaves, roots, seeds and stems are used by traditional healers to make medical remedies (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997). The excellent quality of glasshouse cucumbers (e.g. cultivars 'Telegraph', 'Petita F_1', 'Superator' and 'Hayat') has made the cucumber popular in markets where they are sold at premium prices (Robinson & Decker-Walters, 1997).

***Cucumis melo* L.** (Melon)

This cucurbit species has many other common names including muskmelon, cantaloupe, nutmeg

melon, winter melon, sweet melon, rock melon, and snap melon (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The stems of melon plants are round and pubescent, and occur as a vine in most cases, but compact cultivars are available. The nodes bear single, unbranched tendrils, and the usually subcordate leaves are less lobed than those of *C. sativus*. The fruit in most instances is round or oval, but cultivars such as 'Banana' have elongated fruit. Few fruit are produced per plant (1-2), with the fruit being pubescent in the immature stages and glabrous in the mature state. The fruit contains oblong to elliptical seeds which are white to tan in colour. There is huge variety in the morphology of the fruit rind (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The centre of origin of the melon is uncertain with arguments for and against India, Iran, and Africa, with the majority of authorities supporting the third region. Melon has been shown by archaeological evidence to have occurred in Egypt and Iran in the second and third millennia B.C. respectively. The cultivation of melon cultivars now occurs worldwide in both temperate and tropical regions, with China, Egypt, France, Iran, Italy, Morocco, Romania, Spain, and Turkey, all being important producers (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

Cucumber mosaic cucumovirus (CMV) resistance has been found in some cultivars belonging to the Common Group of melon cultivars. Attempts are being made to transfer this resistance to members of the Cantalupensis Group which is more widely used for agricultural purposes (Robinson & Decker-Walters, 1997).

In Africa, melon seeds are often eaten, usually after being dried and ground, and the leaves are occasionally cooked as a vegetable. The entire plant is used as a fodder crop. The melon plant has a number of medicinal properties: For example, in China the fruits and roots are used as a diuretic; the roots and flowers as an emetic; haematoma has been treated with leaves and seeds; and dysentery and hypertension with the stems. Elaterin, stigmasterol, spinosterol, and the antitumour cucurbitacin B, and other bioactive compounds have been found in melon plants (Robinson & Decker-Walters, 1997).

***Cucurbita* species** (Squash)

The genus *Cucurbita* has five domesticated species (*C. aryrosperma* Huber, *C. ficifolia* Bouche, *C. maxima* Duch., *C. moschata* Duch. ex Poir., *C. pepo* L.) and about 10 wild species (Robinson & Decker-Walters, 1997).

The vines of all these species are frost-sensitive and bear tendrils. Some cultivars have a compact bush growth pattern where the tendrils are reduced both in size and function. This is particularly apparent in *C. pepo*. In most species and cultivars the leaves are large and vary in shape from almost round to palmately lobed. In many cases the leaves are mottled with white or silvery areas. Fruit size, shape, and colour vary widely among cultivars (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

There is much confusion in the use of common names within this group, with some species sharing the same common name and other names, such as squash, pumpkin, cushaw and gourd, have all been applied to the same species (Robinson & Decker-Walters, 1997).

Mexico was the centre of origin for most *Cucurbita* species, but South America played this role for a few species, including *C. maxima* (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997). In Mexico and the eastern USA, archaeological evidence has indicated that wild populations of *C. pepo* occurred 10 000 and 30 000 years ago respectively (Robinson & Decker-Walters, 1997).

The first squash to be introduced to Europe was *C. pepo*. This species is now cultivated around the globe. In pre-Columbian times *C. moschata* was grown in the southwestern USA, Mexico and South America. From these areas it migrated through the Caribbean islands until it reached Florida where it was adopted by the Native Americans. In several regions of Asia and Africa the additional diversification of cultivars has occurred (Robinson & Decker-Walters, 1997).

C. maxima only reached North America from South America in the eighteenth century. This species is now cultivated worldwide. It can be grown at higher latitudes and altitudes as it has better cold tolerance than most other domesticated species of this genus (Robinson & Decker-

Walters, 1997).

C. ficifolia (Malabar gourd) is also cold tolerant and has been found growing at elevations of up to 2600m. The fruits can be stored for up to two years, making them ideal for long-term storage. In Latin America this species is used as a vegetable and as a feed for livestock (Robinson & Decker-Walters, 1997).

Boiling or frying are the usual methods of preparing summer squash, while baking, boiling, or microwaving are favoured for winter squash. The soluble solids content is generally low in the white flesh of summer squash. However, orange, carotenoid-rich flesh high in soluble solids has been selected and bred for in winter squash (Robinson & Decker-Walters, 1997).

Where *C. ficifolia* is grown, the mature fruits are used as winter squash, and the immature fruits as summer squash. There is a proteolytic enzyme in the bland fruits which has potential commercial value in the food industry. The seeds of this species are roasted and eaten, the flowers are used as a condiment, while the stem tips and leaves are prepared as greens (Robinson & Decker-Walters, 1997).

In Mexico, squash has been important for many centuries. There are various preparations of the fruit, the seeds are used as snacks, the flowers are stuffed or fried, and are used to add colour and flavour to soups, stews and salads. Some of the African tribes cook the leaves and shoot-tips as a vegetable. Medicinally the seeds have been used as a diuretic, antipyretic and anthelmintic (Fox & Young, 1982; Robinson & Decker-Walters, 1997).

The following is a classification of the cultivars of *C. pepo*:

C. pepo ssp. *pepo*

① "Pumpkin - fruit orange, round or oval;

② Vegetable marrow - fruit short, cylindrical, tapering from the broad blossom end to the narrow peduncle end;

③ Cocozelle - fruit long, cylindrical, tapering away from the large blossom end, with a

length to width ratio of 3.5 or more;

④ Zucchini - fruit long, cylindrical, with little or no taper." (Robinson & Decker-Walters, 1997).

C. pepo ssp. *ovifera*

① "Scallop - fruit small, flattened, typically with scalloped edges;

② Acorn - fruit small, top-shaped, furrowed, pointed at the blossom end;

③ Crookneck - fruit elongated with a curved neck;

④ Straightneck - fruit cylindrical with a straight slightly constricted neck" (Robinson & Decker-Walters, 1997).

C. moschata (butternut) is divided into three groups in North America:

① "Cheese - fruit variable, but usually ablate with a buff-coloured rind;

② Crookneck - fruit round at the blossom end with a straight or curved neck;

③ Bell - fruit bell-shaped to almost cylindrical" (Robinson & Decker-Walters, 1997).

C. maxima has been divided informally into the following groups:

① "Banana - fruit long, pointed at both ends, with a soft rind and brown seeds;

② Delicious - fruit turbinate, shallowly ribbed, with a hard rind and white seeds;

③ Hubbard - fruit oval, tapering to curved necks at both ends, with a very hard rind and white seeds;

④ Marrow - Fruit oval to pyriform, tapering quickly at the apex and gradually towards the base, with white seeds;

⑤ Show - fruit large, orange, with a soft rind and white seeds;

⑥ Turban - fruit turban-shaped as a result of fruit tissue at the blossom end not being covered with receptacle tissue" (Robinson & Decker-Walters, 1997).

Squash is highly susceptible to attack by pathogenic fungi, bacteria and viruses. Thus many breeding programmes have focused on introducing resistance by using interspecific hybridizations and genetically engineering transgenic plants (Robinson & Decker-Walters, 1997).

Citrullus lanatus (Thunb.) Matsumura & Nakai (Watermelon) (syn. *C. vulgaris* Schrad. ex Eckl. & Zeuh.; *Colocynthis citrullus* (L.) Kuntze)

This cucurbit species (also known as bitter melon, colocynth, desert melon, khama melon and wild watermelon) is an important crop in areas where the growing season is long and warm (e.g. Africa, China, India, USA). This is the world's favourite cucurbit, and it flourishes on fertile, sandy soils in hot, sunny, dry environments, and is moderately drought resistant (Fox & Young, 1982; Robinson & Decker-Walters, 1997).

There are three recognised *Citrullus* species: *C. lanatus*, *C. colocynthis* (L.) (egusi melon; wild watermelon) and *C. ecirrhosus* Cogn. A potential fourth species is the recently described *C. rehmii* De Winter. *C. lanatus* is divided into two varieties: var. *lanatus* (domesticated plants) and var. *citroides* (wild populations). Two species which are closely related to those in *Citrullus* are *Praecitrullus fistulosus*, cultivated in India and Pakistan, and *Acanthosicyos naudinianus* (Sond.) C. Jeffrey, a native of southern Africa (Robinson & Decker-Walters, 1997).

Watermelon can be distinguished from other commonly cultivated cucurbits in that it has pinnatifid leaves. There are branched tendrils on the thin, angular, grooved, hairy stems. The root system is shallow but extensive, and the highly branched vines can measure up to 10 m long (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

All members of the *Citrullus* genus originated in Africa. The domesticated *C. lanatus* var. *lanatus* probably arose from wild populations of the var. *citroides*, common in the central regions of Africa. It has been an important crop in Egypt for thousands of years, and in China and south Russia since the tenth century A.D. (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

The fruit of this species is commonly eaten fresh as a dessert. In some parts of Africa the fruit is cooked. In arid areas of Africa, such as the Kalahari Desert, watermelons have served as an important source of water. The fruit is either consumed fresh or dried. The rind of the fruit may be candied or pickled. A fermented drink is made from the juice in the fruit in regions of the former USSR, or it is boiled down to a heavy, sweet syrup. In India the seeds are powdered and

baked like bread, while in the Orient and Middle East, the seeds are roasted and then eaten (Fox & Young, 1982; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

***Luffa acutangula* Roxb. and *Luffa cylindrica* L.** (Loofah) (syn. *L. aegyptiaca*)

Although the fruits of this genus resemble those of *Cucumis*, the two genera are unrelated. Three wild species are native to South America, while the other two wild species and the two domesticated species are indigenous to the Old World (Robinson & Decker-Walters, 1997).

L. acutangula, referred to as the angled loofah, Chinese okra, ribbed gourd or silk gourd, is divided into three varieties:

① var. *acutangula* is cultivated in southeastern Asia and other tropical areas;

② var. *amara* (Roxb.) C.B. Clarke is confined to India and is a wild form; and

③ var. *forskalii* (Harms) Heiser & Schilling is a wild form from Yemen (Robinson & Decker-Walters, 1997).

L. cylindrica, known as the smooth loofah, dishcloth gourd or sponge gourd, has a wild variety which ranges from southern central Asia to north-eastern Australia and the South Pacific, as well as a domesticated variety which is grown in Asia, Africa, the Caribbean, and some tropical American countries (Robinson & Decker-Walters, 1997).

The loofah plant is a vine which may be more than 10 m in length. The five-angled stem is slightly hairy with 3-5 branched tendrils. The leaves of *L. cylindrica* are large with shallow to deep lobes and a silvery mottle when young, while those of *L. acutangula* are not mottled and only shallowly lobed to angled (Robinson & Decker-Walters, 1997).

The majority of *Luffa* species are monoecious, with relatively large and conspicuous yellow flowers. The solitary female flowers are often found together with the long-stalked raceme of male flowers in the same leaf axil (Robinson & Decker-Walters, 1997).

The fruit of the wild species and varieties is small and bitter. Those of the domesticated varieties are usually not bitter and range from 15-60 cm in length. The fruit of *L. acutangula* can be

distinguished from that of *L. cylindrica* by the 10 pronounced longitudinal ribs. As the fruit matures it changes from bright green to yellow-brown (Robinson & Decker-Walters, 1997).

The immature fruits of domesticated varieties are harvested when they reach 10 cm in length, and then used in curries either peeled or intact. When used as a vegetable, the fruit is prepared in a manner similar to that for summer squash, or is eaten fresh like cucumber. The taste is similar to that of cucumber, but the texture is like that of zucchini. The mature seeds are sometimes roasted, while the leaves can be used as greens (Robinson & Decker-Walters, 1997).

Different parts of the plant have been used for medicinal purposes, usually as a purgative, in Asia, Africa and South America. In Japan a respiratory medication is made from the stem sap (Robinson & Decker-Walters, 1997).

The mature fibrous endocarp of the fruit of *L. cylindrica* is used as a sponge as well as insulation, sandals, slipper soles, padding for saddles, pillow stuffing, and many other uses (Robinson & Decker-Walters, 1997).

Other cucurbits

The cucurbits which are briefly discussed in the following section can all be found in KwaZulu-Natal, and are used as food plants by the local population:

Acanthosicyos naudiniana (Sond.) C. Jeffrey (syn. *Citrullus naudinianus* (Sond.) Hook f.).

The Herero cucumber, or wild melon, has a wide distribution. The fruit is used as a food item, as well as a source of moisture. The flesh of the fruit is either consumed raw or roasted after the skin and seeds have been removed. These are roasted separately and used to make a meal used for baking (Fox & Young, 1982).

Citrullus lanatus is discussed above.

Coccinia adoensis (Hochst. ex A. Rich.) Cogn. (syn. *C. jatrophaefolia* Cogn.)

The leaves are used as a spinach-like green vegetable, and the fruit, either unripe or mature, is eaten raw. The root may be eaten after it has been boiled (Fox & Young, 1982).

Coccinia rehmannii Cogn. (syn. *C. ovifera* Dinter & Gilg)

The leaves of the wild cucumber are used as a herb and the tuber as a source of water. The rather tasteless tuber must be cooked (roasted or boiled) before being eaten. The fruit, although edible, is reported to have little flavour (Fox & Young, 1982).

Cucumis africanus L. f. (syn. *C. hookeri* Naud.) and *Cucumis metuliferus* E. Mey. ex Naud.

The cooked leaves of the horned cucumber (bitter wild cucumber; jelly melon) may be used as a spinach or as a side dish. The bitter fruit is seldom eaten outside times of extreme need (Fox & Young, 1982).

Cucumis myriocarpus Naud.

The fruit of the bitter apple (small thorny cucumber; wild cucumber) is poisonous, although it has been reported that the seed, rind and juice are edible if they are free of pulp. The leaves are eaten only after they have been boiled (Fox & Young, 1982).

Cucurbita pepo is discussed above.

Mormodica balsamina L.

A spinach preparation is made from the leaves of the balsam apple (African cucumber; balsamina; balsam pear). The fruit is said to be poisonous, but the seeds may be eaten raw (Fox & Young, 1982).

Mormodica foetida Schumach. (syn. *M. mokorro* A. Rich).

The leaves are used as a spinach and the fruit is eaten raw (Fox & Young, 1982).

These species which grow outside of formal cultivation may act as virus reservoirs by overwintering and acting as a food source for potential vectors.

For a more comprehensive list of cucurbits and their common names see Appendix III.

1.1.2 Production

Figures from the Food and Agriculture Organization (FAO) of the United Nations indicate that the watermelon was the world's favourite cucurbit, with 1 824 000 ha under cultivation in 1994 yielding 29 360 000 metric tonnes of fruit. Cucumber, melon, squash and pumpkin followed watermelon (in descending order) in total world production (Robinson & Decker-Walters, 1997).

In commercial production, monoculture of cucurbits is prevalent. In the past and in rural areas in the Americas, squash has been interplanted with maize; in Africa cassava and yams have been planted in conjunction with fluted pumpkin, and yams and grain crops are interplanted with *Colocynthis vulgaris* (egusi) (Robinson & Decker-Walters, 1997).

1.1.3 Uses of Cucurbits

There is a wide variety of uses for cucurbits. They are eaten at both the immature and the mature stage. Preparations include the following: baking, boiling, covering with chocolate, frying, pickling, stuffing, or eaten fresh in salads or as desserts. The fruits can be eaten soon after harvest, or they may be stored 'on the shelf' (canning; freezing; pickling). Alcoholic beverages are brewed from the juices of some species. Other parts of the plant (seeds (e.g. *Cucurbita*, *Cucumeropsis*); flowers (squash); leaves and stem tips (e.g. *Telfairia*, *Mormodica*); and roots) are used for food. In Africa, the seeds of watermelon, oysternut (*Telfairia pedata* (Sims) Hook), and !nara (*Acanthosicyos horridus* Welw. ex Hook), as well as other cucurbits, are important dietary items (Robinson & Decker-Walters, 1997). In KwaZulu-Natal the growing tips and newly expanded leaves are eaten as a 'spinach' preparation (T. Adcock, *pers. comm.*, 1997).

Apart from the high vitamin A content of some squash and melon cultivars, and the oil and protein in the seeds of some species, the fruits of cucurbits are not very nutritious. Despite this, they are an important component of the human diet, especially in the tropical areas. Fruits that have an orange flesh-colour often contain high concentrations of β-carotene, a provitamin A carotene. The green parts of the plant have some nutritional attributes as they usually have higher concentrations of ascorbic acid, calcium, iron and phosphorus than the fruits, as well as containing some vitamin A. This can be important as in some tropical areas the leaves, stems and growing tips are eaten. The seeds are often highly nutritious, with the seed proteins of some cucurbits

having comparable nutritive values to legume seeds. Many species (e.g. squash, pumpkin, melon and watermelon) have seeds which can be used as sources of arginine, aspartic acid, calcium, glutamic acid, iron, magnesium, methionine, niacin, phosphorus, protein, and thiamin (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

Other uses of cucurbits include bottles, floatation aids for fishing nets, jewellery, masks, musical instruments, oil for cooking, candles, soap and illumination, ornaments and decoration (with Halloween perhaps being the best known example in the western world), penis sheaths, smoking pipes, storage containers, and utensils (Robinson & Decker-Walters, 1997).

Cucurbits have also been used in the treatment of various ailments. They have been used as purgatives, emetics, anthelmintics, and vermifuges - this is due to the action of cucurbitacins. The fruit and its extracts are the most commonly used part for medicinal purposes. Bitter melon is widely used, and research has shown that mild to moderately chronic cases of diabetes mellitus can be controlled by this plant. It is also being investigated as a treatment for human immunodeficiency virus (HIV), as is Chinese snake gourd (Robinson & Decker-Walters, 1997).

The bitter, toxic cucurbitacins are repellant to aphids, spider mites, and many other pests. Extracts from *Luffa* and *Mormodica* are used in China as a spray to control spider mites and other agricultural pests on crops. Cucumber beetles however are attracted to plants with high concentrations of cucurbitacins (Robinson & Decker-Walters, 1997).

1.1.4 Diseases

Modern agriculture, through its use of crop monocultures, has created artificial ecosystems which require constant attention. The end result of these unstable systems is a breakdown which is visible as recurrent pest outbreaks, pollution of water systems, salinisation, soil erosion, and others. These systems are heavily dependent on chemical inputs to try and maintain a balanced system (Altieri, 1994).

There are a number of bacterial, fungal, phytoplasmal and viral agents which attack cucurbits from the time of germination up to consumption at the dinner table. Thus the control of these

pathogens is vital in order to maintain reliable productivity and quality (Robinson & Decker-Walters, 1997). The diseases caused by viral agents are discussed in section 1.2. Those caused by other organisms are listed in Appendix I.

Genetic resistance to these pathogens is regarded as being the best control measure, however, to achieve this requires time and in its stead a variety of preventative and control measures are employed. These include cultural methods, such as crop rotation and seed treatment, and the application of chemicals (bactericides, fungicides, insecticides and nematicides). In some cases, susceptible cultivars are protected by grafting them onto resistant rootstocks. The prevailing environmental conditions can affect disease development. If the plants are stressed, they have a higher susceptibility to the initial infection and subsequent disease development (Robinson & Decker-Walters, 1997).

1.2 THE VIRUSES

There are in excess of 30 viruses which infect cucurbits worldwide. The most important probably being zucchini yellow mosaic potyvirus (ZYMV), watermelon mosaic 2 potyvirus (WMV2), cucumber mosaic cucumovirus (CMV)*, papaya ringspot potyvirus - Type W (PRV-W) and squash mosaic comovirus (SMV)*. Watermelon mosaic potyvirus - Morocco (WMV-M) is important in Morocco and South Africa. Others include the following: A strain of arabis mosaic nepovirus (AMV)* called cucumber stunt mottle; bean yellow mosaic potyvirus (BYMV)*; beet curly top intergeminivirus (BCTV); beet pseudo-yellow closterovirus (BPYV); bryonia mottle potyvirus (BMV); bryony viruses PI, PII, and PIII; clover yellow vein potyvirus (ClYVV); cucumber green mottle mosaic tobamovirus (CGMMV); cucumber necrosis tombusvirus (CNV); cucumber vein yellowing virus (CVYV); cucumber yellows virus (CYV); french muskmelon necrotic spot virus (FMNSV); lettuce infectious yellows closterovirus (LIYV); melon leaf curl begomovirus (MLCV); melon necrotic spot carmovirus (MNSV)*; melon ourmia virus (MOV); melon rugose mosaic tymovirus (MRMV); melon variegation cytorhabdovirus (MVV); melon vein-banding mosaic potyvirus (MVMV); muskmelon necrotic spot virus (MNSV); muskmelon vein necrosis carlavirus (MVNV); squash leaf curl bigeminivirus (SLCV); squirting cucumber mosaic virus (SCMV); telfaria mosaic potyvirus (TeMV)*; tobacco mosaic tobamovirus (TMV); tobacco necrosis necrovirus (TNV); tobacco ringspot nepovirus (TRSV)*; tomato black ring

nepovirus (TBRV)*; tomato ringspot nepovirus (ToRSV)*; tomato spotted wilt tospovirus; white bryony mosaic carlavirus (WBMV); wild cucumber mosaic tymovirus (WCMV); zucchini yellow fleck potyvirus (ZYFV) (Lovisolo, 1980; von Wechmar *et al.*, 1995; Brunt *et al.*, 1996; Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997). Nine of these are seedborne (marked with '*') (Robinson & Decker-Walters, 1997). Two recently described tospoviruses have been reported from cucurbits, namely watermelon bud necrosis tospovirus and watermelon silver mottle tospovirus (Singh & Krishnareddy, 1996; Chu & Yeh, 1998)

1.2.1. The Potyviridae

Of all the plant virus groups, the Potyviridae is probably the largest and most economically important, as the members of the group are capable of causing disease in a wide range of plants (Moghal & Francki, 1976; Moghal & Francki, 1981; Jordan & Hammond, 1991; Lindbo & Dougherty, 1994). This importance is increased by the relationship of many of these viruses with aphids in their dissemination (Zitter, 1977).

Viruses belonging to this group are flexuous rod-shaped particles measuring 12-15 nm in diameter and 680-900 nm in length (Moghal & Francki, 1981; Jordan & Hammond, 1991; Lindbo & Dougherty, 1994; Shukla *et al.*, 1994). The exception to this rule is the bymovirus subgroup which has a bipartite genome and particle lengths of 200-300 nm and 500-600 nm respectively (Lindbo & Dougherty, 1994; Shukla *et al.*, 1994).

The family has been divided into three subgroups according to their vectors:

1. those members that are aphid-transmitted (*Potyvirus*);
2. those members that are fungus-transmitted (*Bymovirus*); and
3. those members that are mite-transmitted (*Rymovirus*) (Lindbo & Dougherty, 1994; Shukla *et al.*, 1994).

Two further taxa, provisionally called '*Ipomovirus*' (transmitted by *Bemisia tabaci* Genn.) and '*Macluravirus*' (aphid-transmitted), also occur in this family (Shukla *et al.*, 1994).

The type member of the group is potato virus Y potyvirus (PVY) (Jordan & Hammond, 1991; Shukla *et al.*, 1994). The group now contains 198 viruses, 180 of which are found in the

Potyvirus genus (Shukla *et al.*, 1994).

Infection of a cell with a member of the Potyviridae is typified by the formation of cylindrical or pinwheel inclusion bodies in the cytoplasm. Nuclear inclusion bodies and/or amorphous cytoplasmic inclusion bodies are induced by some members of the group (Moghal & Francki, 1981; Lindbo & Dougherty, 1994; Shukla *et al.*, 1994). The virus particles are found scattered or in loose aggregates in the cytoplasm of the cell (Shukla *et al.*, 1994). Plants infected with a potyvirus are usually induced to produce a proteinaceous transmission factor. The proper retention, protection or release of particles from the gut or mouthparts of vectors seems to be dependent on this factor (Harrison, 1981). The host ranges of most potyviruses are limited, usually to just one plant family (Zitter, 1977).

Disease symptoms caused by potyvirus infection are often clearly visible, and usually commence six to eight days after inoculation. Common symptoms include vein clearing, leaf chlorosis, necrosis, leaf distortion, stunting, distorted fruit, and distorted seeds. These can lead to substantial crop losses. These may take the form of yield loss and/or quality impairment (Lindbo & Dougherty, 1994; Shukla *et al.*, 1994).

The majority of potyviruses are vectored by aphids, although some are transmitted by fungi, eriophyid mites, and the whitefly *Bemisia tabaci*. Where the aphids are concerned, the transmission is of the non-persistent type. The virus is acquired by short probes of only a few seconds, is retained for several hours, and can be inoculated into healthy plants during feeding probes of under 60 seconds. A single virus species is often vectored by several aphid species. Two viral proteins, the 52 kD HC-PRO and the capsid protein are involved in the transmission process (Zitter, 1977; Atreya *et al.*, 1990; Jordan & Hammond, 1991; Lindbo & Dougherty, 1994; Shukla *et al.*, 1994). The loss of aphid transmissibility by potyviruses is relatively common, and may be due to deficiencies either in the coat protein or the helper component (Bourdin & Lecoq, 1991). In some cases, strains which produce active helper component are not capable of being transmitted by aphids. This is due to a point mutation in the coat protein (Atreya *et al.*, 1990).

Diseases caused by potyviruses can be controlled by roguing primary sources of inoculum (weeds, volunteers, infected crop plants), using virus-free seed, and the use of plants resistant to the virus and/or the vectors. The use of transgenic plants is a promising form of specific field control (Lindbo & Dougherty, 1994; Shukla *et al.*, 1994). The use of insecticides to control the vectors is contraindicated as it increases virus spread by irritating the vectors causing them to probe more often, as well as for ecological reasons (Shukla *et al.*, 1994).

The following are the major viruses affecting commercial cucurbit production:

1.2.1.1 Watermelon Mosaic 2 Potyvirus (WMV2)

This virus, which has a worldwide distribution, was first discovered in *Citrullus lanatus*, and was described by Webb & Scott in 1965 and Purcifull & Hiebert in 1979 (Purcifull *et al.*, 1984a; Purcifull, 1990). This virus was reported to be present only in the Western Cape Province region of South Africa by van der Meer and Garnett (1985).

In the past, this virus has been referred to as watermelon mosaic virus (a general description which includes watermelon mosaic virus 1, now known as papaya ringspot virus Type W strain) (Purcifull *et al.*, 1984a; Purcifull, 1990), as well as melon mosaic, cantaloupe mosaic, and muskmelon mosaic virus (Lovisolo, 1980).

Isolates of WMV2 with different host ranges and symptoms have been reported (Purcifull *et al.*, 1984a; Chala *et al.*, 1987). These strains are generally serologically closely related, but distinguishable. A variant that was not aphid transmissible has been reported from Europe. 'Thermal' resistance-breaking strains have been obtained by maintaining inoculated pea plants at 30°C (Purcifull *et al.*, 1984a).

WMV2 has been isolated from a wide range of plants from a number of families - the Cucurbitaceae (*C. pepo*; *C. melo*; *C. sativus*; *C. lanatus*), Leguminaceae (*Pisum sativum* L. for example), Chenopodiaceae, and Malvaceae. Van der Meer (1985) reported that 24 plant families were susceptible to this virus. In many cases the plants involved are weeds. The symptoms, leaf distortion, mosaic, mottling, and fruit distortion, persist in naturally infected plants (Purcifull &

Hiebert, 1979; Al-Musa & Mansour, 1982; Purcifull *et al.*, 1984a; van der Meer, 1985; Purcifull, 1990). This virus may be the cause of a cupping of the leaves of infected plants. These leaves may also have a mosaic pattern and be deformed in other ways. Such symptoms were noted by Wyman *et al.* (1979) in a study where all plants were infected with WMV2.

In experimental work, the following species have been used as diagnostic hosts:- *Chenopodium amaranticolor* Coste & Reyn., *C. pepo*, *P. sativum* cultivar Alaska or Ranger, *Nicotiana benthamiana* Domin., and *C. lanatus* (Purcifull *et al.*, 1984a; Purcifull, 1990).

Aphids are responsible for vectoring this virus in nature. *Myzus persicae* Sultzer and *Aphis craccivora* Koch are the most common, but at least 27 other species are capable of vectoring the virus (Al-Musa & Mansour, 1982; Purcifull *et al.*, 1984a; Purcifull, 1990). In addition to the above mentioned species, Al-Musa & Mansour (1982), Purcifull *et al.* (1984a) and Lamont *et al.* (1990) include *Aphis citricola* Van der Goot, *A. gossypii* Glover, *A. fabae* Scopoli, *A. illinoisensis* Shimer, *A. middeltonii* Thomas, *Aulacorthum solani* (Kaltenbach), *Macrosiphum euphorbiae* Thomas, and *Toxoptera citricidus* (Kirkaldy). Castle *et al.* (1992) found that *M. persicae*, *A. gossypii* and *Acyrthosiphon pisum* Harris transmitted this virus with 18, 16 and 16% efficiencies respectively. Transmission of the virus requires the presence of a helper factor (Lovisolo, 1980; Purcifull *et al.*, 1984a). *Macrosphoniella sanborni* (Gillette), *Rhopalosiphum padi* L., and *Semiaphis dauci* (Fabricius) are also mentioned by van der Meer (1985) and Lovisolo (1980) as the major vectors of this virus.

Liriomyza sativae Blanchard (Diptera: Agromyzidae (a leafmining fly)) transmitted two isolates from squash to squash, but this was very inefficient (Purcifull *et al.*, 1984a; van der Meer, 1985). The virus can be spread by mechanical inoculation. Seed transmission does not occur (Purcifull *et al.*, 1984a; Purcifull, 1990). Dodder (*Cuscuta pentagona* Engelmann) is unable to transmit the virus (Purcifull *et al.*, 1984a).

Where frost occurs WMV2 tends to disappear during winter due to a lack of hosts and the reduction of vector populations. As such, in these areas WMV2 is usually not found until mid-December (in South Africa). Once it is present in a field it spreads very rapidly. In areas with

warm winters, hosts (volunteers; crops; weeds) and vectors are present all year round. Thus outbreaks of WMV2 tend to occur earlier in these areas (Trench *et al.*, 1992).

The virus particles are non-enveloped flexuous filaments measuring 730-765 nm in length. The axial canal and basic helix are obscure. Only a few particles are present in plant sap (Purcifull & Hiebert, 1979; Purcifull *et al.*, 1984a; Chala *et al.*, 1987; Purcifull, 1990).

Virus preparations are strongly immunogenic. In standard gel diffusion tests the particles do react. When serological tests are employed, the condition of the capsid protein should be monitored before immunization to determine if proteolysis has occurred. Flocculent precipitates result from mixed liquid tests. ELISA can be used to detect the virus (Purcifull, 1990). Sodium dodecyl sulphate (SDS) immunodiffusion tests and immunoelectron microscopy (IEM) techniques have been used for studying virus relationships (Purcifull *et al.*, 1984a).

1.2.1.2 Watermelon Mosaic Potyvirus - Morocco Strain (WMV-M)

This virus has been found in Morocco and in South Africa, where it is probably the most commonly occurring virus in cucurbits (van der Meer, 1985). It is now also known to occur in Spain, Algeria, Niger, and possibly in Yemen (Quiot-Douine *et al.*, 1990). A WMV strain was reported in South Africa by Eulitz (1977a). This virus was probably WMV-M.

Infected plants are stunted, the leaves exhibit mosaic, dark green blisters, filiformity, and malformation, and the fruit is malformed and has colour break (Fischer & Lockhart, 1974; van der Meer, 1985; van der Meer & Garnett, 1987).

The only plants outside the Cucurbitaceae to be infected with this virus are *Chenopodium album* L. (lambsquarters), *C. amaranticolor*, *C. quinoa*, and *Gomphrena globosa* L. (van der Meer & Garnett, 1987).

In experimental work the following species have been used for diagnostic purposes: *C. pepo*, *C. sativus* cultivar National Pickling, and *C. amaranticolor* (van der Meer, 1985).

M. persicae vectors the virus in a stylet-borne manner (van der Meer & Garnett, 1987).

The flexuous, rod-shaped virus particles range in length from 706-770 nm (Fischer & Lockhart, 1974; van der Meer & Garnett, 1987).

1.2.1.3 Zucchini Yellow Mosaic Potyvirus (ZYMV)

ZYMV was first isolated from *C. pepo* in Italy (Lesemann *et al.*, 1983). Since then it has been reported from Algeria, Australia, Egypt, France, Germany, Israel, Italy, Japan, Jordan, Lebanon, Mauritius, Morocco, South Africa, Spain, Taiwan, Turkey, the UK, and the USA (Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990; von Wechmar *et al.*, 1995). Within five years it was reported from most major cucurbit growing regions (Desbiez & Lecoq, 1997). It is suspected that the actual distribution of the virus is much wider (Al-Shahwan *et al*, 1995). The presence of this virus in South Africa was first reported in 1995 by von Wechmar *et al* (1995).

ZYMV has also been called muskmelon yellow stunt virus (Lesemann, *et al.*, 1983; Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990; Desbiez & Lecoq, 1997). The virus shows a high degree of variability (Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990), and isolates from different geographic localities often exhibit diversity in their host range, symptomology, and aphid transmission (Desbiez *et al*, 1996).

Crop plants which are susceptible to this virus include zucchini squash (*C. pepo*), cantaloupe (*C. melo*), cucumber (*C. sativus*), and watermelon (*C. lanatus*). These hosts exhibit mosaic, chlorosis, shoestringing, stunting, and fruit and seed malformation. The weed *Melothria pendula* L. has been recorded as a host in Florida, USA, showing chlorosis and a mosaic symptom. In naturally infected plants, the expressed symptoms persist (Lovisolo, 1980; Lisa *et al.*, 1981; Lesemann *et al.*, 1983; Lisa & Lecoq, 1984; Purcifull *et al.*, 1984b; Al-Musa, 1989a; Büchen-Osmond & Purcifull, 1990; Ullman *et al.*, 1991; Blua & Perring, 1992a; Fereres *et al.*, 1992; Al-Shahwan *et al.*, 1995; Desbiez *et al.*, 1996; Desbiez & Lecoq, 1997). In addition to the above mentioned symptoms, star-cracking of the fruit has been reported by Nameth *et al.* (1985) and Desbiez & Lecoq (1997). In Hawaii three weed species of the Cucurbitaceae, *Mormodica charantia* L., *Cucumis dipsaceus* Ehrenb. ex Spach, and *Lagenaria siceraria* (Molina) Standl.,

were infected (Ullman *et al.*, 1991). Other plants which may act as reservoirs for this virus are *Ranunculus sardous* (buttercup), *Lamium amplexicaule* L. (henbit), *Sesamum indicum* L. and *Moluccella laevis* L.. Old cucurbit crops, volunteers and plants in private gardens can act as sources and reservoirs of ZYMV (Desbiez & Lecoq, 1997).

Yields may drop by 40-50% or more (Nameth *et al.*, 1985; Al-Shahwan *et al.*, 1995). It has been shown that when plants are infected during the vegetative stage they produce significantly less fruit than when they are infected at the perfect flower stage (Perring *et al.*, 1989). One to two weeks after infection plants usually stop producing marketable fruit (Lecoq *et al.*, 1991).

Under experimental conditions members of the Aizoaceae, Amaranthaceae, Chenopodiaceae, Compositae, Cucurbitaceae, Labitae, Leguminosae, Ranunculaceae, Scrophulariaceae, Solanaceae, and Umbelliferae have been infected with ZYMV (Lisa & Lecoq, 1984). In diagnostic trials *C. amaranticolor*, *C. quinoa*, *C. melo*, *Cucurbita okeechobeensis*, *C. pepo*, *G. globosa*, *L. acutangula* and *R. sardous* have been used (Lisa & Lecoq, 1984; Purcifull *et al.*, 1984b; Nameth *et al.*, 1985; Büchen-Osmond & Purcifull, 1990).

ZYMV is vectored by at least 10 aphid species in the stylet-borne manner, of which *A. gossypii* and *M. persicae* are probably the most important. *A. craccivora* has been shown by Yuan and Ullman (1996) to be more important than *A. gossypii*. Desbiez *et al.* (1996) showed the transmission efficiency of *M. persicae* to be 80%, and that of *A. gossypii* to be 73%. These two aphids and *A. pisum* have been found to transmit this virus with 41, 35 and 4% efficiencies respectively (Castle *et al.*, 1992). Other species include *Acyrtosiphon kondoi* Shinji, *A. pisum*, *Aphis armoraciae* Cowen, *A. citricola*, *A. middletonii*, *A. spireacola* Patch, *Hydaphis* (*Lipaphis*) *erysimi* Kaltenbach, *M. euphorbiae*, *T. citricidus* and a *Uroleucon* sp. Transmission is only possible with the presence of a helper component. The virus can also be transmitted by mechanical means (Lisa & Lecoq, 1984; Purcifull *et al.*, 1984b; Nameth *et al.*, 1985; Al-Musa, 1989b; Büchen-Osmond & Purcifull, 1990; Blua & Perring, 1992b; Fereres *et al.*, 1992; Desbiez *et al.*, 1996; Yuan & Ullman, 1996; Desbiez & Lecoq, 1997).

The virus can be acquired by the vector in 10-60 seconds, and transmitted within 60 minutes of

access to a susceptible host (Lisa & Lecoq, 1984). Three to five hours after the acquisition (at 21 °C) time the virions lose their viability. It is thus possible for aphids caught in a cool wind current to carry the virus for great distances. *M. persicae* was able to retain and transmit the virus over a wide range of environmental conditions, whereas *A. gossypii* was unable to transmit the virus under low- temperature/low-humidity conditions (Fereres *et al.*, 1992).

According to some authors (Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990), no seed transmission occurs, but others (Al-Musa, 1989b; von Wechmar *et al.*, 1995; Desbiez *et al.*, 1996) report a very low rate of transmission by this means. Davis & Mizuki (1986) found 18% seed transmission in *C. pepo* 'Black Beauty', although the symptoms were not as severe as those in plants infected by other means. Aphids were able to acquire and transmit the virus from these plants. Gleason (1990) was unable to show any seed transmission in *C. melo*.

In a study conducted by Blua & Perring (1992a; b) it was found that the properties of a plant in the early stages of infection stimulated wing formation in aphids (especially *A. gossypii*) feeding on it. This would mean that the virus could be dispersed at a greater rate and with greater efficiency. When a plant is systemically infected with a virus there are changes in the concentrations of amino acids, sugars, nucleic acids, and proteins within the plant. These changes may act as cues to the aphids to change their behaviour, i.e. to produce more alate morphs (Blua & Perring, 1992a, b). However late stage infection of the host with ZYMV results in slow colony growth and the production of fewer alatae, and incoming aphids did not recognise (i.e. landed, tested and rejected) these plants as viable hosts. This would increase the number of viruliferous insects (Blua & Perring, 1992b).

The virus particles are non-enveloped, flexuous filaments. The particles measure 750 nm in length by 11 nm wide. Both the axial canal and the basic helix are obscure (Lisa *et al.*, 1981; Lisa & Lecoq, 1984; Purcifull *et al.*, 1984a; Nameth *et al.*, 1985; Büchen-Osmond & Purcifull, 1990; Desbiez & Lecoq, 1997). The virus usually induces cylindrical inclusions of type I, however some isolates induce inclusions of types III and IV (Desbiez & Lecoq, 1997).

Preparations of ZYMV are strongly immunogenic. Dissociation with sodium dodecyl sulphate

(SDS) is required to perform gel diffusion tests. ELISA and IEM can be used to detect the virus (Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990; Desbiez & Lecoq, 1997).

As the symptoms of ZYMV are very similar to those caused by other cucurbit viruses, a diagnosis based solely on symptoms is uncertain. The virus can easily be identified by means of standard serological techniques. ZYMV is often found in combination with other cucurbit viruses (Lisa & Lecoq, 1984; Büchen-Osmond & Purcifull, 1990; Desbiez & Lecoq, 1997). Any diagnosis based solely on symptoms should not be regarded as conclusive.

1.2.1.4 Papaya Ringspot Potyvirus (PRSV)

The disease caused by this virus was first identified in Hawaii in *Carica papaya* L. (papaya) plants, but the causal organism could not be identified. It was only later shown to be caused by a virus (Purcifull *et al.*, 1990). The Type W strain has been identified from the USA, Mexico, Caribbean countries, Germany, France, India, Italy, Taiwan, the Middle East, and South America (Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990). The Type P strain has been found in nearly all the tropical and subtropical areas where papaya is cultivated (Purcifull *et al.*, 1984c). No strains of this virus have been found so far in South Africa.

Synonyms of this virus are Papaya distortion ringspot virus, Papaya mosaic virus, Watermelon mosaic virus 1 (Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990), and Specific watermelon mosaic virus (Purcifull *et al.*, 1984c).

The Type P strain infects papaya as well as cucurbits. The Type W strain (previously referred to as Watermelon mosaic virus 1) only infects cucurbits. Antigenically these strains cannot be distinguished. A strain variant in Guadeloupe is distinct from, but related to, the Type P and Type W isolates (Purcifull & Hiebert, 1979; Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990). There is variation within the strains. The severity of the symptoms induced by Type P isolates varies. It may be possible that some of the milder strains could be used in cross-protection against more severe isolates. A strain isolated in Taiwan causes wilting in papaya (Purcifull *et al.*, 1984c).

In cucurbits, the Type W Strain induces mottling and distortion of both the leaves and the fruit.

Weeds belonging to the Cucurbitaceae, such as *M. pendula* and *M. charantia*, can act as reservoirs of the Type W strain. These particular plants can provide a large reservoir of the virus prior to the spring aphid migration as well as a site of overwintering. Other weeds which are susceptible to infection are *C. dipsaceus* and *L. siceraria.* This virus has a narrow host range (Adlerz, 1974a,b; Purcifull & Hiebert, 1979; Purcifull *et al.*, 1984c; Ullman *et al.*, 1991). The following plants can be used for diagnostic purposes: *C. papaya* (only Type P isolates), *C. pepo*, *C. metuliferus* cultivar Accession 2459, and *L. acutangula* (Purcifull *et al.* 1984c; Purcifull *et al.*, 1990). The Type W isolate has been shown to infect various cucurbit species (Purcifull *et al.*, 1984c).

The natural vectors of this virus are aphids, which transmit the virus in the stylet-borne manner. Twenty-one species, including *M. persicae* and *A. gossypii*, vector Type P isolates. Twenty-four species, including *M. persicae*, *A. solani*, *A. craccivora*, and *M. euphorbiae*, transmit the Type W strain. An amorphous inclusion protein is the helper component which is required for transmission. The virus can be acquired in acquisition probes of 10-60 seconds, and transmitted within 60 minutes of access to susceptible hosts. *Liriomyza sativae*, a leafmining fly, transmitted Type W isolates with a low frequency from squash to squash in greenhouse trials (Purcifull *et al.*, 1984c). The virus can also be transmitted by mechanical inoculation. The virus is not spread in the seeds (Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990).

The virus particles are flexuous, non-enveloped filaments. The particles measure 760-800 nm in length by 12 nm wide. Many particles can be found in leaf sap (Purcifull & Hiebert, 1979; Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990).

Preparations of the virus are strongly immunogenic. There is no reaction of the particles in standard gel diffusion tests. A reaction does occur in SDS-immunodiffusion tests after dissociation. This is unreliable unless 5% ascorbic acid is added to the leaf extract. All serological techniques require the addition of SDS or pyrollidine in order to disrupt the particles into diffusible fragments. Flocculent precipitates result from mixed liquid tests. ELISA can be used if the leaf extracts are prepared in 0.25M potassium phosphate and 0.1M EDTA at pH 7.5. ISEM can be used. SDS-immunodiffusion and indirect ELISA can be used to detect the pinwheel

inclusion protein. SDS-immunodiffusion tests, Western blot tests, and immunofluorescence *in situ* can be used to detect the amorphous inclusion proteins (Purcifull *et al.*, 1984c; Purcifull *et al.*, 1990).

1.2.2 The Cucumoviridae

1.2.2.1 Cucumber Mosaic Cucumovirus (CMV)

In the past this virus has been referred to as Cucumber virus 1, *Cucumis* virus 1, *Marmor cucumeris*, Soybean stunt virus, Spinach blight virus, and Tomato fern leaf virus (Francki & Habili, 1990).

CMV exists as a number of different strains which have been identified by using biological properties and amino acid sequence data of the coat protein (Francki & Habili, 1990). Members of the A-CMV strain-group are not pathogenic to members of the Cucurbitaceae. The B-CMV group and the C1-CMV subgroup cause systemic symptoms in *C. maxima* and *C. moschata* (Lovisolo, 1980).

This virus, which now has a worldwide distribution, was first found in the USA in *C. sativus* plants (Francki & Habili, 1990). It is now considered to be one of the most common viruses attacking cucurbits, particularly in the temperate regions (Lovisolo, 1980).

The symptoms, which persist in naturally infected plants, are variable and depend on the host and the strain of the virus involved. The hosts are found mainly in the Cucurbitaceae, Fabaceae, and Solanaceae. For a comprehensive list of hosts consult Francki & Habili (1990). CMV is often carried in weed seeds, and this may be an important factor in the epidemiology of this virus (Zitter, 1977). The weeds *M. charantia*, *C. dipsaceus* and *L. siceraria* have been recorded as harbouring the virus (Ullman *et al.*, 1991). The weed *Stellaria media* L. (chickweed) has been implicated in the overwintering of the virus. Members of the Cruciferaceae, Compositae, and Leguminosae are also susceptible to this virus (Lovisolo, 1980).

The presence of weeds infected with CMV is not always a threat to cucurbit crops in the area. In certain instances where this has occurred, there has been no increase in disease in the crop

(Lovisolo, 1980).

Species which have been used in diagnostic tests include *C. amaranticolor*, *C. quinoa*, *C. sativus*, *Vigna unguiculata* (L.) Walp., *Lycopersicon esculentum* L., *Nicotiana x edwardsonii*, *Nicotiana glutinosa*, and *Nicotiana tabacum* L. (common tobacco) (Francki & Habili, 1990).

Numerous dicotyledonous plants have been infected with the virus. Monocotyledons are not susceptible to this virus (Francki & Habili, 1990).

In nature the virus is vectored by more than 60 species of aphids in the stylet-borne manner. However, the vectoring of CMV by aphids is often inefficient and erratic. In some studies aphids have failed to transmit the virus when it is still detectable within the plant. Transmission is not dependent on a helper virus. It can be transmitted mechanically. Seed transmission has been shown to occur in 19 host species (Stimmann & Swenson, 1967; Francki & Habili, 1990).

The isometric particles have a diameter of 29 nm, are not enveloped, and have a rounded profile. The arrangement of the capsomeres is not readily seen. Variable numbers of particles can be found in the plant sap (Francki & Habili, 1990).

Although virus preparations are generally poorly immunogenic, this can be improved by aldehyde fixation. The virus particles react in standard gel diffusion tests. Non-specific precipitated particles in saline are required for serological tests. Granular precipitates result from mixed liquid tests. ISEM and ELISA can be used to detect the virus (Francki & Habili, 1990).

The hexagonal inclusions of CMV can be distinguished from those of tobacco mosaic virus (TMV) in that the CMV inclusions are hollow, show no evidence of stacked plate structures, are found mainly in the mesophyll cells and are resistant to Triton X-100 (Francki & Habili, 1990).

1.2.3 The Comoviridae

1.2.3.1 Squash Mosaic Comovirus (SqMV)

The virus was first isolated from *Cucurbita pepo* plants in California in the USA (Campbell,

1990). When compared with WMV2 and CMV this virus is considered to be of minor importance (Lovisolo, 1980).

The virus has been found to spread in Argentina, Australia, Brazil, Canada, China, Israel, Japan, Jamaica, Mexico, Morocco, the USA and Venezuela. It is presumed that it is present in Europe, India, and the former USSR It has been identified in New Zealand but it is not known to spread in this country (Campbell, 1990). SMV has been reported from quarantine plantings in South Africa (Trench *et al.*, 1992).

This virus has been referred to as Muskmelon mosaic virus, Pumpkin mosaic virus, and Cucurbit ring mosaic virus (Lovisolo, 1980; Campbell, 1990).

Two serological groups (I and II) of this virus are recognised (Campbell, 1990). Infection of cantaloupe with members of SMV-I results in severe symptoms, but these isolates only cause mild symptoms on pumpkin and watermelon. SMV-II isolates cause severe symptoms on pumpkin, only mild symptoms on cantaloupe, and do not infect watermelon (Lovisolo, 1980).

SMV causes a systemic mosaic, often in conjunction with ringspots and leaf deformation, in *C. melo*, *C. sativus*, *C. pepo*, *C. moschata*, and *C. maxima*. A mild yellow mosaic has been reported in *Ecballium elaterium* from Israel. There is a single report from Morocco of an infection of *C. album* (Campbell, 1990). The experimental host range, i.e. species used for diagnostic purposes, includes *C. melo*, *C. pepo* and *C. metuliferus* (Campbell, 1990).

The natural vectors of this virus are cucumber beetles (Order Coleoptera: Coccinellidae). The species *Acalymma trivittata* (Mannerheim), *A. thiemei thiemei* (Baly), *Diabrotica undecimpunctata undecimpunctata* Mannerheim, *D. bivittula* (Kirsch), *Epilachna chrysomelia*, and *E. paenulata* (Germar) have been shown to transmit the virus in a non-persistent manner. No helper virus is required for transmission to occur. It can be transmitted mechanically and by seed. Pollen is not a means of virus spread in this instance (Lovisolo, 1980; Campbell, 1990).

The non-enveloped isometric virus particles measure 28 nm in diameter. The profiles of the

particles are angular. The arrangement of the capsomeres is not readily visible (Campbell, 1990).

Preparations of this virus are strongly immunogenic. The particles will react in standard gel diffusion tests. Serological tests do not require special conditions. ELISA can also be used to detect the virus (Campbell, 1990).

1.2.4 Virus Spread

The type of virus, the crop, the environment, and the mode of transmission all interact to affect the spread of virus between plants (Thresh, 1974; Thresh, 1976; Harrison, 1981). Stylet-borne viruses for example, tend to spread in a very localized fashion around the primary infection foci. It is advantageous for viruses and vectors that exploit transitory hosts and habitats to spread rapidly and become established in new areas. Mild winters which allow the survival of crop and weed hosts as well as vectors can result in a great deal of early season virus spread (Thresh, 1974; Thresh, 1976).

As the distance from infection foci increases, the spread of viruses tends to decrease (Thresh, 1976). How readily a particular virus will be transmitted by aphids depends to a large degree on the number and size of virus sources (Zitter, 1977). Populations of infected plants are less important than the abundance and proximity of individual virus source plants (Zitter & Simons, 1980). Several plants can be infected by one aphid after a single acquisition feed (Yuan & Ullman, 1996). The disease gradient becomes shallower the stronger and more active the vector is, and the longer the virus is retained by the vector. Aphid behaviour and the distribution of virus sources are more important in the spread of stylet-borne viruses than the number of vectors present. In other areas, the majority of the virus spread may be due to migrants that fail to settle and colonise, or to a small number of very active aphids (Thresh, 1976). As far as WMV2 and ZYMV are concerned, primary virus introduction into melon fields from an external source(s) takes place at a low frequency. However, after the initial infection secondary spread both within and between fields takes place rapidly provided there is a great deal of vector movement (Castle *et al.*, 1992).

The overall plant condition can affect the spread of a virus, as in general the greatest numbers of

aphids are usually found on plants with distinct yellow symptoms. The yellow hue may be due to virus infection, a nutrient deficiency, or some other factor (Zitter & Simons, 1980). As the quality of the host declines, the aphids may be stimulated to search for a new host (Thresh, 1976).

Environmental conditions play a large role in determining the interactions between hosts, vectors and viruses, especially where overwintering is concerned. They also affect vector activity. Here the important factors are temperature, wind speed, wind direction, and the amount of air turbulence (Harrison, 1981). Wind is an important factor in virus spread, but the distances involved are often difficult to determine (Thresh, 1976; Harrison, 1981; Fereres *et al.*, 1992).

The occurrence of field grown plants which are infected with two or more viruses is common, especially when stylet-borne potyviruses are involved. The resulting effect of a dual infection on the viruses varies. There may be an increase in the transmission of one virus and a decrease in the transmission of the other; there may be an overall decrease in the transmission of both viruses; there may be no effect at all (Zitter, 1977).

1.3 THE VECTORS

1.3.1 Systematics

Aphids fall within the order Hemiptera, suborder Homoptera, series Sternorrhynca, which also includes the psyllids, scale insects and whitefly. The series is divided into superfamilies, with the aphids in the superfamily Aphidoidea, which is divided into three families, the Adelgidae, Phylloxeridae, and the Aphididae (Blackman & Eastop, 1984). Only the Aphididae will be considered.

The Aphididae is represented by such genera as *Aphis, Callaphis,* and *Lachnus*. The siphunculi are present or are secondarily absent, and the cauda are often developed. The antennae of the alates are usually five- or six-segmented. The parthenogenetic forms of these aphids are viviparous. The life cycle is variable, but is usually annual, with some groups having host alternation. A wide variety of plants serve as hosts for the members of this family (Blackman & Eastop, 1984).

Synonyms can be a problem in identifying some aphids, particularly those with a cosmopolitan distribution and those that are important crop pests such as *Aphis gossypii* (41 synonyms), *Myzus persicae* (36 synonyms) and *Aphis fabae* (33 synonyms) (Ilharco & van Harten, 1987).

The classification of an organism as a pest is a subjective exercise. A pest is generally a species which interferes with human activities. This makes the quality of being a pest an anthropocentric and circumstantial one. Many factors are considered in the ranking of a species as a pest (Pedigo, 1989). The major factors are shown in Figure 1.1.

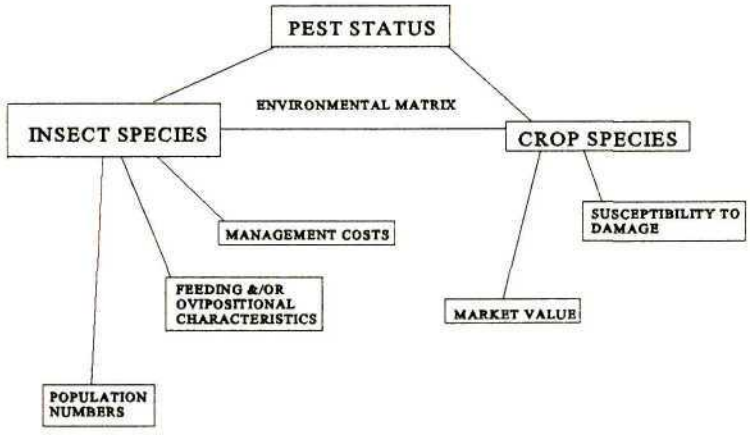

Figure 1.1 Factors used to class a species as a pest (Pedigo, 1989).

1.3.2 The Life Cycle and Polymorphism

The parthenogenetic phase of reproduction is common to all species of aphids, although in some it may be abbreviated to just two or three generations. On the other hand the loss of the sexual cycle is relatively widespread. This however, often occurs only in certain populations of a species which occur in an environment which favours this situation (Blackman & Eastop, 1984).

Some species exhibit heteroecy (host alternation), with the sexual stage occurring on one host, termed the primary host, and the asexual stage occurring on a different species, termed the

secondary host. The two different hosts are often distantly related (Blackman & Eastop, 1984). Cyclical parthenogenesis and heteroecy allow aphids to exploit their hosts, especially annual herbaceous plants, to their full potential (Blackman & Eastop, 1984).

There is a marked polymorphism in aphids with different types (or morphs) occurring depending on the stage of the life cycle (the change from the sexual generation to the asexual generation) and the season (the seasonal change of hosts). This may occur in either the larvae or the adults, with as many as five adult female morphs for a single clone (Miyazaki, 1987).

The fundatrix emerges from the egg and is a parthenogenetic female morph. This morph gives rise to a parthenogenetic line of females which ultimately ends with the production of sexuals. One of two morphs may arise from the fundatrix, either an alate or an apterous viviparous female. While the alate females tend to be rather uniform, there may be substantial variation amongst the apterous females. Towards the end of the season parthenogenetic females produce the alate sexuals, with different females producing the males and females. Some of the parthenogenetic females may produce gymnopara, which fly to the primary host and then deposit the sexual female. A similar situation may arise with males, where an andropara flies to the secondary host and deposits the sexual male. The sexual female is often referred to as the oviparous female (Miyazaki, 1987).

Parthenogenetic reproduction, which is important in determining the population structure of aphids as well as their high rates of increase, arose in the group 200 million years ago in the Permian. It allows for the telescoping of generations as the embryos of a viviparous female can have developing embryos within them. This allows for the tremendous rate of increase. Aphids have four larval instars in developing from birth to adult. The time required for this is dependent on four factors: food quality and temperature (extrinsic factors) and birth weight and if it is an alate or apterous morph (intrinsic factors). The peak of reproduction is usually achieved early on in adult life (Dixon, 1987a).

The egg stage is usually the stage of hibernation for aphids, however viviparous females of anholocycylic species are commonly reported to hibernate. Where winters are mild these females

may continue to reproduce, but at a much lower rate than during warmer periods. Some species produce a specialized hibernation stage at one of the larval instars which hibernates in concealed sites on a host plant. The larva moults in spring and development continues in the normal fashion (Swenson, 1968; Miyazaki, 1987). The following diagram, Figure 1.2, shows a typical aphid life cycle.

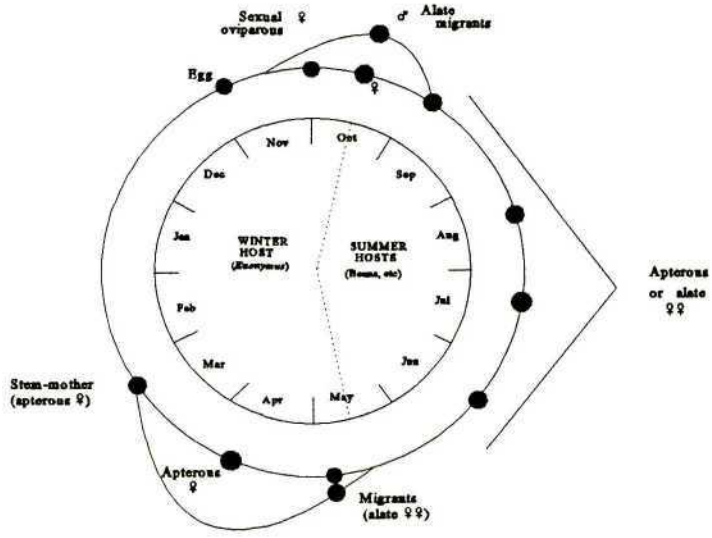

Figure 1.2 The life cycle of *Aphis fabae* (Black Bean Aphid). The black circles represent separate generations of insects (Davies, 1988). Note: this diagram represents the situation in a Northern Hemisphere context.

1.3.3 Host Plant Relationships

In general the primary host is the host with which the aphid first formed a relationship, with the secondary host being a later acquisition. This is suggested by the fact that many secondary hosts belong to more advanced plant families than the primary host. There does not necessarily have to be any relationship between the primary and the secondary hosts (Blackman & Eastop, 1984; Dixon, 1987b).

The aphid genera tend to be associated with specific plant families, and each species tends to be restricted to specific genera or species within that plant family. Aphid species which are considered to be pests on agricultural crops generally have a wider host range than other aphids

which are regarded as being economically unimportant. This however does not mean that they are polyphagous in the sense that they are totally non-specific, but rather that they are capable of feeding on many different families. Even *M. persicae*, the most polyphagous aphid, has not been recorded from even one percent of the flowering plant species. There are some plants which seem to have no aphids which are specific to them, but are attacked by a range of aphid species which use them as 'reserve' hosts. Exotic plants, such as imported agricultural crop plants, are the most likely candidates to act as reserve hosts (Blackman & Eastop, 1984; Dixon, 1987b). Members of the Aphidoidea are phloem feeders and thus have a diet which is high in sugars but low in amino acids. Factors which can inhibit aphids in one way or another are plant secondary metabolites (toxins) and the presence and type of hairs and hooks present on the host surface (Dixon, 1987b).

There is a succession of stages in the location of an acceptable host by an aphid. These are, in broad terms, the finding of the host habitat, the finding of a host within that habitat, the acceptance of the host, and the suitability of the host. There are a variety of stimuli to which the aphid responds during the process, but not all of these are of equal importance and may change with time and the stage in the life cycle (Klingauf, 1987).

When a host plant becomes stressed or the colony population becomes too dense the older larvae and the apterous adults leave the host. Wing and wing muscle development is controlled by five environmental factors: food, crowding, photoperiod, temperature, and time (Taylor, 1968). Once the alate morph has undergone its last moult its response to plant stimuli decreases while its response to 'sky' stimuli (light of wave lengths 300 to 500 μm) increases. During this time, known as the teneral period, the aphid is characterized by a 'resting mood'. This is followed by the distance flight which may cover hundreds of kilometres. The aphid then changes to an 'attack mood' and starts searching for a host. During the attack phase the aphids are increasingly attracted to the relatively longwave radiation reflected from the soil and vegetation. Yellow and green surfaces (wavelength 500 - 580 μm) are particularly attractive. Host plants may have subtle variations of tint and hue which influence their selection by certain aphids (Swenson, 1968; Klingauf, 1987). At wind speeds in excess of 1.5 mph (2.42 kph) aphids have no control over their direction of flight and can only settle when they collide with an obstacle, when they enter the

boundary layer due to air turbulence, or when the wind speed drops to below 1.5 mph (2.42 kph) (Swenson, 1968).

Visual cues used by foraging insects may include the spectral quality, the dimensions, and the pattern of individual plants or their component parts. Moving up to the plant population level, the distributional characteristics of a host plant population may be just as important to detection as the morphology of individual plants. Other aspects which may have an affect include the size of the vegetational patch, the degree of difference in morphological characters between hosts and non-hosts, the density and distribution pattern of host individuals in the patch, the degree of contrast between the boundary of the patch and the surrounding area or horizon, and the overall ease of detection of host plants in space and time. It is probable that non-visual signals are more important for detecting host plants than are visual stimuli (Prokopy & Owens, 1983).

Many of the diurnal herbivorous insects are attracted to yellow, which suggests that they may be able to distinguish between the wave-lengths of light reflected by foliage (which peak at 500 to 580 nm) and those reflected by other objects (which peak at less than 500 nm or at greater than 580 nm). It has been suggested that yellow is a "super-stimulus" of the foliage-type stimulus. Contrast between the plant and its surroundings appears to be important, as more aphids have been found to alight on plants that are surrounded by bare soil than those surrounded by other vegetation (Prokopy & Owens, 1983).

Once an alate arrives on a potential host, host acceptance/rejection is based on responses to many stimuli. The basic procedure is as follows: ① the aphid is attracted to a plant, ② the plant surface and outer tissues are tested, ③ the host tissues are penetrated, and ④ the phloem is tested. This is done by walking around the leaf surface and probing. The factors which affect host selection include: "gravity, light, temperature, relative humidity, barometric pressure, and wind; the host's shape, its colour and odour, structure of the surface and texture of the tissues; chemical composition of the surface, the outer and inner tissues including phloem sap, and amount of food; as well as plant spacing, neighbouring plants, the presence of weeds or bare soil in the vicinity of a host" (Klingauf, 1987). If the plant is suitable the aphid moves into the settlement mood and the following flights become progressively shorter. The qualitative and quantitative qualities of

the phloem sap ultimately determine the suitability of a plant as a host. If the plant is an unacceptable host, the following flight will be longer (Klingauf, 1987). The possible patterns of aphid movement are shown in Figures 1.3 - 1.6. Non-colonizing aphids can be important in the ecology of PRV-W and WMV2 (Zitter, 1977). This is probably true for all stylet-borne potyviruses.

Figure 1.3 The sedentary behaviour of the 'non-flyer' alate (after Moericke, 1955; Shaw, 1970; Harrewijn *et al.*, 1981).

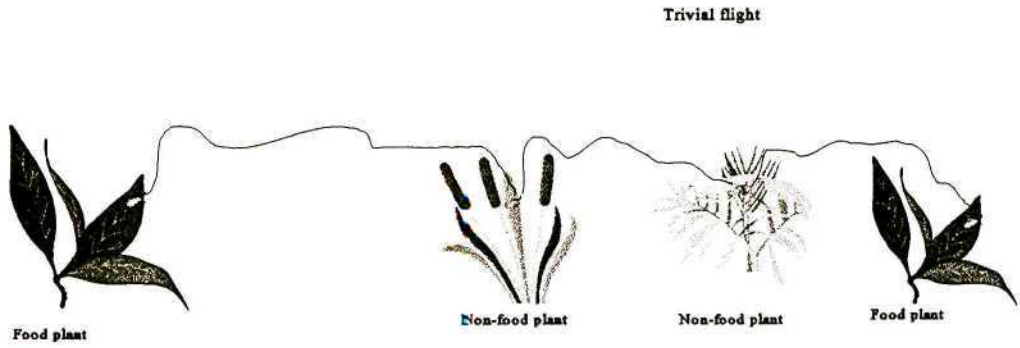

Figure 1.4 The flight behaviour of the 'flyer' alate (after Moericke, 1955; Shaw, 1970; Harrewijn *et al.*, 1981).

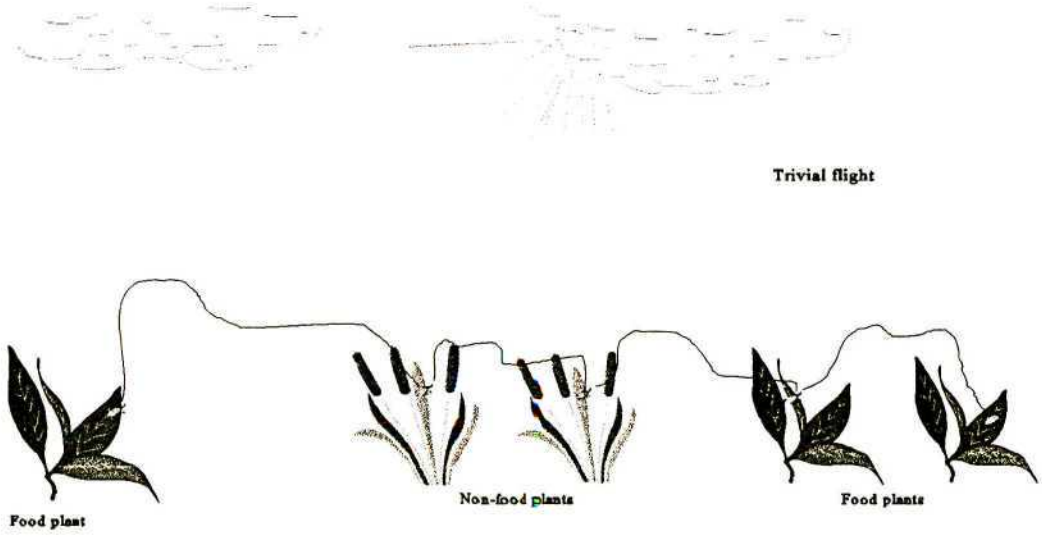

Figure 1.5 The behaviour of the 'migrant-non-migrant' alate (after Moericke, 1955; Shaw, 1970; Harrewijn *et al.*, 1981).

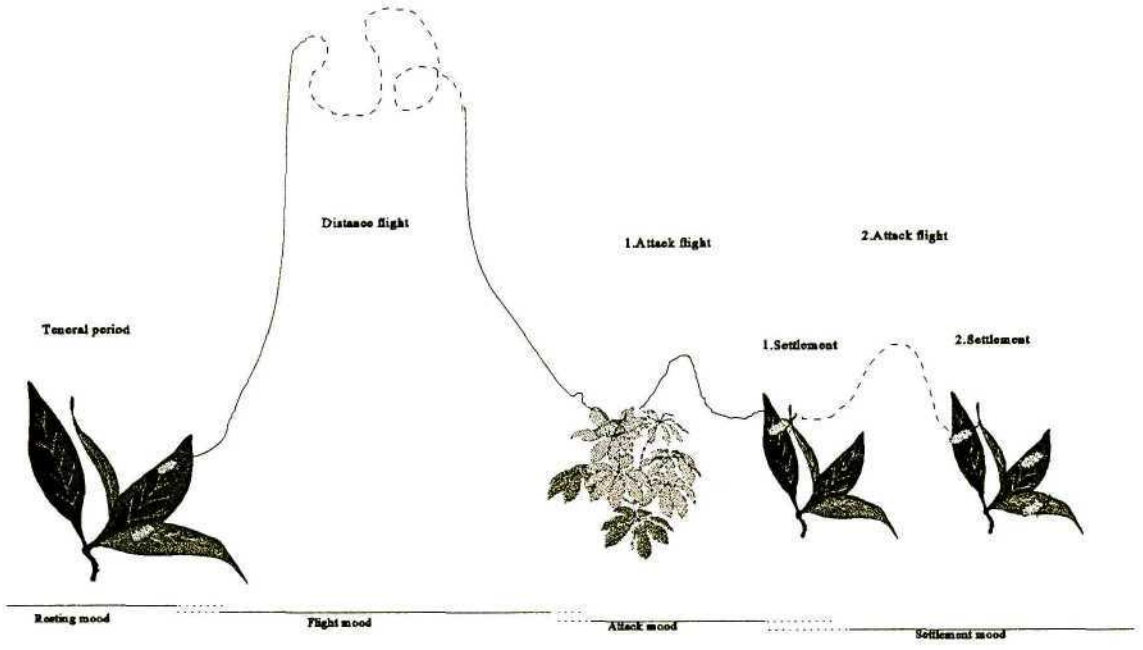

Figure 1.6 The flight pattern of an alate migrant aphid (after Moericke, 1955).

Olfactory stimuli may also assist in host location by aphids. Secondary plant substances and a variety of other factors can influence plant selection/rejection (Klingauf, 1987). In some cases it has been noted that diseased plants seemed to be more favourable for rapid vector development than healthy plants (Swenson, 1968).

Seasonal recolonization of an area is probably due to progressive and continuous movement over several generations rather than a single migration flight. This indicates that middle distance migrations of 1-100 km may be more important than long distance flights (Taylor, 1968).

1.3.4 Geographical Distribution

There is a characteristic aphid fauna occurring on indigenous plants in each of the major zoogeographical regions of the world. The majority of the major agricultural crops are exotics in the countries where they are grown, and their aphid pests are usually also exotic to the region (Blackman & Eastop, 1984).

Few of the aphid species which are regarded as pests in an area are native to that area, and it can be said that certain species will eventually be found wherever their crop host plants are cultivated. Many of the cosmopolitan pest species are anholocyclic, which enables them to survive all year round by parthenogenetic reproduction. This allows them to spread to areas where their primary host does not occur (Blackman & Eastop, 1984).

1.3.5 Morphology

The taxonomy of aphids is based predominantly on the morphology of the adult viviparous female. In general these insects are plump, soft-bodied, and ovoid in shape. The head and prothorax are usually easily distinguished from the rest of the body. Specimens which measure less than two millimetres in length are regarded as being small, while those greater than 3 mm are regarded as being large (Millar, 1990).

The eyes may consist either of just three facets, or be multi-faceted (compound). A three-faceted ocular tubercle is often present on the posterior margin of the compound eye. Three ocelli are always present in alate morphs and absent in apterous forms (Millar, 1990).

The antennae are four to six segmented. There are five segments in the rostrum which is positioned below the head. It has a groove in the dorsal side which houses the stylets (Millar, 1990).

The tarsi are usually two-segmented, with the first segment being much shorter than the second one. At the end of each tarsus are two claws (Millar, 1990).

Alate aphids have two pairs of wings which are relatively poor in venation. The hind wing is smaller than the fore wing, and has a set of hamuli (hooklets) which allow the hind and fore wings to be linked during flight. The wings are usually pale, except for the pterostigma (Millar, 1990).

The abdomen is comprised of nine visible segments. In most cases the siphunculi are present. These structures may be secondarily lost in some groups. They vary greatly in structure and are very important taxonomically (Millar, 1990).

An important taxonomic feature is the number of hairs on and the shape of the cauda. The cauda can be used to flick away drops of honeydew which are excreted from the anus. The droplets of honeydew present on the cauda and anal plate are often removed by ants (Millar, 1990).

1.3.6 Virus Transmission

Aphids transmit more plant viruses than any of the other invertebrate groups (Swenson, 1968). Aphids are an important factor in the spread of viruses both within a field, and over long distances. This transmission can fall into one of three broad categories: non-persistent or stylet-borne, semi-persistent, and persistent or circulative transmission. Circulative transmission can be further divided into two subdivisions: propagative and non-propagative (Swenson 1968; Sylvester, 1989). As this study focuses on stylet-borne viruses and their vectors this is the only type of transmission that will be considered further.

Stylet-borne viruses only have a temporary relationship with their vector(s). The virus can be acquired from and inoculated into a plant in a very short space of time, often requiring less than one minute. The virus is lost in about an hour by feeding aphids and after several hours by non-feeding aphids. The efficiency of acquisition and transmission varies with vector species. In some cases the virus requires a helper factor in order for the vector to acquire it. There are several groups of viruses which fall into this group: the carlaviruses, the caulimoviruses, the cucumoviruses, the potexviruses, and the potyviruses (Swenson, 1968; Zitter, 1977; Sylvester, 1989). An increase in the acquisition time does not increase the probability that an aphid will acquire a stylet-borne virus. The host species can have an affect on the amount of virus available to the vector which will affect the amount of spread. For example, cucumber is generally a poor host for cucumber mosaic virus (CMV) (Zitter & Simons, 1980). The majority of acquisition studies indicate that the virus is acquired from the epidermal cells during the brief sensory probes (Blua & Perring, 1992a).

In order for a vector to spread a plant virus the following sequence of events must occur:
① a primary source of the virus is present either as an infected plant or as an over-wintering vector (this later situation would not be a factor in stylet-borne virus epidemiology); ② the vector forms an association with the source plant; ③ the vector tests the plant to assess its suitability as

a food plant by probing, and may feed on the host plant; ④ the virus is acquired by the vector during probing or feeding; ⑤ the vector may become viruliferous immediately after probing or feeding (e.g. stylet-borne viruses, or there may be some delay (e.g. circulative viruses); ⑥ the vector moves from the source plant to a healthy host plant; ⑦ this plant is then inoculated by the vector (either by probing or feeding, or some other manner); and ⑧ the plant becomes infected with the virus, and can thus act as a source plant (Irwin & Ruesink, 1986). Factors which are important in the spread of viruses by aphids are: the number of aphids present, aphid activity, the timing of aphid flights relative to the age of the susceptible crop, the species of aphid concerned, and the availability (distribution and abundance) of virus sources. Temperature is an important factor in the spread of aphid-borne viruses. A mild winter may result in early spring flights of some aphid species which were able to overwinter on secondary hosts (Zitter, 1977). Aphids are incapable of directional flight when the wind speed exceeds 1.5 mph (2.42 kph). When the wind speed is in excess of this and is unidirectional, unidirectional aphid dispersal in the same direction as the wind can be expected (Adlerz, 1974a).

The relative importance of alate and apterous aphids appears to vary depending on the virus involved and location of the pathosystem in space and time (Thresh, 1976).

There are many factors of aphid behaviour which favour the spread of stylet-borne viruses. These include random alighting of aphids on hosts and non-hosts, the dominance of dispersal over host-finding, and the combination of the high intensity and the long duration of aphid migrations (Swenson, 1968). If and when a crop is initially infected is usually determined by migrant vector flights. For some aphids the plant to plant movements involved in host selection is the only time during which they are efficient vectors. When a continuous parthenogenetic cycle is combined with a continuum of virus-susceptible, vector-host plants, there is the possibility of a virus reservoir building up over successive seasons. Crowding causes an increase in the number of alate aphids produced. This leads to a flood of emigration that ultimately leads to the decline of the colony (Taylor, 1986).

When studying virus transmission by aphids the differentiation must be made between the aphid's ability to transmit the virus, and its importance in the spread of that virus. Thus the importance

of a particular vector species is dependent on its abundance and efficiency as a vector. Therefore a vector which is inefficient but is present in large numbers may be the most important vector (Raccah, 1986).

1.3.7 The Vectors of Cucurbit Viruses

A number of aphids have been recorded, either as colonizers or as transitory visitors, from various members of the Cucurbitaceae. Those that are commonly recorded are shown in Table 1.1. This is not a complete list as other aphids do attack cucurbits.

The following list contains those aphid species which have been recorded from cucurbit plants in sub-Saharan Africa: *A. gossypii* (*C. lanatus*; *Coccinia adoensis* (A. Rich.) Cogn.; *Cucumis hirsutus* Sond.; *C. melo* L.; *C. myriocarpis* Naud.; *C. sativus*; *C. maxima*; *C. pepo* L.; *Lagenaria* sp.; *Momordica charantia* L.; *Sechium edule* (Jacq.) Sw.; *Zehneria scabra* (L.f.) Sond.); *A. craccivora* (*S. edule*); *A. fabae* (*C. pepo*); *M. persicae* (*C. lanatus*; *Cucumis* sp.; *C. maxima*); *M. euphorbiae* (*Cucumis* sp.; *C. maxima*; *C. pepo*) (Millar, 1994).

Table 1.1 Aphids commonly recorded as feeding on cucurbits (Blackman & Eastop, 1984).

Aphids	Cucumber	Melon	Pumpkin	Watermelon
Aphis citricola	+	-	-	-
A. craccivora	-	+	+	+
A. fabae	+	-	+	-
A. gossypii	+	+	+	+
A. maidiradicis	-	-	+	-
A. nasturtii	+	-	-	-
Aulacorthum solani	+	-	-	-
Macrosiphum euphorbiae	+	-	+	-
Myzus persicae	+	+	+	+
Smynthurodes betae	-	-	+	-

It must always be remembered that the most common aphid species or biotype is not necessarily the most important vector species, as vector efficiency may be more important. On the other

hand, the most efficient virus vectors are not always the most important vectors, as the development of large populations may be more important (Swenson, 1968; Thresh, 1976).

1.3.7.1 *Aphis gossypii* Glover (Melon Aphid; Cotton Aphid)

The appearance of the apterous morph is highly variable. The larger individuals (1.8 mm) are dark green, but when an individual matures in crowded surroundings at high temperatures it may be less than one millimetre and pale yellow to almost white. Usually they are light green with a dark green mottling, dark siphunculi, and a pale or dusky cauda. This species is often attended by ants. The alate morph varies in length from 1.1 to 1.8 mm (Blackman & Eastop, 1984).

This species is very polyphagous and is considered to be a major pest of both cotton and cucurbits. In addition to this it is known to vector more than 50 viruses. It is found virtually world wide but is limited to glasshouses in temperate areas. The taxonomy of the species is difficult, as throughout the world it has a number of anholocyclic lines, each of which has many unique characters of its own (Blackman & Eastop, 1984).

1.3.7.2 *Macrosiphum euphorbiae* Thomas (= *solanifoli* Ashmead) (Potato Aphid)

The adult apterous morph ranges in size from 1.7 to 3.6 mm, and are may be spindle- or pear-shaped. The alate morph varies in size from 1.7 to 3.4 mm. The eyes are reddish in colour, and the body is usually a shade of green and is often shiny. The immature insects are paler than the adults with a dark spinal stripe, a light dusting of a whitish wax, and are long-bodied (Blackman & Eastop, 1984).

Rosa species act as the primary hosts for this species. It is very polyphagous, using more than 200 plant species in over 20 families as secondary hosts. It is known to vector more than 40 stylet-borne viruses and five circulative viruses. The species appears to have originated in North America, but is now almost world wide in its distribution. Outside of North America it exists in an anholocyclic manner, although sexual morphs are sometimes produced in small numbers (Blackman & Eastop, 1984).

1.3.7.3 *Myzus persicae* Sulzer (Green Peach Aphid; Peach-Potato Aphid)

This aphid is small to medium in size (1.2 - 2.3 mm). The apterous morph occurs in a variety of colours ranging from a whitish green through mid-green to pink or red. This colouring is uniform and the insect is not shiny. Adults of the alate morph have a black dorsal patch in the centre of the abdomen. It is often found in dispersed colonies on the older leaves of a wide range of plants (Blackman & Eastop, 1984).

The primary host is usually *Prunus persica* (L.) Batsch (peach), but the aphid may also use *P. nigra* Ait. (Canadian plum), *P. tanella* Batsch (dwarf almond), and possibly *P. serotina* Ehrh. (black cherry) for this purpose. The secondary hosts range over more than 40 plant families, and include many economically important species (Blackman & Eastop, 1984). Although this species is highly polyphagous in relation to other aphids, it has been recorded from less than one percent of all vascular plants. Thus in terms of the usual meaning of polyphagy, i.e. a total lack of specificity, *M. persicae* is quite specific (Blackman, 1976). *C. lanatus* is not colonized by *M. persicae*, which can thus be expected to make numerous short flights, increasing the transmission of viruses (Adlerz, 1974b).

This aphid, which is probably of Asian origin but is now spread worldwide, is the most important virus vector. It has been shown to vector in excess of 100 plant viruses. Although this species is normally heteroecious between *Prunus* and secondary host plants, it is capable of an anholocyclic existence on secondary hosts in climates that permit survival over the winter period (Blackman, 1976; Blackman & Eastop, 1984).

The question "How important is it to measure the abundance of a vector species?" has been asked.....When modelling the epidemiology of a virus, vector density only indirectly influences the spread of a virus. The degree of vector movement and associated activity can change with a change in the abundance of the vector species (Irwin & Ruesink, 1986).

Other insects which attack cucurbits are listed in Appendix II.

1.4 VECTOR AND VIRUS CONTROL

1.4.1 Aphid-Borne Virus Management

Plant virus diseases are difficult to control in the tropics for a number of reasons: Winter temperatures are not severe enough to break the disease cycles; reservoirs of insect vectors are present at all times; resistant and tolerant cultivars are not generally available; and the crops are usually grown in many small, scattered plots making isolation difficult if not impossible (Gonsalves & Garnsey, 1989). These problems are exacerbated under extreme epidemic conditions (Raccah, 1985).

After the Second World War, disease control moved its emphasis from the pathogen to the pathosystem, and now the focus is shifting towards the entire production system. With the realization that chemical pest and pathogen control has detrimental side effects, there have been changes in crop protection: ① it is acceptable to keep pathogen populations below the economic threshold rather than eradicate them, ② cultural methods of crop protection such as biological control and crop rotation were developed or improved, and ③ the realization that the use of more than one control method was better than total reliance on one, usually chemical, control measure (Rabbinge & van Oijen, 1997).

An important point to take note of at this point is that healthy plants are usually not as attractive to aphids as virus-infected plants (Zitter, 1977). It has been noted that in some cases diseased plants appeared to be better suited to rapid vector development than healthy plants (Swenson, 1968).plants in the late stage of infection with ZYMV are initially more attractive to incoming vectors, but are rejected after probing (Blua & Perring, 1992a).

A thorough understanding of the vector(s), the hosts, the virus, and the environment is required. For example is the virus and/or the vector present in weeds surrounding the crop? It must also be kept in mind when conducting vector studies that the most common species caught may not be the most important vector (Zitter & Simons, 1980; Sylvester, 1989).

In the epidemiology of plant viruses the date that the virus is first introduced to a field or area is extremely important, as the earlier that the virus is present the greater the damage to the crop

(Thresh, 1974).

As viruses cannot be chemically controlled they can be a serious problem. Control is best achieved with genetic resistance. However, this is not always available, so other means are required (Robinson & Decker-Walters, 1997). These are discussed in the following text.

There are farming practices which ultimately result in a better crop as well as reduce the impact of virus diseases by affecting the efficiency of virus vectors and crop management strategies (Zitter & Simons, 1980). The most common management practices are: ① virus source elimination, ② crop isolation from virus sources, ③ crop manipulation, and ④ a variety of methods which aim to reduce the number of active vectors either regionally or locally (Sylvester, 1989).

1.4.1.1 Virus source elimination

The most vulnerable point in the epidemiological cycle of viruses is the virus source. These can be reduced by seed certification programmes and the roguing of volunteer plants (weeds and crops) (Zitter, 1977).

The initial infection of a field can be the result of infected planting material or the plants have been fed upon by viruliferous vectors which have immigrated from other infection sites (Maelzer, 1986).

1.4.1.2 Quarantine and certification programmes

Quarantine measures aim to prevent the introduction of a virus into an area from which it has not previously been reported. This may be effective against seed-borne or vegetatively transmitted viruses, but with the potential of long-distance dissemination of aphids these measures are unlikely to be effective (Sylvester, 1989).

Certification measures aim to limit (hopefully eliminate) the amount of infected propagation material. This will then delay the entry of the virus into the crop which will hopefully allow for a better yield (Thresh, 1974; Sylvester, 1989).

1.4.1.3 Control of weeds and volunteers

Before entering into control measures, the importance of weeds in the pathosystem will be discussed. A weed is a plant that has harmful or objectionable characteristics, and is growing where it is not wanted (Duffus, 1971). Roadsides, irrigation ditches, perennial plantings such as orchards and vineyards, railroads and abandoned or neglected crops are places where weeds can be found in large numbers (Duffus, 1971; Thresh, 1976). Previous studies have shown that weeds, volunteers and wild plants are often infected with agronomically important viruses such as CMV. This virus is often symptomless in its weed hosts. Once a virus is introduced to an area, weeds may serve as permanent reservoirs of the virus in that area (Duffus, 1971; Orsenigo & Zitter, 1971). The prevalence of viruses in a crop can be affected by the composition of the weeds in which they persist when cucurbits are not being cultivated (Al-Musa, 1989b). Weed plants can act as reservoirs for aphids, and in some cases may serve as reservoirs of both aphids (vectors) and viruses (Zitter, 1977). Many of the domesticated cucurbit species can be problematic weeds (Robinson & Decker-Walters, 1997). The removal of weeds would probably be of little benefit in the control of ZYMV as this virus has few known reservoirs (Desbiez & Lecoq, 1997).

In a study conducted by Al-Musa (1989b) CMV was only found in *Lablab purpureus* (L.) Sweet (= *Dolichos lablab* L.) and *Solanum nigrum* L., WMV2 in cucurbit weeds and *Malva parviflora* L. (cheeseweed), and ZYMV in cucurbit weeds (except *Ecballium elaterium*), *Sysimbirium irio* L. (London rocket) and *Crepis aspera*. WMV2 and ZYMV were often found in mixed infections in the cucurbit weeds.

Certain weeds may serve a beneficial purpose by being involved in the biology of beneficial insects and by reducing crop apparency. By managing weed populations, some pest problems may be reduced. Weed flowers, especially those of the Umbelliferae, are used by adult hymenopteran parasitoids to bridge periods when their host(s) is unavailable. Flowers of the Compositae are preferred by the predaceous lacewings (Neuroptera), while pollen is important in egg production for many Syrphidae (Diptera), and is used as a food source for some coccinellids (Coleoptera) which prey on aphids. The extrafloral nectaries of peach trees are reportedly used by a number of aphidophagous coccinellids such as *Coccinella* spp., *Adalia bipunctata* L. and *Cycloneda*

sanguinea L. Weeds may also provide a habitat where non-pest herbivorous insects can increase in number, thus aiding the survival and reproduction of beneficial species. Although beneficial insects usually move from weeds into the crop, the presence of alternative food sources on the weeds may prevent or delay this movement. It is possible to force the insects into the crop by cutting down the weeds, but the timing of such an action should be planned to coincide with optimal phases in the biology of the beneficial insects (Altieri & Whitcomb, 1979, Bowie *et al.*, 1995). The use of companion planting, herbs or novel crops are alternatives to weed management (Bowie *et al.*, 1995).

When crops are grown in the same or adjacent fields on consecutive years volunteer plants can serve as major sources of virus for primary infection (Duffus, 1971; Zitter, 1977; Zitter & Simons, 1980; Maelzer, 1986). Plants which are related to the crop in question can also serve as a source of virus (Zitter, 1977). The importance of volunteers can be reduced by crop rotation and cultivation of fields (Zitter & Simons, 1980).

Weed eradication is usually aimed at the control of stylet-borne viruses. On the negative side, it is expensive, and in areas where there is a high density of both weed hosts and vectors it may not be worthwhile to plant susceptible crops. Either herbicides or cultivation can be used. When susceptible crops are absent from the area, herbicides belonging to the chlorophenoxy group can be used. The use of cultivation may have a negative effect by forcing the vector off the preferred weed host and onto the non-preferred crop host (Duffus, 1971; Orsenigo & Zitter, 1971; Sylvester, 1989). The potential benefits of this practice can be nullified if neighbouring fields or farms are not treated in a similar manner (Zitter & Simons, 1980). Apart from acting as over-wintering sites for both vectors and viruses, alate migrants develop earlier in the season on secondary hosts than on primary hosts. The secondary hosts are also likely to be hosts of the virus, thus allowing for the development of viruliferous migrants (Swenson, 1968; Duffus, 1971).

Many plant diseases caused by insect-vectored viruses can be successfully controlled by efficient weed control strategies. This may be as good as, or better than, chemical control of the vectors (Duffus, 1971; Zitter & Simons, 1980; Sylvester, 1989; Jones, 1991). With cucurbit crops it is important to remove weed hosts from surrounding areas before the crop is planted (Zitter, 1977).

While weeds are in themselves a problem, their presence can affect the behaviour and efficiency of both pests and beneficial insects. The presence of weeds near a field can in some instances be beneficial by providing alternate prey/hosts, pollen or nectar, and microhabitats that the weed-free crop stands do not offer. In some instances the presence of alternate prey in the weed stands prevents or delays the dispersal of beneficial insects into the crop. When this occurs, the weeds can be cut, forcing the beneficial insects to move. This however is not usually required. The composition of the weed communities is also important to ensure the presence of species which will attract beneficial insects (Altieri, 1994).

1.4.1.4 Roguing

This is the removal of diseased material from a field. In annual crops it is not of much use in controlling virus diseases, especially those caused by stylet-borne viruses, as by the time symptoms are expressed the plant has already been infectious for a few days. It has been shown to be effective in some perennial crops such as banana (Thresh, 1976; Zitter, 1977; Sylvester, 1989; Jones, 1991). The removal of old infected crops could however reduce the sources of viruses in the immediate area of new young crops (Desbiez & Lecoq, 1997).

1.4.1.5 Crop isolation from virus sources

Time:

Time is a control technique that requires a lack of a significant endemic cycle in weed hosts, and uses crop-free periods, i.e. periods when the fields are not planted with the susceptible crop. This technique can also be used to avoid the major migration times of aphid vectors. It is particularly effective against viruses with a limited host range (Zitter & Simons, 1980; Harrison, 1981; Sylvester, 1989).

Space:

An example of this technique is that potato seed crops are planted at a place remote from commercial crop stands, or at the very least upwind from the commercial plantings (Sylvester, 1989).

A plant-soil mosaic is more attractive to aphids than a continuous green 'carpet' of foliage.

Denser plantings and fast growing cultivars may result in lower aphid densities (Gibson & Plumb, 1977).

The use of cover crops and barrier crops also fall into this category. A cover crop is a crop which is interspersed with the crop which is to be protected. The plants of the cover crop are generally taller than the main crop, and thus cover it. Barrier crops are usually planted around the crop to be protected. In both cases the aphids are 'cleaned' of any stylet-borne virus before they reach the main crop (Thresh, 1976; Zitter & Simons, 1980; Maelzer, 1986; Sylvester, 1989; Jones, 1991).

Windbreaks and hedges can be used as a form of barrier. In addition, beneficial insects can use windbreaks and hedges for overwintering. However, pest species can also use them for this purpose. There may also be some competition between the windbreak and the crop for light, water and nutrients (King & Olkowski, 1991). It may also be beneficial to actively create and/or manage so-called 'island' habitats within and around crop stands. By doing this the structural diversity of the agroecosystem is increased which results in community stability and the movement of predators from the 'islands' into the crop, thus markedly improving pest control (Thomas *et al.*, 1992; Altieri, 1994). The diversity of the vegetation can alter the movement of insect herbivores, thereby influencing their abundance (Coll & Bottrell, 1996). Increased biodiversity can be used by subsistence farmers to assist them in achieving a stable, year-round food supply. The diversification of vegetation can result in pest regulation, optimal nutrient recycling, soil conservation, energy conservation and less dependence on external inputs which are often expensive and scarce (Altieri, 1991; 1994).

Trap crops protect target crops from attack by pest organisms such as insects and nematodes by attracting the pests to them. The protection is exercised by preventing the pests from reaching the crop, or by enabling control measures to be concentrated in certain areas. The trap crop may be the same cultivar as the target crop, or an entirely different species, but must be more attractive than the target crop. The use of trap crops reduces the cost of insecticide use and often increases yields. In certain instances they have been shown to attract natural enemies of insect pests to the area, thus enhancing natural biological control. It is thus a method which is of potential benefit

to both commercial and subsistence farmers (Hokkanen, 1991; Dent, 1995). Trap crops are more effective when they are a different species and taller than the target crop. Thus maize and sunflowers are popular choices. The trap crop may serve merely to dilute the incoming vectors, thus reducing numbers in the main crop, or it may be treated with an insecticide to kill the vectors (Maelzer, 1986).

When setting up a trap crop the following points must be kept in mind: ① the number of pests expected, ② the mobility of the insect, and ③ the direction(s) of influx of insects. Thus the behaviour of the insect must be known for a trap crop to be effective. The effectiveness of trap crops has often been enhanced by the use of sex and aggregation pheromones (Hokkanen, 1991). When planted outside the main crop trap crops are easier to manipulate, though they often perform better when they are interplanted with the main crop. In addition to their beneficial characteristics when planted around a crop, an interplanted trap crop can screen the main crop from migrating vectors, reduce colonization due to increased plant density, and increase the distance between susceptible plants thus reducing the rate of spread (Maelzer, 1986).

Net increases in profits of 10 to 30% have been recorded, mainly due to reduced use of insecticides, reduced pest attack, or a combination of the two. The greatest benefit will be derived when the target crop is a non-preferred host of the pest, and the trap crop has another beneficial use such as human consumption, animal feed, or green manure (Hokkanen, 1991). Virus spread due to insect vectors has been significantly reduced by the use of trap crops (Dent, 1995).

A form of trap crop practice is the strip intercrop method. In strip intercropping "two or more crops are grown simultaneously in strips wide enough to permit independent cultivation, but narrow enough for the crops to interact agronomically" (Grossman & Quarles, 1993).

In the 1930's Marcovitch (1935) had favourable results in fields where rows of turnips were planted in every third row after two rows of watermelons and other food crops. This was believed to be due to turnips encouraging beneficial insects as well as hosting aphids. In Marcovitch's experiment, the turnip crop was planted two weeks prior to the planting of the

watermelons, and was used as a sacrificial crop. The planting of kale, mustard or rape around melon fields has also been advocated in the past. Coccinellid beetles could build up their populations on the crucifer by feeding on the cabbage aphid, and would be able to move into the melon field to control the melon aphid. A combination of turnips, melons and cabbages has also been used to control aphids (Grossman & Quarles, 1993).

Marcovitch (1935) also recommended using cotton, corn, cowpea, sorghum and various *Crotolaria* species as intercrop plants. The plants chosen for strip intercropping have to be carefully selected to ensure that there is no competition between the plants, and that the trap crop is not deleterious to beneficial insects (Grossman & Quarles, 1993). Plants belonging to the Umbelliferae, Leguminosae, and Compositae have been shown to harbour and support a complex of predators and parasitoids which are useful in suppressing populations of pests (Altieri, 1994).

Letourneau (1990) found that when squash (*C. pepo*) was intercropped with corn (*Zea mays* L.) or with cowpea (*Vigna sinensis* (L.)) the generalist predator *Orius tristicolor* (White) (Hemiptera: Anthocoridae) showed greater colonization rates. This was also true of densely planted monocultures and monocultures with artificially enhanced complexity. Bugg *et al.* (1991) used cool-season cover crops (vetches, clovers, or Brassicaceae) as relay intercrops to enhance populations of the bigeyed bug *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae). Other predators which find these cover crops attractive are the insidious flower bug (*Orius insidiosus* (Say) (Hemiptera: Anthocoridae)), lady beetles (Coleoptera: Coccinellidae) and other bigeyed bugs (*Geocoris* spp.).

By using strip intercropping, less pesticides are used and soil conservation is promoted. This makes the technique more desirable from an ecological and environmental view point (Grossman & Quarles, 1993). By increasing the diversity of a system it is possible to improve the abundance and efficiency of beneficial insects by: "① providing alternative hosts/prey at times of pest host scarcity; ② providing food (pollen and nectar) for adult parasitoids and predators; ③ providing refuges for overwintering, nesting and so on; ④ maintaining acceptable populations of the pest over extended periods to ensure continued survival of beneficial insects." Studies have shown that there are fewer herbivores in polycultures than in monocultures. This may be due to

improved insect diversity in polycultures or increased search efficiency of herbivores where monocultures are involved (Altieri, 1991; 1994). Coll & Bottrell (1996) found that diverse stands of plants tend to reduce the emigration rate of beneficial insects from an area. The diverse habitats created by diversified agroecosystems may provide more microhabitats which are attractive to beneficial insects. (Altieri, 1991).

Once a stylet-borne virus has been acquired by an aphid, it will be lost if the aphid probes an intermediate plant prior to feeding on another host plant of the virus (Raccah, 1986). However, there are some problems associated with trap crops. Highly mobile pests may not remain within the trap crop; some aphid species which are passively dispersed cannot be attracted to a trap crop; in some cases more pests than normal may be attracted to the area by the trap crop; natural enemies may not aggregate in the trap crop, or they may be killed when the trap crop is treated with insecticides which may have a negative impact on the overall situation. In the absence of pests trap crops are useless. Reliable forecasting techniques will probably increase the attractiveness of this technique to farmers (Hokkanen, 1991). An increase in the diversity of vegetation may cause emigration of parasitoids from an area, and may also interfere with the trivial movement of parasitoids by altering air movement or creating physical barriers. Plants of different heights are the most problematic in this regard. By keeping a monoculture format, parasitoids which use plant cues to locate their hosts can be encouraged (Coll & Bottrell, 1996).

1.4.1.6 Crop manipulation

The delaying of planting to avoid the spring migration of aphids is one method of crop manipulation. Field size has an effect on crop vulnerability. It has been shown that if vector population is limited and remains constant in size, then the proportion of diseased plants will decrease as field size increases (Sylvester, 1989). Early season spread of viruses is more important than late season spread because seedlings have a greater susceptibility to infection than older plants, and the effect on yield is greater. These plants will also act as important sources of virus for secondary spread of the virus later in the season (Swenson, 1989). A crop that is planted upwind from major virus sources can avoid severe infestation when windborne vectors are the major means of spread (Thresh, 1976).

The age of the plant, the leaves at the time of inoculation, as well as any genetic variation can affect the susceptibility of a given host plant (Zitter, 1977). The use of resistance breeding also falls under crop manipulation. The use of resistant varieties can be beneficial in reducing losses due to virus diseases (Sylvester, 1989; Desbiez & Lecoq, 1997). Beck (1965) defined host plant resistance as the "collectable, heritable characteristics by which a plant species, race or clone, or individual may reduce the possibility of successful utilization of that plant as a host by a pest species, race, biotype or individual."

In cucumber there are a number of different alleles which have been reported to confer disease resistance: e.g. *Wmv* for watermelon mosaic virus and *Cmv* for cucumber mosaic virus. In the later case more alleles of additional genes are needed to obtain a high level of resistance. Zucchini yellow fleck virus and zucchini yellow mosaic virus are 'combatted' with the recessive alleles *zyf* and *zymv* respectively, while *prsv* and *Prsv*-2 provide resistance to papaya ringspot virus (Robinson & Decker-Walters, 1997).

Melon contains the *nsv* gene which gives resistance to melon necrotic spot virus. Resistance to papaya ringspot virus is conferred by two alleles, Prv^1 and Prv^2, of a single gene, but their reaction differs with some strains of the virus. The dominant allele *Zym* gives resistance to pathotype 0 of zucchini yellow mosaic virus. The dominant allele of gene *Ag* provides tolerance to the melon aphid (*A. gossypii*), and resistance to viruses transmitted by this insect is conditioned by *Vat* (Robinson & Decker-Walters, 1997).

The *Zym* gene in squash has been reported to play a role in resistance to zucchini yellow mosaic virus (Robinson & Decker-Walters, 1997).

Resistance to zucchini yellow mosaic virus in watermelon is provided by the single dominant allele *Zym* (Robinson & Decker-Walters, 1997; Desbiez & Lecoq, 1997).

The *C. pepo* cultivars 'Tigress' and 'Jaguar' have had ZYMV resistance derived from the *C. moschata* cultivar 'Nigerian Local' bred into them (Lecoq *et al.*, 1991; Robinson & Decker-Walters, 1997). It is likely that this resistance will be broken down in a relatively short space of

time (Lecoq *et al.*, 1991).

Of all the *Cucurbita* species *C. ecuadorensis* Cutler & Whitaker and *C. foetidissima* have been proved to have resistance to the greatest number of viruses (CMV, WMV1, WMV2, and others). The resistance from *C. ecuadorensis* has been bred into *C. maxima*. Using the same cross the 'Redlands Trailblazer' cultivar, which is resistant to ZYMV, WMV, and PRSV (papaya ringspot virus), has been developed in Australia (Provvidenti *et al.*, 1978; Gilbert-Albertini *et al.*,1993; Robinson & Decker-Walters, 1997). Although the above mentioned species are possibly the best sources of resistance, other species which show resistance to viruses should not be ignored, as the mode of inheritance and their incompatibility with cultivated species may differ (Provvidenti *et al.*, 1978). *C. gracilior* has been described as being either resistant or tolerant to WMV2. *C. moschata* is the only cultivated member of this genus to have resistance to WMV2. It also has the dominant *Zym* gene which gives resistance to ZYMV. The resistance to the two viruses is due to the *Zym* gene or perhaps two closely linked genes (Gilbert-Albertini *et al.*, 1993). In a review by van der Meer and Garnett (1985), resistance to WMV was reported to have been found in *Citrullus ecirrhosus* Cogn., *Coccinia sessilifolia* (Sond.) Cogn., *Cucumis metuliferus* E. Mey, ex Naud., *Luffa cylindrica* L. and several traditionally cultivated forms of *C. lanatus*

Resistance often increases with age, thus the use of speedlings or some other type of live material may be better than the use of seed (Sylvester, 1989). Resistance breeding could be extended to include characteristics which make the plant unattractive or repellent to the vector before landing, or which inhibit probing and penetration (Blackman, 1976; Klingauf, 1987). This method could provide an effective and inexpensive means of controlling virus movement into and within fields (Jones, 1990). For example, aphids are primarily attracted to yellows, greens and oranges in decreasing order of preference. Thus breeding plants with a different colour hue may reduce aphid colonization. The breeding of plants with special hairs on the epidermis of leaves which can either injure or immobilize aphids may be useful. This mechanism is non-specific and may aid in the control of other insect pests. Conversely, some beneficial insects may be harmed (Gibson & Plumb, 1977; Jones, 1990). The presence or absence of pubescence or surface waxes and tissue toughness are commonly sought morphological characteristics in breeding programmes. These can affect plant colour, architecture and anatomy (Jones, 1990; Dent, 1995). Other resistance

mechanisms are the interference with the sustained feeding behaviour of the vectors concerned, and the resistance exhibited by some plants which specifically interferes with the transmission of the virus by the vector (Jones, 1990). Depending on the mechanism of resistance, insects on resistant plants are often smaller, slower breeding, more restless, more stressed, and may retain higher levels of secondary plant metabolites. This can affect the pest insects' susceptibility to both insecticides (toxicity of a chemical is related to body size), thereby reducing chemical input, and biological control agents. (van Emden, 1990).

The distribution and availability of a virus within plants can affect the vectoring capabilities of the aphids (Zitter, 1977). A combination of vector and virus resistance within a plant could give a good level of control (Blackman, 1976), and is more stable than would be expected (Harrison, 1981). The centre of origin of a crop species is the most likely place to find specific resistance genes if a vertical resistance breeding programme is being used (Gibson & Plumb, 1977). Although not a solution on their own, resistant plants may make some contribution when used as part of an integrated management program (Zitter & Simons, 1980).

When breeding for resistance or tolerance to viruses it must be kept in mind that strong non-preference of the plant by the vector may appear to be the sought after character (Bohn *et al.*, 1972).

Resistant varieties do not always inhibit virus spread (Swenson, 1968), but may make aphids more restless which will result in increased alate production and plant-to-plant movement (Gibson & Plumb, 1977). Other problems involved with resistance breeding are ① the formation of new vector biotypes by the continued use of resistant cultivars. This is most likely to occur where the vector is monophagous; ② the use of a mechanism which enhances resistance to one vector species may increase susceptibility to another (Jones, 1990).

A diverse population of plants can limit the spread of viruses within an area, as not all the plants present are likely to be suitable hosts due to their different genetic make-up. However, not a great deal is known about the specifics of the interaction between the plants and the vectors. For example, does genetic diversity affect the feeding behaviour of the vector, or does it affect the

population dynamics of the insect? The former situation may affect the rate of transmission of a virus, whilst the latter situation may affect the abundance of insects due to changes in colonization, emigration, reproduction, or mortality. In studies conducted by Power (1991) the genetic diversity was found to affect aphid abundance only at high insect densities. Where there are reductions in vector densities, increased rates of dispersal could be responsible. If this is the case, genetic diversity could increase virus spread if the virus concerned was of the stylet-borne type. Genetic diversity within a crop may reduce the evolution of resistance-breaking strains (Power, 1991).

Coat protein-mediated protection (CPMP), where the gene for the coat protein of a virus(es) is inserted into the genome of a plant to protect it, is usually only effective against the specific virus or related strains of the expressed coat protein (CP) (Namba *et al.*, 1992; Desbiez & Lecoq, 1997). In their work, Namba *et al.* (1992) showed that *Nicotiana benthamiana* plants containing the CP of WMV2 and ZYMV were also protected against bean yellow mosaic virus (BYMV), clover yellow vein virus (CYVV), pepper mottle virus (PeMV), tobacco etch virus (TEV), potato virus Y (PVY) and pea mosaic virus (PMV). It thus appears that the CP gene of potyviruses offers wider protection than most other viruses. In a study involving cantaloupe and yellow crookneck squash transformed with the CP of ZYMV and WMV, Clough & Hamm (1995) showed that the use of transgenic plants reduced disease incidence and increased the yield of marketable fruit.

The size of the field, the plant density and the stage of plant development can affect virus spread in a field. Insects tend to land on the edges of fields creating a distinct edge effect. This can be reduced by increasing the size of the field. An increase in the plant density can decrease the incidence of virus diseases by affecting the host recognition response of the vector (Zitter & Simons, 1980).

Agroecosystems with the following characteristics can be expected to have low pest potentials: "① High crop diversity through mixtures in space and time; ② Discontinuity of monoculture in time through rotations, use of short maturing varieties, use of crop-free or preferred-host-free periods, etc.; ③ Small scattered fields creating a structural mosaic of adjoining crops and

uncultivated land which potentially provides shelter and alternative food for natural enemies. Pests also may proliferate in these environments depending on plant species composition. However, the presence of low levels of pest populations and/or alternate hosts may be necessary to maintain natural enemies in the area; ④ Farms with a dominant perennial crop component; ⑤ High crop densities or presence of tolerable levels of weed background; ⑥ High genetic diversity resulting from the use of variety mixtures or several lines of the same crop" (Altieri, 1994).

1.4.1.7 Vector control

Control of insect virus vectors, such as aphids which are highly dispersive, is especially difficult as they can cause economic damage at low population levels (Blackman, 1976). However, where annual crops are concerned, peaks of virus spread can often be linked to peaks of migration of alate vectors (Maelzer, 1986). This makes vector control an important part of virus control.

1.4.1.8 Chemical control of the vector

A variety of chemicals have been used to control aphids. Prior to the Second World War nicotine and arsenic-containing compounds were used as contact insecticides. After the War chlorinated hydrocarbons, such as DDT, and organophosphates were used. However, the persistence of these compounds in the environment and their accumulation within food chains has led to these products being withdrawn from the market in most countries (Devonshire, 1989; Edwards & Stinner, 1990).

The development of the carbamates, the organophosphorous compounds, and the synthetic pyrethroids which have a systemic mode of action added a new dimension to aphid control (Schepers, 1989). However, without careful use and management, resistance to these chemicals will develop, and the control is only effective if the timing of application is perfect. In the past resistance problems have been overcome by introducing a new chemical (Blackman, 1976; Gibson & Plumb, 1977; Zitter & Simons, 1980; Devonshire, 1989).

This means that new strategies have to be developed which will include the use of behaviour-altering substances, such as antifeedants and pheromones, and improved forecasting methods which will limit the use of insecticides (Devonshire, 1989). Some pyrethroids have been used in

combination with mineral oils. The combination allows the use of oils at concentrations which are not phytotoxic, the oil extends the useful life of the insecticide, and the combination gives better results than either treatment on its own (Raccah, 1985).

Antifeedants have been shown to reduce virus spread in the laboratory. However the range of insects against which they are effective varies greatly, and their most effective period spans only a few hours, or at most a few days. Semiochemicals can be broadly divided into two groups (Figure 1.7). Aphid sex and alarm pheromones are being studied to capture male aphids and to make aphids move to make them more vulnerable to contact insecticides, as greater movement increases the chance of the insect coming into contact with the chemical. Pheromones can also act as kairomones: it is thought that syrphid flies may be attracted by the aphid alarm pheromone. Kairomones can be placed in trap crops to attract pests to and hold them in this crop. The crop can then be treated with an insecticide. The main crop could be treated with allomones and kairomone inhibitors to prevent pests entering, but would not be treated with insecticides (Griffiths, 1990). Aphid pheromones tend to make the insects stop feeding, or even drop to the ground. However, these compounds have not proved to be very useful in preventing virus spread and are very expensive to produce (Zitter & Simons, 1980).

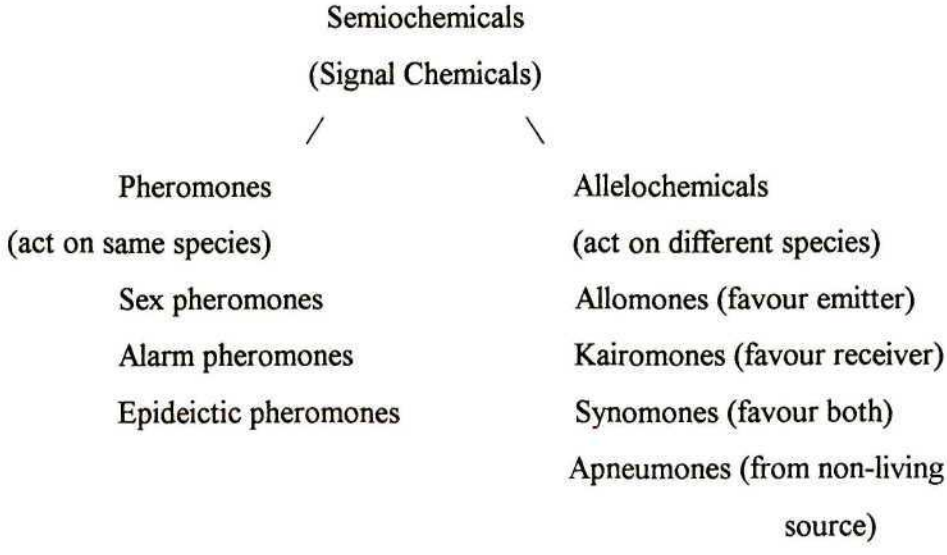

Figure 1.7 Classification of semiochemicals (Griffiths, 1990).

The use of toxic allelochemicals as a means of plant resistance can be damaging to populations

of natural control agents by being toxic to immature parasitoids within their hosts, or killing the host before the parasitoid can emerge. Some of these allelochemicals are also toxic to predatory insects such as coccinellids, syrphid fly larvae and neuropterans (van Emden, 1990). Conventional chemicals can also have negative effects on beneficial insects such as honey bees and predatory species (Brown & Stephenson, 1990).

A further negative aspect of many chemicals is that they stimulate aphid movement. This facilitates an increase in virus transmission where the stylet-borne viruses are concerned (Swenson, 1968; Zitter & Simons, 1980; Maelzer, 1986; Gibson & Rice, 1989; Pinese *et al.*, 1994; Yuan & Ullman, 1996; Desbiez & Lecoq, 1997). Another reason for the inefficiency of aphicides is that the major vectors are often transient inhabitants moving through the crop, and are thus not the targets of routine sprays (Lamont *et al.*, 1990; Yuan & Ullman, 1996; Desbiez & Lecoq, 1997). Chemical control of the vectors can thus be regarded as ineffective (Clough & Hamm, 1992).

1.4.1.9 Classical biological control

Reliance on natural enemies of aphids as an alternative to chemicals is not practical as the populations of these insects take time to increase and offer effective control, and the damage is done to the crop in a short space of time. Predators are the only natural enemies which have impact on the populations of *M. persicae*, and then only if these are present in large numbers early in the season, or the aphids are not reproducing at their full potential (Blackman, 1976). In crop monocultures there is also a lack of adequate resources for the effective performance of beneficial insects (Altieri, 1994). Some parasitoids can transfer from one species to another, indicating the potential value of non-pest aphids as reservoir hosts (Powell *et al.*,1990). Insects which are deleterious to aphids fall into three broad groups: the polyphagous predators (carabid and staphylinid beetles, and spiders); those predators specific to aphids (coccinellid beetles, syrphid fly larvae and neuropterans); and parasitoids (ichneumonid and braconid wasps) (Brown & Stephenson, 1990).

Natural enemies may prove to be useful if they can be encouraged into the field or its margins. Usually direct damage by aphids and other insects is the result of a lack of predators and

parasitoids when the pest first arrives in the field. This may be averted if there are large numbers of beneficial insects already present in the field. It has been suggested that the planting of a crucifer such as kale, mustard or rape around a melon field is useful in controlling aphids. The beneficial insects multiply on the crucifers by feeding on the cabbage aphid, and then move into the cucurbit crop and feed on the melon aphid, *A. gossypii*. Where companion planting of this nature is used care must be taken that the companion plant, or a common plant in the area, does not have sticky leaves which may remove beneficial insects, such as parasitic wasps, from the system. Some plants, such as cotton, attract insects by having an abundant supply of nectar and/or pollen (Marcovitch, 1935). This is due to a requirement by some beneficial insects for some plant materials, especially pollen and nectar, at specific stages of their life cycles. The availability of these materials may greatly influence the effectiveness of such insects (Bowie *et al.*, 1995; Jervis & Kidd, 1996). As an example, *Allograpta exotica* (Diptera: Syrphidae), an aphid predator, preferred fennel flowers, as well as those of some other weeds. The presence of these plants in and around a crop would encourage these predators to reproduce (Salto *et al.*, 1991).

As parasitoids can play an important role in reducing aphid numbers after mild winters, it is important to protect populations of these beneficials within the farmland ecosystem. This requires detailed knowledge of the ecology and behaviour of the parasitoids. The usefulness of parasitoids switching from one host to another depends on their ability to do so. This has been shown to be strongly influenced by the genotype of the parasitoid (Powell *et al.*, 1990).

An indirect method of making better use of beneficial insects is the control of ants in and around the field. When ants are foraging for honeydew or are tending a honeydew source, they interfere with other types of predators and parasitoids by either killing them or causing them to disperse (Kidd & Jervis, 1996).

In using natural enemies to combat pests, it is probably better to encourage unspecialized polyphagous predators. Spiders, ants, beetles, parasitic hymenopterans and chilopods are the main groups of predaceous arthropods. Spiders probably fulfill this role the best. Most spiders tend to prey on phytophagous and detritophagous insects rather than pollinating and predaceous species (Nentwig, 1988).

Where vector resistance is used as a means of control, pests feeding on these plants may be more susceptible to pathogens, and the increased restlessness may increase their exposure to these pathogens (van Emden, 1990). These strategies are unlikely to prevent primary virus spread but they could limit secondary spread by keeping aphid numbers low, thus preventing overcrowding and the production of alate morphs.

1.4.1.10 Behaviour modification

A flying aphid may be attracted to or repelled by certain wavelengths of light. The particular wavelength varies according to what stage of the migratory flight the aphid is in. When it starts flying it is attracted to blue, and when searching for a host it is attracted to the orange-yellow-green wavelengths reflected by the leaves. This relationship between aphid behaviour and light has been exploited to control viruses by controlling the vector. For example, fields may be surrounded by strips of yellow polyethylene coated with a clear adhesive which will trap incoming aphids. Another method is to use a mulch of a substance, such as aluminium (aluminium foil; aluminium coated paper; aluminium-painted polyethylene sheeting), which reflects short wave light, i.e. a reflective mulch. These mulches often have other benefits such as preventing weed growth within the field, increasing soil temperature, the prevention of attack by soil pathogens and therefore fruit rot, conserving water, and reducing fertilizer leaching (Zitter, 1977; Wyman *et al.*, 1979; Zitter & Simons, 1980; Prokopy & Owens, 1983; Maelzer, 1986; Klingauf, 1987; Gibson & Rice, 1989; Jones, 1991; Robinson & Decker-Walters, 1997). Other insects repelled by an aluminium mulch include the thrips *Frankliniella tritici* (Fitch), the banded cucumber beetle (*Diabrotica balteata* LeConte), the spotted cucumber beetle (*Diabrotica undecimpunctata howardi* Barber) and a leafminer (*Liriomyza* sp.). As *Diabrotica* spp. are repelled, it may be possible that the *Acalymma* sp. responsible for vectoring bacterial wilt and SMV may also be repelled, thus offering some control of these diseases (Schalk *et al.*, 1979). The aluminium mulch decreased the rate of introduction of virus from external sources, the spread of virus within the field, and the number of plants that ultimately became infected (Jones, 1991). This method has been shown to give effective control of CMV and the W-strain of PRV, with aluminium mulches giving the best control. The use of organic mulches, such as straw and sawdust, have also been effective in reducing aphid populations and the incidence of virus diseases. Yellow plastic sheets which are covered with a long-lasting glue and placed outside the fields can be used to trap alate

aphids and reduce viral spread (Zitter & Simons, 1980).

Silver plastic can be used as an alternative to aluminium as a reflective mulch. In a comparative study of different colour mulches, silver outperformed white, yellow, and black with yellow edges. This mulch delayed the appearance of foliar virus disease symptoms (caused by CMV, PRV-W, WMV2, ZYMV, and SMV) by 10 to 13 days, and resulted in a significant increase in yield (Zitter, 1977; Brown *et al.*, 1993; Pinese *et al.*, 1994). These benefits were quantified by Wyman *et al.*, (1979). Reductions in virus infection of 94 and 77% respectively were obtained from the use of aluminium and white mulches, and total fruit yield was increased by 43%. Similar results were obtained by Smith *et al.* (1964). Successful trials with aluminium mulches have been conducted in South Africa (Daiber & Donaldson, 1976; Eulitz, 1977b). In a study conducted by Summers *et al.* (1995) the use of a silver spray mulch or a silver polyethylene film mulch increased marketable fruit by 70% in spring plantings, and by 80% and 75% respectively in autumn plantings when compared to an unmulched control. A combination of a mulch and trickle irrigation significantly reduced the number of plants infected with WMV and increased yields by 77-270% (Maelzer, 1986).

Blue and grey mulches are not effective in repelling aphids. The black mulch resulted in heat stress, and this treatment gave worse yields than the control. However plants grown over the silver mulch grew quicker than the control plants (Pinese *et al.*, 1994). This positive growth reaction may be due to several reasons such as sunlight enhancement and soil temperature adjustment (Wyman *et al.*, 1979). The painting of aluminum strips onto a black mulch is one way of reducing the cost of silver mulches (Lamont *et al.*, 1990).

Problems involved with the use of synthetic mulches include difficulty in laying the mulch, disposal after the growing season, and the high initial cost of the material. The effectiveness also decreases with time as the plants cover the reflective surface (Zitter, 1977; Zitter & Simons, 1980; Lamont *et al.*, 1990; Summers *et al.*, 1995; Desbiez & Lecoq, 1997). Jones (1991) found that the efficiency of the reflective mulch was reduced during overcast periods at the peak periods of aphid migrations. With time, white mulches become dulled or slightly yellowed due to the accumulation of soil and dust and general weathering. This may affect the repellency of the mulch

(Summers *et al.*, 1995). These mulches do not prevent virus infection of the plants, but they usually extend the harvesting period which should increase the returns (Lamont *et al.*, 1990; Summers *et al.*, 1995; Desbiez & Lecoq, 1997).

Plastic mulches present a disposal problem at the end of the crop. They cannot be disked in as the resulting pieces of plastic can become a nuisance during future farming practices. Removing the mulch from the field is labour intensive and the mulch still has to be disposed of. Degradable mulches may also be less expensive to apply than conventional plastic mulches (Summers *et al.*, 1995). A degradable mulch, such as the ones developed by Earthguard, Continental Products Co., Euclid, Ohio, or by BASF Corp., Charlotte, N.C. could solve the disposal problem (Russo, 1995; Summers *et al.*, 1995).

Another alternative to synthetic mulches is to use a straw mulch. Rummel *et al.* (1995) showed that the use of a wheat straw mulch retarded aphid development on the cotton plants. This was probably due to increased light intensity on the lower side of the leaves. Other researchers, de Oliveira *et al.* (1990) for example, have shown that a straw mulch can be effective against other insect pests as well. These authors also mention the added advantages of reduced water loss and the inhibited growth of weeds due to the presence of a straw mulch. More than 50-60% of the soil surface must be covered in order for a mulch to be effective (Maelzer, 1986).

With high value crops such as zucchini the high cost of a mulch is offset by an increase in yield (Adlerz & Everett, 1968; Pinese *et al.*, 1994). A further complication is that while some insects are repelled by the reflective surface, others are attracted to it. In the case of aluminum *Apis melifera* L. (Honey bees), a beneficial insect, and *Diaphania nitidalis* (Stoll) (pickleworm), a destructive species, were attracted. Unfortunately the beneficial hymenopteran family Braconidae is also repelled (Schalk *et al.*, 1979). Adlerz & Everett (1968) found that more aphids were trapped over white plastic than bare ground. This is probably due to some aphids being attracted to white rather than yellow. The attractiveness of white over yellow can also be affected by the physiological state of the aphid concerned (Summers *et al.*, 1995).

Living mulches, also known as intercrops or smother crops, can be used to reduce soil erosion

and weed invasion, as well as improving water infiltration and soil structure. However, careful choice of plants is necessary as competition with the main crop can lead to a reduction in yield. The reasons for the success of living mulches in controlling insect pests are many and varied. They include: the confusion or repulsion of pests by changes in reflected light; the physical hiding of the crop or the emission of volatile masking odours; the encouragement of beneficial insects; the impediment of movement of the pest (Altieri *et al.*, 1985; Wiles *et al.*, 1989; Grossman, 1993). Clover has been found to be important in providing food and shelter to insects, both beneficial and potential pests (Altieri *et al.*, 1985).

Another aspect of behaviour modification is to mimic important recognition patterns of the host. The mimic which is most attractive to the insects is not necessarily the closest copy of the natural pattern, but rather one that incorporates "super-normal" stimuli. This mimic can then be used for either direct control or for monitoring of the insect. Whenever visual traps are used the influence of the background and optimal trap positioning must be remembered in order to achieve the best results (Prokopy & Owens, 1983).

Perring *et al.* (1989) used floating row covers, usually used to protect plants from frost to hide cantaloupe plants from the incoming vectors. They obtained excellent results when the covers were left on until the perfect flowering stage. They do warn that when ambient light is declining the covers may have a negative effect by reducing plant growth and cancelling out the effects of reduced virus infection. Summers *et al.* (1995) found this technique to offer no advantage over the unmulched control.

Studies on the effectiveness of using plant odours and insect pheromones have, and are being, conducted. Plant breeding is also being investigated as certain properties of the host surface, such as hairs and trichomes, make the surface unattractive to aphids. Other aspects of resistance include the presence or absence of certain chemical compounds in the plant. These measures are useful against the semi-persistent and circulative viruses (Klingauf, 1987 ; Gibson & Rice, 1989). Many plants have pesticidal properties which may be used as an alternative to synthetic chemicals. These compounds are generally used in one of two ways: ① they are produced artificially by chemical companies, or ② they are used directly after the crude plant sap has been extracted

(Yang & Tang, 1988). Briggs *et al.* (1996) found a garlic barrier in combination with fish oil to give sufficient control of insects for home gardeners, and commercial farmers wanting to avoid synthetic chemicals and accept some damage.

Whatever the treatment being applied, a statistical difference in the abundance of vectors may not result in a difference in the number of plants infected with a virus (Summers *et al.*, 1995).

1.4.1.11 Mild strain protection

This is "the use of a mild virus isolate to protect plants against economic damage caused by infection with a severe challenge strain(s) of the same virus." Points made by this definition are: the availability of a mild strain is the most important requirement; the economic benefit derived from the use of the strain determines its effectiveness; and the use of cross protection may not restore production to that of virus free plants, but may be considered effective if an economic benefit is derived from its use (Gonsalves & Garnsey, 1989).

A variety of techniques can be used to obtain a mild strain:
① selection of a naturally occurring mild strain;
② induced mutation of a naturally occurring strain by the use of a mutagenic agent such as nitrous acid followed by selection; and
③ passage through selective hosts or vectors (Gonsalves & Garnsey, 1989).

Two methods are commonly employed to inoculate plants with protecting viruses:
① the plants are inoculated as soon as possible after germination. Infection should be verified either visually or by an indexing test (e.g. ELISA); and
② a healthy propagating source plant is inoculated with the protecting strain and then used once it has become systemically infected. This technique is used for clonally propagated plants (Gonsalves & Garnsey, 1989).

Variable results have been obtained using a mild ZYMV strain to protect zucchini and marrow plants against severe ZYMV strains. Inoculated plants gave yields ranging from 38% less to 7% more than the uninoculated plants depending on the cultivar used and the site of the trial. In all

cases the harvested fruit was indistinguishable from the uninoculated fruit, and leaf symptoms were mild. Inoculated plants did show some stunting and were about 20% smaller than healthy uninoculated plants and there was a 10-day delay in flowering. However, there was no significant difference in the total fruit weight per plant between the uninoculated control and the inoculated plants. There has been no indication of instability in the mild strain under commercial growing conditions. Where severe strains invaded the plots the inoculated plants yielded symptomless fruits while uninoculated plants yielded unmarketable fruit. Indications from growers were that a 10-20% loss was preferable and acceptable, provided that the mild strain prevented the 100% loss which might occur in the event of a severe ZYMV strain entering their fields (Spence *et al.*, 1996). The use of the strain ZYMV-WK has proved to be effective in protecting plants against severe strains in field trials (Lecoq *et al.*, 1991; Desbiez & Lecoq, 1997). Rezende & Pacheco (1998) successfully used two mild strains of PRV-W (PRV-W-1 and PRV-W-2) to protect zucchini squash (*C. pepo*) against severe strains (PRV-W-C; PRV-W-B; PRV-W-P) of this virus.

It is unlikely that the use of cross protection as the only control measure will give lasting control of disease throughout the life of the crop. It is therefore desirable to use other virus management techniques as well, as the longer the plants can be protected from a severe challenge(s), the longer the cross protection will be effective (Gonsalves & Garnsey, 1989). Gonsalves and Garnsey (1989) suggested the following methods: the timing of planting to avoid high vector populations; isolation from severe sources of inoculum by means of windbreaks or positioning the crop upwind of disease reservoirs; roguing infected plants from nearby plantings; and the use of virus-tolerant varieties.

Bourdin & Lecoq (1991) have shown a potential problem with the use of cross protection. Where ZYMV-NAT (a non-aphid-transmissible isolate) was used to protect plants from potyvirus infection, it was found to be transmitted *in vitro* by aphids when it was in a plant co-infected with PRSV-E2. This was due to ZYMV-NAT RNA being encapsidated partially or totally by the coat protein of PRSV-E2. The epidemiological consequences of this, if it occurs in the field, are vast. It is a possible explanation why non-aphid-transmissible isolates persist and are transmitted in the field. More importantly this phenomenon could change the vector specificity of the viruses involved.

1.4.1.12 Integrated control

In the 1990s and beyond, pest control must move towards a system where the interactions between all the "inputs of cultivations, fertilizers, rotations, related cultural practices and chemical and biological pest control are considered in a systems context" (Edwards & Stinner, 1990). This is particularly true of the stylet-borne viruses such as ZYMV (Desbiez & Lecoq, 1997). The interactions between crop protection and farm practices are shown in Figure 1.8. Integrated pest management (IPM) is an idealized, crop-oriented management strategy which considers all control possibilities of all crop pests, and selects the options which allow for both the short and long term maximization of profit (Maelzer, 1986). In order to effectively deal with virus diseases and their vectors an integrated approach involving several different techniques may be required (Zitter & Simons, 1980). A short-coming in the implementation of IPM is a the lack of cooperation between entomologists, plant pathologists, weed specialists, agronomists and plant breeders which would lead to sound IPM programmes being formulated. Such programmes should control animal pests, diseases and weeds by using the available alternative, ecologically and environmentally desirable techniques (Edwards & Stinner, 1990). The potential of IPM is shown in Figure 1.9.

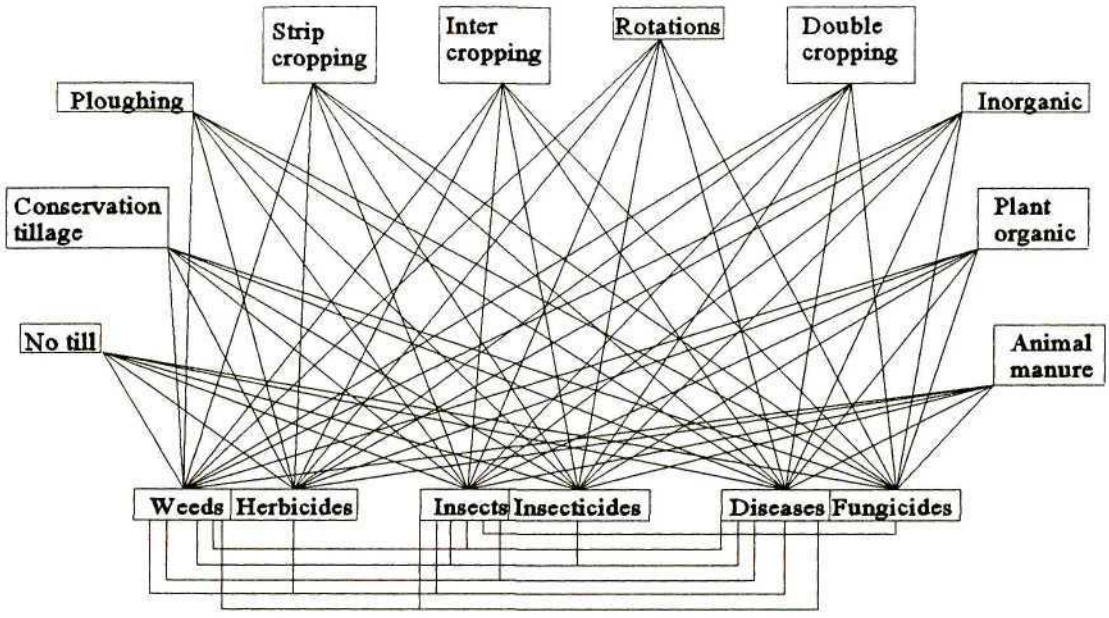

Figure 1.8 The interactions between crop protection and farm practices (Edwards & Stinner, 1990).

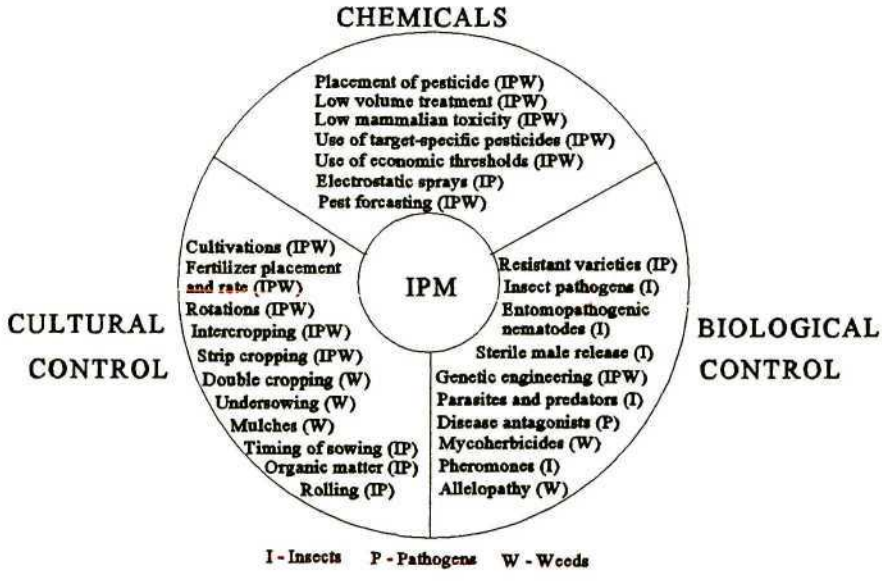

Figure 1.9 The potential of integrated pest management (Edwards & Stinner, 1990).

IPM has a number of advantages, including the minimization or avoidance of pesticide resistance, the reduction of pesticide use, and the prevention of secondary pests becoming a problem (Harris,

1975).

Pinese *et al.* (1994) combined the use of a silver reflective mulch with applications of a mineral oil and an insecticide to almost double the yield of zucchini marrow. The yield increase was mainly due to lower virus incidence which allowed for an extended cropping period.

Integrated control programmes often have a trap cropping component. Biopesticides, biocontrol agents, resistant cultivars, and rotations are often used in combination with trap crops (Hokkanen, 1991).

When fully developed, integrated farming systems could:
① "Maximize profits by lower costs of purchased chemicals";
② "Minimize food contamination";
③ "Decelerate the development of resistance to chemicals";
④ "Lessen the environmental impact of pesticides on beneficial organisms, wildlife and man" (Edwards & Stinner, 1990).

1.4.1.13 Other methods

It has been shown that some aphids are strongly attracted to yellow. Thus the use of crops which are not of this hue may be less attractive to passing vectors. For example, some cucurbits have a silvery leaf which reflects more ultra-violet and blue light than do the normal cultivars, and thus escape infection with aphid-vectored viruses (Prokopy & Owens, 1983; Sylvester, 1989). The control of nitrogen application can play a role as plants with a high nitrogen content have an increased susceptibility to viruses (Gibson & Plumb, 1977; Sylvester, 1989).

Mineral oils, which are sprayed onto the crop, have had some success in controlling the spread of stylet-borne viruses, especially with frequent applications below phytotoxic levels (Vanderveken, 1977; Wyman *et al.*, 1979; Sylvester, 1989; Pinese *et al.*, 1994; Clough & Hamm, 1995), but there are problems with phytotoxicity in some cases. Oils appear to act at the level of virus-vector relationships rather than at the plant-virus level. The shape and size of the virus particles does not seem to have a bearing on the inhibitory effect of oils (Vanderveken, 1977).

The success of mineral oil treatments seems to be dependent on the application pressure, the best being 400 psi (Raccah, 1985). JMS Stylet-Oil™ has been shown to give good control of viruses on crookneck squash and tomato without any signs of phytotoxicity (Zitter & Simons, 1980). Other effective oils include Albarol™ and Lovis™ (Pinese *et al.*, 1994). Virol™ has been shown to be effective at a 5% concentration, but this is may be phytotoxic in hot climates (Raccah, 1985). A treatment which combines insecticide (e.g. demeton-S-methyl) and mineral oil applications has been shown to give better protection than just mineral oil. In low value crops such as watermelons and pumpkins, this is a good alternative to reflective mulches (Pinese *et al.*, 1994). A study by Wang and Pirone (1996) has presented evidence that mineral oils act by interferring with the retention of virus particles in the aphid mouthparts.

As the number of aphids on a plant increases, the rate of inhibition caused by mineral oils decreases. This can be counteracted by increasing the oil concentration of the emulsion (Vanderveken, 1977).

Clough and Hamm (1995) stated that the beneficial effect of cultural practices is short lived. It must be remembered when experimenting with new techniques with a view to these replacing existing techniques, that the new procedure must be at least as effective as the one it would replace (Russo, 1995).

1.5 MOTIVATION FOR THE STUDY

This study was conducted to determine if six of the important viruses (cucumber mosaic cucumovirus (CMV), squash mosaic comovirus (SqMV), papaya ringspot potyvirus (PRSV), watermelon mosaic potyvirus - Morocco (WMV-M), watermelon mosaic 2 potyvirus (WMV2), zucchini yellow mosaic potyvirus (ZYMV)) were present in KwaZulu-Natal, and in what combinations they occurred. Differences in distribution of these viruses was recorded, as this could influence the combinations of viruses in multiple infections, and thus the potential severity of disease in these plants. The presence of these viruses in weeds occurring within and around areas of cultivation was investigated.

As virus diseases of cucurbits cause severe crop losses in KwaZulu-Natal to both commercial and

resource-poor farmers, a variety of cultural control techniques were used to determine which showed the most potential for use in the field. The techniques used could be applied either on commercial farms or on subsistence or market garden-type farms. Some methods were more practical for commercial farms, while others would be better applied on market garden type farms due to the cost or labour intensity required. The effectiveness of these treatments in controlling aphids (Hemiptera: Homoptera: Aphididae), the vectors of the viruses, and fruit flies (Diptera: Tephritidae), a major pest of cucurbits in the region, was also monitored.

The separate chapters are written as distinct scientific papers. This has required the duplication of some information and data.

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CHAPTER 2: THE VIRUSES WHICH COMMONLY INFECT CUCURBITS IN KWAZULU-NATAL

2.1 INTRODUCTION

There are in excess of 30 viruses which infect cucurbit species (Robinson & Decker-Walters, 1997). Of these, watermelon mosaic 2 *Potyvirus* (WMV2) (Purcifull, 1990), watermelon mosaic *Potyvirus* - Morocco strain (WMV-M) (van der Meer, 1985), zucchini yellow mosaic *Potyvirus* (ZYMV) (Desbiez & Lecoq, 1997), papaya ringspot *Potyvirus* - Type W (PRV-W) (Purcifull *et al.*, 1990), cucumber mosaic *Cucumovirus* (CMV) (Lovisolo, 1980), and squash mosaic *Comovirus* (SMV) (Lovisolo, 1980) are probably the most important. These viruses cause a variety of symptoms including distortion and mosaic of the leaves and fruit (Lovisolo, 1980; Purcifull et al., 1984; van der Meer, 1985; Büchen-Osmond & Purcifull, 1990; Francki & Habili, 1990; Purcifull, 1990).

In South Africa WMV-M has been regarded as the most widespread and important virus in cucurbit crops (van der Meer & Garnett, 1987), although other viruses (e.g. WMV2) do occur (van der Meer, 1985; van der Meer & Garnett, 1985). CMV and ZYMV have also been reported from the country (Trench *et al.*, 1992; von Wechmar *et al.*, 1995), while SMV has only been reported from plant material in quarantine (Trench *et al.*, 1992). With the exception of SMV, which is spread by cucumber beetles (Coleoptera: Coccinellidae) (Campbell, 1990), all the viruses affecting cucurbits in South Africa are vectored in the stylet-borne manner by aphids (Hemiptera: Homoptera: Aphididae) (Shukla *et al.*, 1994). It is common to find field grown plants which have been infected with more than one virus. This can affect the severity of disease in the plant (Zitter, 1977).

Other factors affecting virus spread are the overall condition of the plant (Zitter & Simons, 1980), wind, and the overall interaction between the prevailing environmental conditions, the hosts, the vectors and the viruses (Harrison, 1981).

This study was conducted to determine which cucurbit viruses were present in KwaZulu-Natal, and how common infections by these viruses are in the field. The occurrence of multiple

infections was also investigated.

2.2 MATERIALS AND METHODS

2.2.1 Collection Of Material From The Field

Infected plant material was collected from various locations (Assagai, Bayne's Drift, Inanda, Jozini, KwaMbonambi, Msinga, Nkwaleni Valley, Pietermaritzburg, Port Edward, Sheffield Beach, Stanger, Tala Valley, Thornville) around KwaZulu-Natal. These localities are shown in Figure 2.1. In general only symptomatic leaves were taken, but where possible the whole plant was collected. The samples were cut with either a knife or a pair of scissors. The cutting instrument was cleaned after each sample by wiping it with a cloth soaked in a 1:9 sodium hypochlorite solution to prevent contamination of the next sample. To inhibit plant compounds from destroying the virus particles, the material was stored in a cooler bag until it could be placed in a refrigerator at the laboratory. Afterwards the sample was placed in a freezer to prevent plant enzymes from destroying the virus particles. This is not always an ideal situation as "freezing leaf samples infected with some potyviruses may decrease detectability by ELISA (Enzyme-linked immunosorbance assay)" (Barnett, 1986).

Each individual sample was designated a number which indicated the locality, date, and type (weed (W) or crop (C)) of plant. Each area was given a two letter code (AS = Assagai; BD = Bayne's Drift; IN = Inanda; JZ = Jozini; KM = KwaMbonambi; MS = Msinga; NK = Nkwaleni Valley; PB = Pietermaritzburg; PD = Port Edward; SB = Sheffield Beach; ST = Stanger; TA = Tala Valley; TV = Thornville), and each site in that area was given a number (1,2,3,....). For example AS1-FEB14/97-C1 shows that the sample was collected at Assagai, Site One on the 14th of February, 1997, was a crop plant, and was the first sample collected at this locality. Areas where small scale production plots were tested included Assagay, Jozini, KwaMbonambi, Msinga, Nkwaleni, Port Edward, and Pietermaritzburg. Commercial fields were sampled at Bayne's Drift, Tala Valley, Thornville, Sheffield Beach and Stanger. Samples were taken as regularly as possible. A more thorough representation could have been gained if more personnel had been involved in sample collection, and security was better in some of the areas. The majority of the samples were collected in the second half of the growing season as this is when virus disease is at its most severe. This information was gained from consultation with farmers.

2.2.2 Enzyme Linked Immunosorbant Assays (ELISAs)

For monitoring the presence or absence of specific viruses ELISA kits were used. This technique is useful as it is quick and accurate (Barnett, 1986). However, it has been noted that at various times in the infection cycle some viruses may not be detectable by the normally highly sensitive ELISA method. DAS-ELISA is more specific than indirect ELISA and most other serological tests (Barnett, 1986).

Figure 2.1. The localities around KwaZulu-Natal from which samples were collected for analysis of viral infection of cucurbits (PMB = Pietermaritzburg).

2.2.2.1 Double Antibody-Sandwich ELISA

This technique was used for the detection of WMV-2, WMV-M, and ZYMV in field samples. This technique was used for the detection of CMV in the samples collected in 1998. PRV and SMV were also tested for in these samples. Specific antibodies, the conjugate, and positive controls for these tests were obtained from Sanofi, Phyto-Diagnostics, France, and were part of a Plantest ELISA kit. The freeze-dried components were rehydrated with sterile distilled water. The required buffers were made by the author (See Appendix IV). The tests were performed in accordance with the manufacturers instructions. For all ELISA tests the microtitre plates were read using a SLT 963 PR plate reader, SLT-Labinstruments Ges.m.b.H, Grödig, Austria, linked to a Seikosha SP-2050s printer.

2.2.2.2 Indirect ELISA

This technique was used for the detection of CMV in the 1997 field samples. The reagents were obtained from Dr G. Pietersen, Plant Protection Research Institute (PPRI), Pretoria. The samples were macerated (one gram of sample to 10 ml of extraction buffer) using a mortar and pestle.

The coating $F(ab')_2$ fragment was diluted 1:2000 in coating buffer. Aliquots of 200 μl of the coating reagent was added to the central 60 wells, and a similar amount of distilled water was placed in the outer wells. The plate was incubated for 4 hours at 30°C. The reagents were discarded and the plate was rinsed with PBS-Tween and then given three 3- minute washes with the same. The plate was dried by striking it against absorbent paper. The samples and controls were added in 200 μl aliquots to the central 60 wells and 200 μl of distilled water was added to the outer wells. Each sample was placed into two wells. The plate was placed in a 4°C incubator for 16 hours. A 1:100 dilution of detecting IgG was prepared in extraction buffer 30 minutes before the end of the incubation period. The plate was given one rinse followed by five 3-minute washes with PBS-Tween, and was dried as before. The detecting IgG was added in 200 μl aliquots to the central wells and distilled water was added to the outer wells. The plate was incubated for 4 hours at 30°C.

Thirty minutes before the end of the incubation period a 1:2000 dilution of goat anti-rabbit-Fc conjugate in conjugate buffer was prepared. After the reagents were discarded, the plate was given one rinse followed by three 3-minute washes with PBS-Tween followed by drying in the above mentioned manner.

The conjugate was added to the central wells in 200 μl aliquots and distilled water was placed in the outer wells. The plate was incubated for 16 hours at 4°C. A 1 $mg.ml^{-1}$ solution of substrate (*p*-nitrophenyl phosphate) in substrate buffer was prepared 30 minutes before the end of the incubation period and placed in a 30°C incubator. The reagents were discarded from the plate which was then given one rinse and five 3-minute washes with PBS-Tween followed by drying in the above mentioned manner. The substrate was added to the central wells in 200 μl aliquots as well as to the entire top row of the plate. This was done quickly to ensure the uniformity of the results. The plate was incubated for 30 minutes at 30°C. The photometer was blanked

against the substrate and the readings were taken within 60 minutes of the substrate reaction to minimize background readings.

2.3 RESULTS

2.3.1 Symptoms Observed in the Field

Figures 2.2 - 2.12 show both leaf and fruit symptoms occurring in field-grown plants infected with viruses.

Figure 2.2. Leaves of *Cucurbita pepo* (zucchini) showing mosaic and leaf distortion.

Figure 2.3. A leaf of *Cucurbita pepo* showing severe leaf distortion (shoe-stringing).

Figure 2.4. Leaves of *Cucurbita moschata* (butternut) showing the mosaic symptom.

Figure 2.5. Leaves of *Cucurbita pepo* (gem squash) showing mosaic and leaf distortion.

Figure 2.6. Leaves of *Cucurbita pepo* (pumpkin) showing mosaic and leaf distortion (blistering).

Figure 2.7. Leaves of *Cucumis sativus* (cucumber) showing the mosaic symptom.

Figure 2.8. Fruit of *Cucurbita pepo* (zucchini) showing mosaic and fruit distortion.

Figure 2.9. A healthy fruit of *Cucurbita pepo* (zucchini).

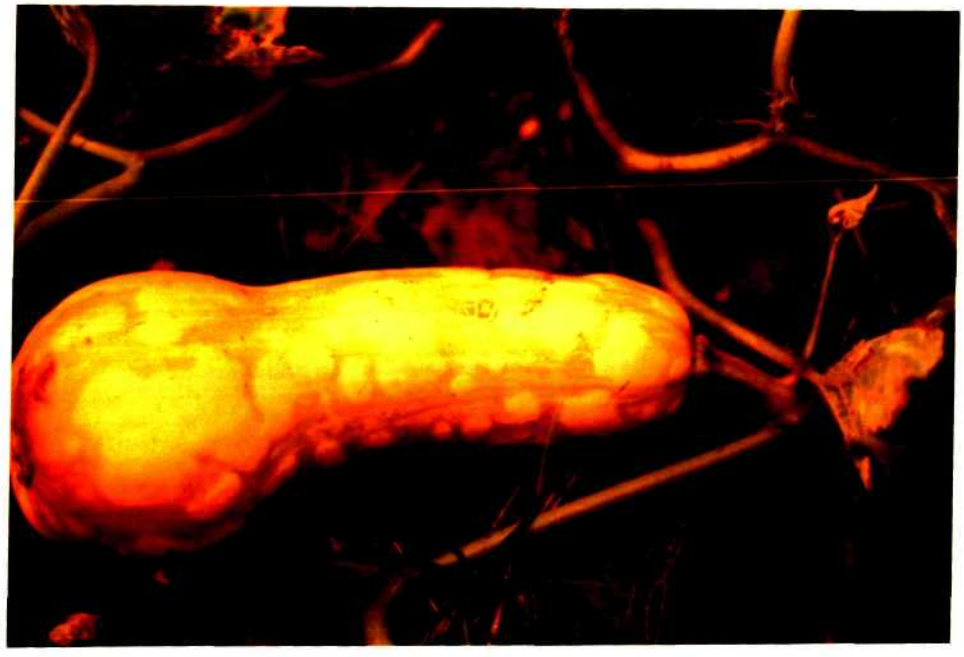

Figure 2.10. Fruit of *Cucurbita moschata* (butternut) showing fruit distortion.

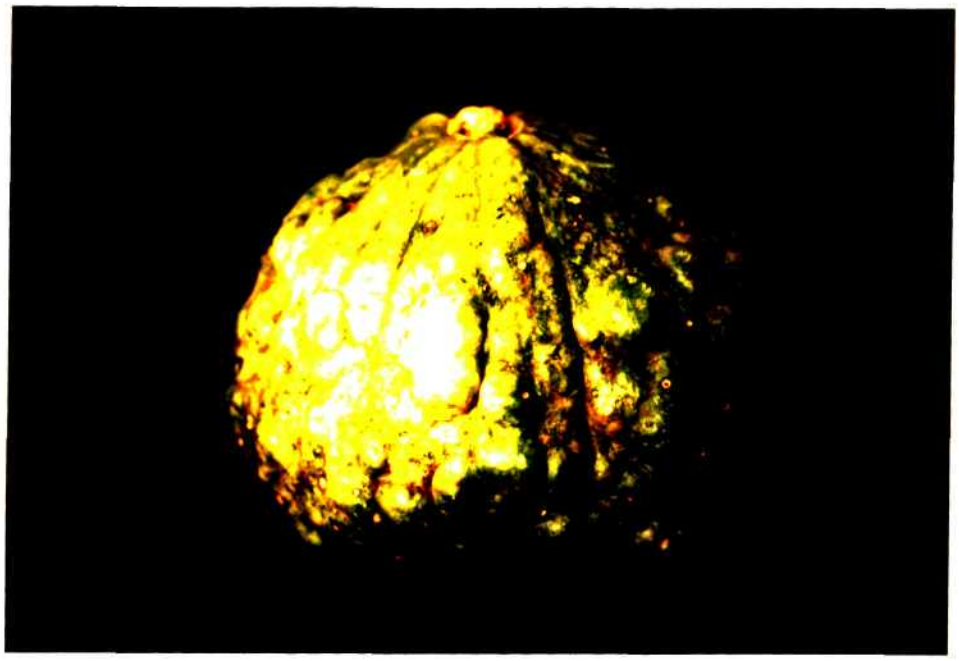

Figure 2.11. Fruit of *Cucurbita pepo* (pumpkin - 'Queensland Blue') showing mosaic and fruit distortion.

Figure 2.12. Fruit of *Cucumis sativus* (cucumber) showing mosaic and fruit distortion.

2.3.2 ELISA tests

The following table (Table 2.1) shows the results of the ELISA tests as well as the symptoms expressed by the plants used in the study. For the ELISA tests, a sample was regarded as being positive if the optical density values were equal or greater than the value of the positive control. In the absence of a positive control, a reading of twice that of the negative control was regarded as being positive. The samples are ordered according to month rather than according to year. Table 2.2 shows the presence of viruses in those weed species tested. All the weed or non-cucurbit species listed above were collected from the borders or from within cucurbit fields. The prevalence of the individual viruses and combinations of viruses are shown in Table 2.3. Of the samples collected, 38.7% were infected with more than one virus. All the samples tested for PRV and SMV (i.e. all samples collected in 1998) were negative for these viruses. A comparison of the prevalence of different viruses in 1997 and 1998 is shown in Table 2.4. The distribution and combinations of the viruses within KwaZulu-Natal is shown in Figure 2.13.

Table 2.1. The results of the ELISA tests showing which viruses were present in which samples, and at what point in the growing season they appear.

SAMPLE	PLANT	ZYMV	WMV-2	WMV-M	CMV	SYMPTOM[1]
BD1-21NOV97-C4	Zucchini	-	-	-	-	Mos; LD.s
BD1-15DEC97-C5	Gem Squash	-	-	-	-	LD
BD1-15DEC97-C6	Pumpkin	+	+	-	-	Mos
BD1-15DEC97-C7	Pumpkin	-	-	-	-	Mos
BD1-15DEC97-C8	Zucchini	-	-	-	-	Mot; LD.s
PB2-15DEC97-C1	Butternut	-	-	-	-	Mot
PD1-30DEC97-C1	Zulu Pumpkin	+	-	-	-	Mot
TV1-10FEB98-C6	Gem Squash	-	-	-	-	Mos; LD
BD1-12FEB98-C9	Butternut	-	-	-	-	Mos; LD
BD1-12FEB98-C10	Zucchini	+	+	-	-	Mos; LD; FD
BD1-12FEB98-C11	Gem Squash	+	+	±	-	Mos; LD
PB1-14FEB97-W1	Horned Cucumber*	-	-	±	-	M Chl
PB1-14FEB97-C3	Italian Pumpkin	-	-	+	-	Mot; LD.b
PB1-14FEB97-C4	Watermelon	-	-	-	-	NONE
PB1-14FEB97-C5	Zulu Pumpkin	+	-	+	-	Mot; M LD

Table 2.1 cont.

SAMPLE	PLANT	ZYMV	WMV2	WMV-M	CMV	SYMPTOMS
PB1-14FEB97-C6	Sweet Melon	-	-	±	-	M Mot
AS1-24FEB97-C1	Butternut	+	-	+	-	Mos
AS1-24FEB97-C2	Butternut	-	-	±	-	S Mos; LD
AS1-24FEB97-C3	Zucchini	+	-	+	-	S Mos; LD.c.s
AS1-24FEB97-C4	Butternut	+	-	-	-	Mot
AS1-24FEB97-W5	*Galinsoga parviflora**	+	-	±	-	Mos; LD
TV1-27FEB97-C1	Butternut	+	-	+	-	Mos; LD
TV1-27FEB97-C3	Butternut	+	-	-	-	S Mos; LD
TV1-27FEB97-C4	Butternut	+	-	-	-	S Mos; LD
TV1-27FEB97-C5	Butternut	-	-	±	-	FD; LD
TV2-6MAR97-C1	Pumpkin	+	-	+	-	S Mos
TV2-6MAR97-W2	*Ipomoea purpurea**	-	-	-	-	Mos; LD
TV2-6MAR97-W3	*Malva parviflora**	+	-	-	-	Mos
TV2-6MAR97-C4	Butternut	+	-	+	-	Mos
NK1-10MAR98-C1	Zulu Pumpkin	-	-	-	-	Mos
NK1-10MAR98-C2	Zulu Pumpkin	-	-	-	-	Mos
SB1-10MAR98-C1	Cucumber	-	-	-	-	Mos; LD; FD
SB1-10MAR98-C2	Cucumber	-	-	-	-	Mos; LD; FD
TA1-13MAR97-C1	Gem Squash	+	-	-	-	LD.c.s
TA1-13MAR97-C2	Gem Squash	+	-	-	-	Mos; LD.b.cup.c
TA1-13MAR97-W3	*Amaranthus* sp.*	+	-	-	-	None
TA1-13MAR97-W4	*Amaranthus* sp.*	-	-	-	-	None
TA1-13MAR97-C5	Butternut	-	-	±	-	Mos
TA1-13MAR97-C6	Butternut	+	-	-	-	Mot; FD
IN1-20MAR98-C1	Zucchini	-	+	-	-	Mos; LD.s
PB1-24MAR98-C7	Pumpkin	-	+	-	-	Mos; LD.cup
BD1-26MAR98-C12	Butternut	+	-	+	-	Mot
AS1-5APR97-W6	*Solanum* sp.*	+	-	±	±	Mot

Table 2.1 cont.

SAMPLE	PLANT	ZYMV	WMV2	WMV-M	CMV	SYMPTOMS
AS1-5APR97-C7	Zucchini	-	-	+	-	Mos; St; LD.b.s
AS1-5APR97-C8	Butternut	+	-	-	-	Mos
AS1-5APR97-W9	*Solanum* sp.*	+	-	-	+	LD
JZ1-17APR98-C1	Pumpkin	-	-	-	-	Mot
BD1-18APR97-C1	Butternut	+	-	+	+	S Mos; LD
BD1-18APR97-C2	Cucumber	+	-	±	-	Mos; LD; FD
BD1-18APR97-C3	Zucchini	+	-	+	±	S Mos; LD.s; FD
ST1-20APR97-C1	Pumpkin	+	-	-	-	LD.b
ST1-20APR97-C2	Pumpkin	+	+	-	-	Mos
ST1-20APR97-C3	Pumpkin	+	-	-	-	Mot; LD.b
ST1-20APR97-C4	Pumpkin	+	-	-	-	None
TA1-23APR98-C7	Butternut	±	+	-	±	Mos
BD1-25APR98-C13	Zucchini 'SQ 228'	+	+	-	-	Mos; LD
BD1-25APR98-C14	Zucchini 'House Zucchini'	-	-	+	-	Mos; LD; FD
BD1-25APR98-C15	Zucchini 'Elite'	-	-	-	-	Mos; LD; FD
BD1-25APR98-C16	Zucchini 'SQ 229'	-	-	-	-	Mos; LD; FD
BD1-25APR98-C17	Zucchini 'Jaguar'	-	-	-	-	Mos; LD; FD
BD1-25APR98-C18	Zucchini 'SQ 197'	+	-	+	-	Mos; LD; FD
BD1-25APR98-C19	Zucchini 'Puma'	-	+	-	-	Mos; M.LD, FD
BD1-25APR98-C20	Zucchini 'Gemma'	-	+	-	-	Mos; LD; FD
BD1-25APR98-C21	Zucchini 'Season Opener'	+	+	-	-	Mos; LD; FD
BD1-25APR98-C22	Watermelon	-	+	+	-	Mos; LD
BD1-25APR98-C23	Zucchini 'Consul'	-	+	-	-	Mos; LD; FD
BD1-25APR98-C24	Zucchini 'Verde'	-	+	+	±	Mos; LD; FD
KM1-12MAY97-C1	Pattipan	+	-	+	±	Mos.l.f; LD
KM1-12MAY97-C2	Zucchini	+	-	+	-	Mos.l.f; LD
KM1-12MAY97-C3	Zucchini	+	-	±	-	Mos.l.f; LD

Table 2.1 cont.

SAMPLE	PLANT	ZYMV	WMV2	WMV-M	CMV	SYMPTOMS
KM1-12MAY97-C4	Pattipan	-	-	-	-	Mos.l.f
MS1-29MAY97-C1	Zulu Pumpkin	+	+	±	+	S Mos; LD
MS1-29MAY97-C2	Zulu Pumpkin	-	+	+	+	Mos
MS1-29MAY97-C3	Zulu Pumpkin	-	+	-	-	LD
MS1-29MAY97-C4	Zulu Pumpkin	+	-	-	+	None

* weed species

[1] b = blistering; Chl = Chlorosis; cup = cupping; c = curling; f = fruit; FD = Fruit Distortion; l = leaf; LD = Leaf Distortion; M = Mild; Mos = Mosaic; Mot = Mottle; S = Severe; s = shoestringing; St = Stunting; - = negative result; ± = uncertain result; + = positive result.

Thirteen samples (BD1-21NOV97-C4; BD1-15DEC97-C7; BD1-15DEC97-C8; TV1-10FEB98-C6; BD1-12FEB98-C9; NK1-10MAR98-C1; NK1-10MAR98-C2; SB1-10MAR98-C1; SB1-10MAR98-C2; BD1-25APR98-C15; BD1-25APR98-C16; BD1-25APR98-C17; KM1-12MAY98-C4) were found not to react with any of the antisera used. The plants from which these samples were collected were all expressing severe symptoms of virus infection. With the exception of the one Thornville sample, all these plants were found in the northern part of the province.

Table 2.2. Weeds from cucurbit fields which were tested using ELISA.

SAMPLE	PLANT	ZYMV	WMV2	WMV-M	CMV	SYMPTOMS
PB1-14FEB97-W1	Horned Cucumber	-	-	±	-	M Chl
AS1-24FEB97-W5	Galinsoga	+	-	±	-	Mos; LD
TV2-6MAR97-W2	*Ipomoea purpurea*	-	-	-	-	Mos; LD
TV2-6MAR97-W3	*Malva parviflora*	+	-	-	-	Mos
TA1-13MAR97-W3	*Amaranthus* sp.	+	-	-	-	None
TA1-13MAR97-W4	*Amaranthus* sp.	-	-	-	-	None
AS1-5APR97-W6	*Solanum* sp.	+	-	±	±	Mot
AS1-5APR97-W9	*Solanum* sp.	+	-	-	+	LD

Table 2.3. The prevalence of infections of plants with the viruses, as indicated by the ELISA tests.

Virus(es) present	Percentage of plants infected [1]
CMV	6.67%+ 6.67%± 86.67%-
WMV-M	24.00%+ 14.67%± 61.33%-
WMV2	22.67%+ 0.0%± 77.33%-
ZYMV	50.67%+ 1.33%± 48.00%-
CMV + WMV-M	2.67%+ 0.0%± 97.33 %-
CMV + WMV2	0.0%+ 0.0%± 100%-
CMV + ZYMV	2.67%+ 1.33%± 96.00%-
WMV2 + WMV-M	4.00%+ 0.0%± 96.00%-
WMV2 + ZYMV	9.33%+ 1.33%± 89.33%-
WMV-M + ZYMV	16.00%+ 6.67%± 77.33%-
ZYMV + WMV-M + WMV2	0.0%+ 1.33%± 98.67%-
ZYMV + WMV-M + CMV	1.33%+ 4.00%± 94.67%-
ZYMV + WMV2 + CMV	1.33%+ 2.67%± 96.00%-
WMV-M + WMV2 + CMV	1.33%+ 1.33%± 97.33%-
WMV-M + WMV2 + ZYMV + CMV	0.0%+ 1.33%± 98.67%-

[1] + = confirmed positive result; ± = uncertain result; - = confirmed negative result.

Table 2.4 A comparison of the prevalence of the viruses in 1997 and 1998 (Only confirmed infections are presented).

Virus	1997	1998
CMV	10%	0%
WMV-M	26%	20%
WMV2	10%	48%
ZYMV	64%	24%
Mixed	32%	32%

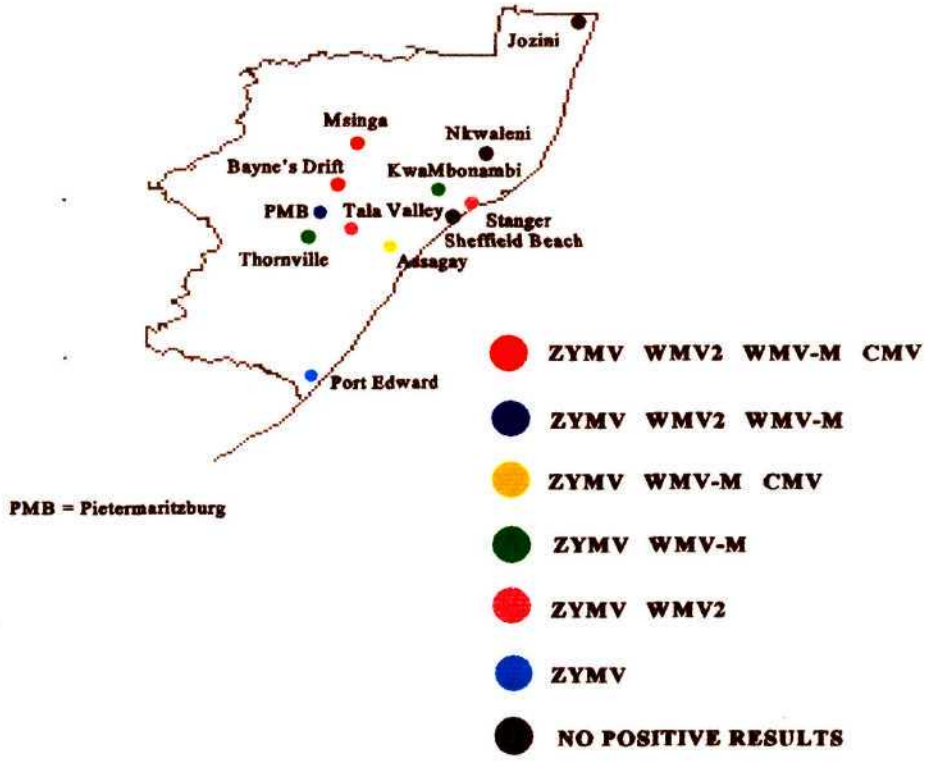

Figure 2.13 The distribution and combinations of cucurbit viruses in KwaZulu-Natal.

2.4 DISCUSSION

Of the four viruses initially surveyed for, ZYMV was found to be the most prevalent over the two year study (Table 2.3). The prevalence of viruses does vary considerably from year to year (Table 2.4). As different aphid species have different transmission efficiencies (Castle *et al.*, 1992), a variation in the species composition of the general aphid population could cause a variation in the prevalence of different viruses in a crop. This survey has also indicated that since its identification in South Africa in 1993 (von Wechmar *et al.*, 1995), ZYMV has replaced WMV-M (van der Meer, 1985) as the most prevalent cucurbit virus infecting cucurbits in the region (Table 2.3).

This study has confirmed the presence of WMV2 in South Africa (Table 2.1). This virus can also have a higher occurrence than WMV-M, making it an important virus in cucurbit crops (Table 2.4). The prevalence of WMV2 increased markedly in 1998 compared to 1997. The survey has also indicated that PRV-W and SMV are absent from KwaZulu-Natal. CMV was found in some samples, but occurred so late in the growing season (Table 2.1) that it cannot be regarded as a serious threat to cucurbit production in the region.

The potential importance of weeds in the virus pathosystem is shown in Table 2.2. It is interesting to note that ZYMV was present in the majority of weed samples tested. The analysis was conducted twice to confirm these results. This result comes from a small sample base, and these results may be due to chance infections. The presence of viruses in weeds indicates the importance of weed control strategies as part of integrated disease control strategy.

A number of samples (32%) were found to be infected with more than one virus (Table 2.4). If the uncertain test results are included, 38.7% of the samples were infected with more than one virus. This situation could have a number of consequences: there could be no interaction between the viruses; the resulting disease could be more severe than if only one virus was present; there could be some protection offered by one virus against the other(s); where two or more viruses belonging to the same family (e.g. the Potyviridae) are involved, transcapsidation could occur (Bourdin & Lecoq, 1991), potentially affecting both the host and vector ranges of the viruses concerned.

The distribution of viruses in KwaZulu-Natal is shown in Figure 2.13. ZYMV was found to occur throughout the region. WMV2 was found in a central band across the province. WMV-M was found in a slightly wider zone, but otherwise had a similar distribution pattern to WMV2. CMV was only detected in a central zone from a limited number of samples, further reducing its importance in the cucurbit cropping system. The variation in distribution of the viruses will affect the possible combinations of viruses in multiple infections within an area. This could affect the severity of disease in these areas. A more thorough sampling of the southern and far northern regions may have changed this pattern. The limited number of samples from some localities (e.g. Jozini, Port Edward) may have reduced the chance of detecting certain viruses (especially ZYMV, WMV2, WMV-M) from these areas.

A number of samples from plants expressing severe symptoms were found not to react with any of the antisera initially used in the survey (i.e. ZYMV, WMV-M, WMV2, CMV). These samples, along with the other samples collected in 1998, were tested for PRV-W and SMV using DAS-ELISA. None of the samples were positive for these viruses indicating that these viruses do not occur in KwaZulu-Natal. This means that either the virus(es) present were at a stage when they

were not detectable by DAS-ELISA (Barnett, 1986), or another virus not considered in the study was infecting the plants. As the samples were stored in a freezer, the detectability of the potyviruses could have been reduced (Barnett, 1986).

A comparison of the DAS-ELISA and indirect ELISA techniques showed that the DAS-ELISA technique was better suited to survey studies than the indirect ELISA technique. This was due to the time requirements of the two methods and the ease of obtaining the reagents. This supports the findings of Barnett (1986).

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CHAPTER 3: THE APHID VECTORS OF CUCURBIT VIRUSES

3.1 INTRODUCTION

Aphids, which are important vectors, belong to the order Hemiptera, suborder Homoptera, superfamily Aphidoidea, family Aphididae (Blackman & Eastop, 1984). Those species which have a cosmopolitan distribution (e.g. *Aphis gossypii* Glover; *Myzus persicae* Sulzer) have a number of synonyms (41 and 36 respectively) which can make the identification of a particular specimen difficult (Ilharco & van Harten, 1987).

Few of the aphid species which are regarded as pests in an area are native to that area, and it can be said that certain species will eventually be found wherever their crop hosts are cultivated. Many of the cosmopolitan pest species are anholocyclic, which enables them to survive all year round by parthenogenetic reproduction (Blackman & Eastop, 1984).

Parthenogenesis (reproduction without fertilization) (Figure 3.1) and heterocy (host alternation) allow aphids to exploit their hosts, especially annual herbaceous plants, to their full potential (Blackman & Eastop, 1984). Parthenogenesis allows for the telescoping of generations, and thus a tremendous rate of increase (Dixon, 1987a).

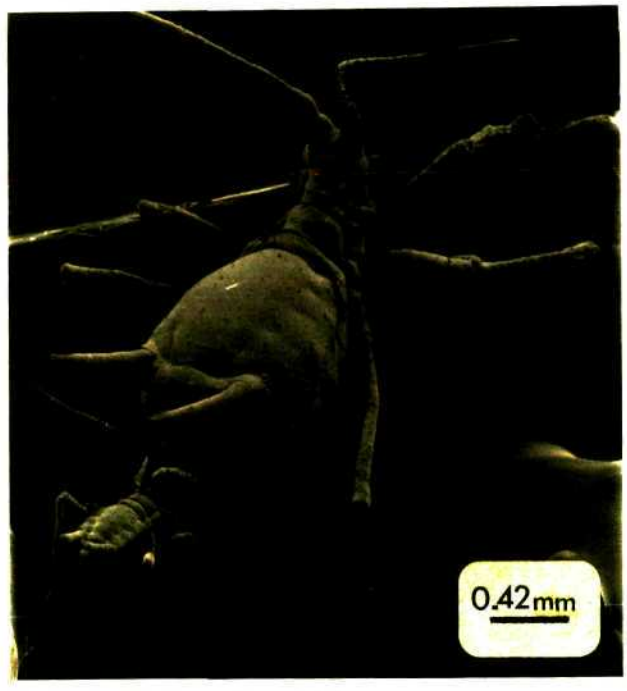

Figure 3.1. Parthenogenetic birth of a juvenile aphid.

Aphid species which are considered to be pests on agricultural crops generally have a wider host range than other aphids which are regarded as being economically unimportant. This however does not mean that they are polyphagous in the sense that they are totally non-specific, but rather that they are capable of feeding on a greater range of host families than the majority of aphid species. Even *M. persicae* (green peach aphid), the most polyphagous aphid, has not been recorded from even one percent of all flowering plant species (Blackman & Eastop, 1984; Dixon, 1987b).

Many of the diurnal herbivorous insects are attracted to yellow. It has been suggested that yellow is a "super-stimulus" of the foliage-type stimulus. Contrast between the plant and its surroundings appears to be important, as more aphids have been found to alight on plants that are surrounded by bare soil than those surrounded by other vegetation (Prokopy & Owens, 1983). Some plant extracts, such as garlic and onion oils can be repellant to aphids (Hori, 1996).

Non-colonizing aphids (i.e. aphids which probe and then reject a plant as a host) can be important in the ecology of papaya ringspot potyvirus - Type W (PRV-W) and watermelon mosaic 2 potyvirus (WMV2) (Zitter, 1977).

Seasonal recolonization of an area is probably due to progressive and continuous movement over several generations rather than a single migration flight. This indicates that middle distance migration of 1-100 km may be more important than long distance flights. When a host plant becomes stressed or the colony population becomes too dense, the older larvae and the apterous adults leave the host (Taylor, 1986). Aphids transmit more plant viruses than any of the other invertebrate groups. These insects are an important factor in the spread of viruses both within a field and over long distances. In some cases it has been noted that diseased plants seemed to be more favourable for rapid vector development than healthy plants (Swenson, 1968).

This study focuses on aphids as vectors of stylet-borne viruses and only this relationship will be mentioned. Stylet-borne viruses only have a temporary relationship with their vector(s). The virus can be acquired from and inoculated into a plant in a very short space of time, often requiring less than one minute. The virus is lost in about an hour by feeding aphids and after

several hours by non-feeding aphids (Swenson, 1968; Zitter, 1977; Sylvester, 1989). The majority of acquisition studies indicate that the virus is acquired from the epidermal cells during the brief sensory probes (Blua & Perring, 1992).

A mild winter may result in early spring flights of some aphid species which were able to overwinter on secondary hosts (Zitter, 1977). Aphids are incapable of directional flight when the wind speed exceeds 1.5 mph (2.42 kmph). When the wind speed is in excess of this and is unidirectional, aphid dispersal in the same direction as the wind can be expected (Adlerz, 1974).

A number of aphids have been recorded as feeding on members of the Cucurbitaceae. The following list covering sub-Saharan Africa was compiled by Millar (1994): *A. gossypii*; *Aphis craccivora* Koch; *Aphis fabae* Scopoli; *M. persicae*; *Macrosiphum euphorbiae* Thomas. It must always be remembered that the most common aphid species or biotype is not necessarily the most important vector species, as vector efficiency may be more important. On the other hand, the most efficient virus vectors are not always the most important vectors, as the development of large populations may be more important (Swenson, 1968; Thresh, 1976). The purpose of this study was to assess the effects of aphid repellant treatments on aphid numbers.

3.2 MATERIALS AND METHODS

3.2.1 Field Studies

The trials were conducted at Bayne's Drift. Trial 1 was conducted from 23 October 1997 - 01 December 1997. Trial 2 was conducted from 02 February 1998 - 17 February 1998. Trial 3 was conducted from 07 March 1998 - 04 April 1998. The fluctuations of aphid numbers have been monitored by using sticky traps (Heathcote, 1974). "Bug Trap" yellow colour traps (AgriBiol, Vlaeberg) (Figure 3.2) coated with a long lasting glue were used. At each trial site one trap was placed in each replication site, i.e. 20 traps per trial. The traps were positioned approxiamately 80 cm above the ground. The traps were collected and replaced every seven days during the trials.

The traps were returned to the laboratory, viewed with a hand lens and the insects trapped were counted. Once this had been done, the traps were stored in a freezer to prevent deterioration of the specimens by decomposition. The results were analysed using the area under the pest

progress curve (AUPPC) adapted from the area under the disease progress curve (AUDPC) analysis (Berger, 1981). The data were also analysed using a two-way analysis of variance (ANOVA).

Figure 3.2. "Bug Trap" yellow sticky trap used to monitor aphid numbers.

3.2.2 Control Methods

The beds were raised. Rows were separated by a space of 1.5 m, and within rows, plants were spaced at 0.5 m intervals. The field was irrigated by sprinkler irrigation. The trial was laid out using a randomised complete block design. The *C. pepo* cultivar 'Elite' (Stark Ayres) was used.

3.2.2.1 Companion Planting

Fennel (*Foeniculum vulgare* L.) seedlings were planted after every two zucchini plants. This meant that each zucchini plant had a fennel plant adjacent to it. The motivation behind this was to encourage natural enemies into the plots which would move from the fennel plants onto the zucchini plants.

3.2.2.2 Garlic Repellant

This substance was an experimental garlic extract (Kombat Chemicals, Greytown) which was

diluted 1:10 in water and sprayed to the point of runoff onto the zucchini plants at seven day intervals. The odour is reportedly unattractive to many insects, and would thus discourage them from landing on the plants treated with the repellant. In the second and third trial a fish oil extract was added to the garlic repellant at a dilution of 1:3 in garlic repellant.

3.2.2.3 Straw Mulch

Straw can be used as an alternative to plastic mulches, and has the added advantage of creating a habitat for terrestrial predators within the otherwise hostile field environment. The reflectance of the straw and the presence of more predators was expected to influence the number of pests visiting the plants. The mulch was spread to a depth of approximately 12 cm and extended approximately 0.5 m on either side of the plants.

3.2.2.4 Reflective Mulch

White Knittex Shade Net® (Knittex, Durban, South Africa) giving 60% shade was used as the reflective mulch. Strips of this material (8 m x 0.5 m) were laid down on both sides of each row of zucchini plants. The reflectance of this material was expected to repel pest insects as well as change the visual appearance of the plants by giving a green against white matrix, as opposed to a green against brown matrix as is normal in crop fields.

3.2.2.5 Resistant/Tolerant Cultivars

In the third trial the fennel treatment was replaced by the use of the tolerant cultivar 'Puma' (ZYMV; WMV2) (Harris Moran Seed Company, California supplied by Hygrotech). This cultivar was also used in conjunction with the other treatments. The tolerance information was obtained from the company catalogue.

3.3 RESULTS

3.3.1 Field Studies

The results from the yellow sticky traps which were used to study the numbers of aphids in the fields is shown in Figures 3.3-3.6. The pest progress curves are shown in Figures 3.7 -3.8. Figure 3.3 shows the pooled data from the replications. Therefore no statistical analysis was possible, and only a trend can be seen.

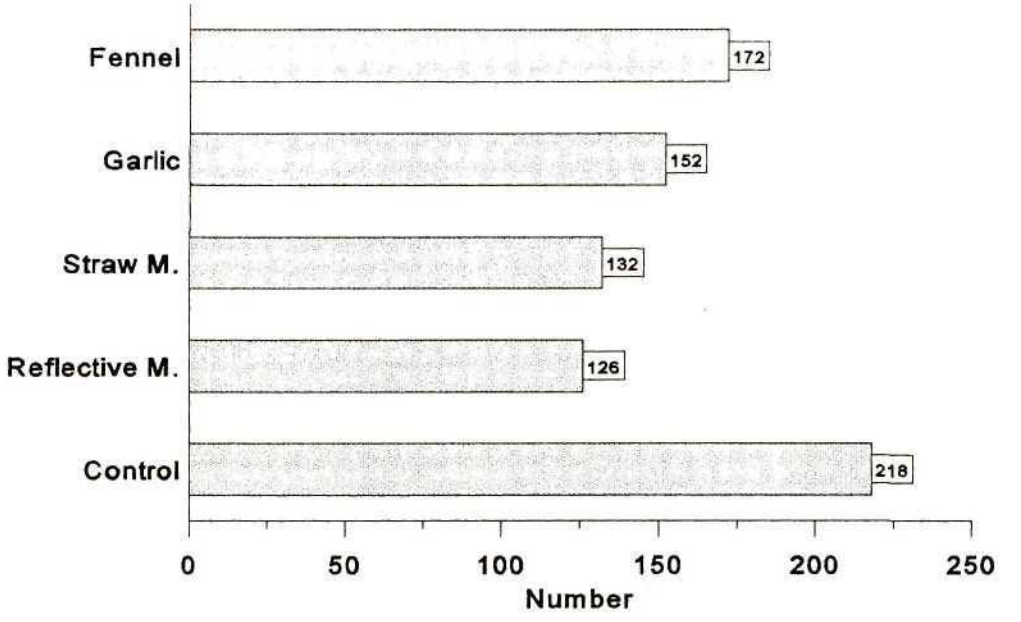

Figure 3.3. Aphid numbers in response to five treatments used at Bayne's Drift during Trial 1 (30 October 1997 - 01 December 1997).

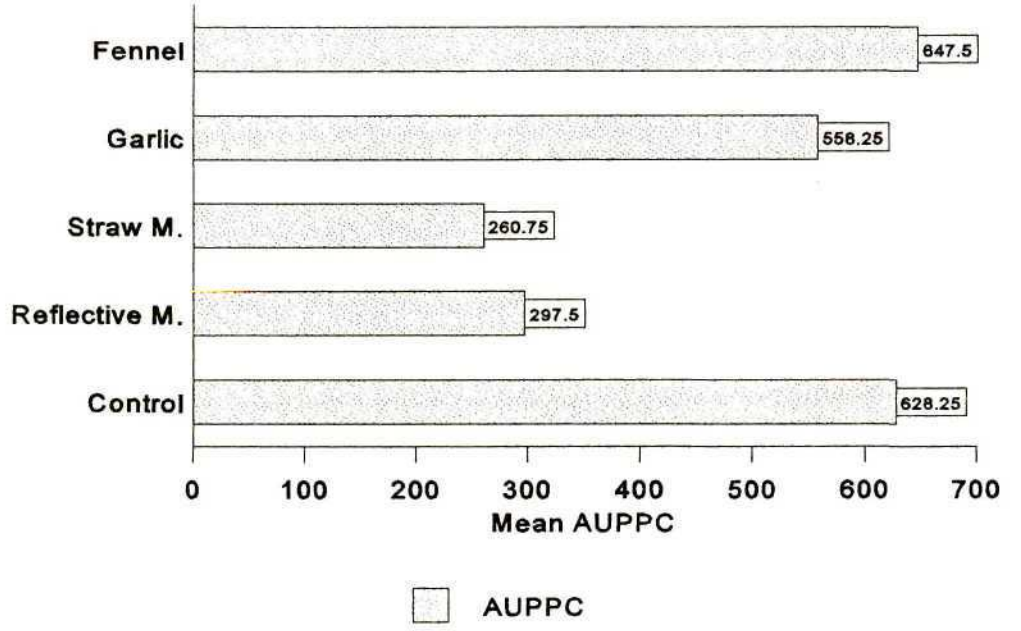

Figure 3.4. Aphid numbers (represented as mean AUPPC) in response to five treatments used at Bayne's Drift during Trial 2 (2 February 1998 - 17 February 1998).

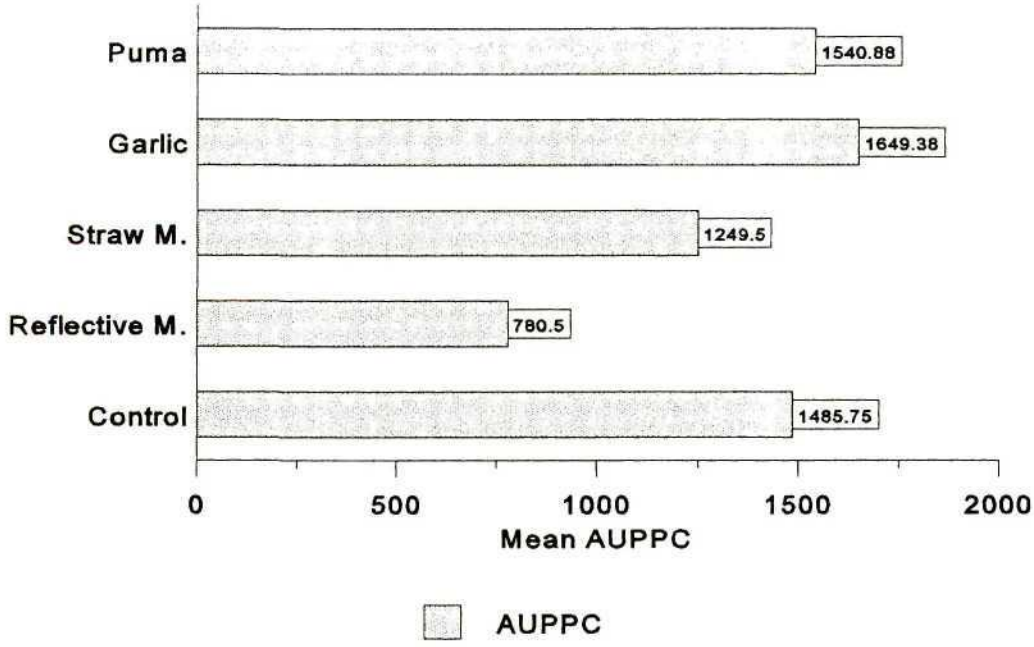

Figure 3.5. Aphid numbers (represented as mean AUPPC) in response to five treatments used at Bayne's Drift during Trial 3 (7 March 1998 - 4 April 1998).

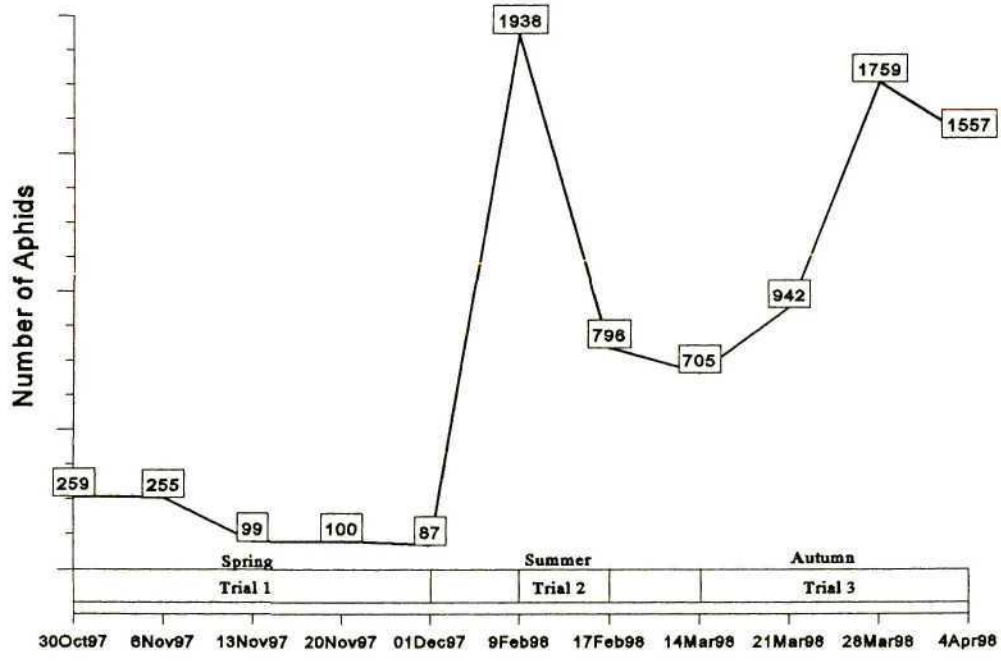

Figure 3.6. The changes in aphid density during the course of the season (30 October 1997 - 4 April 1998).

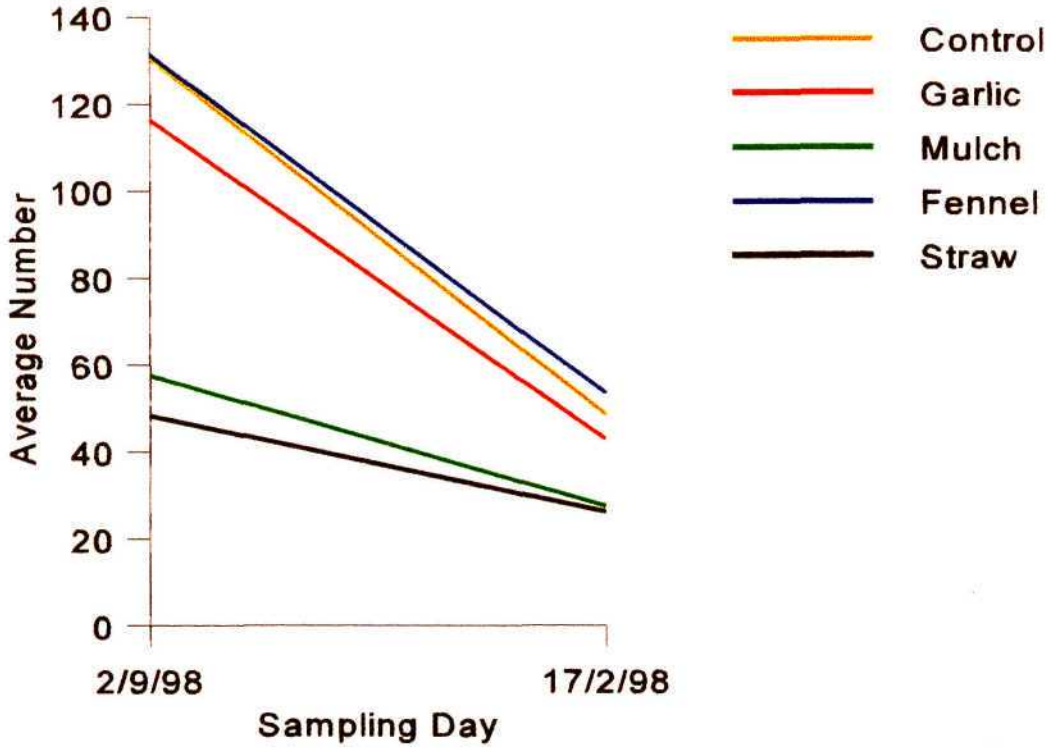

Figure 3.7. The pest progress curves of aphids in response to five treatments (Trial 2) at the Bayne's Drift site (2 February 1998 - 17 February 1998).

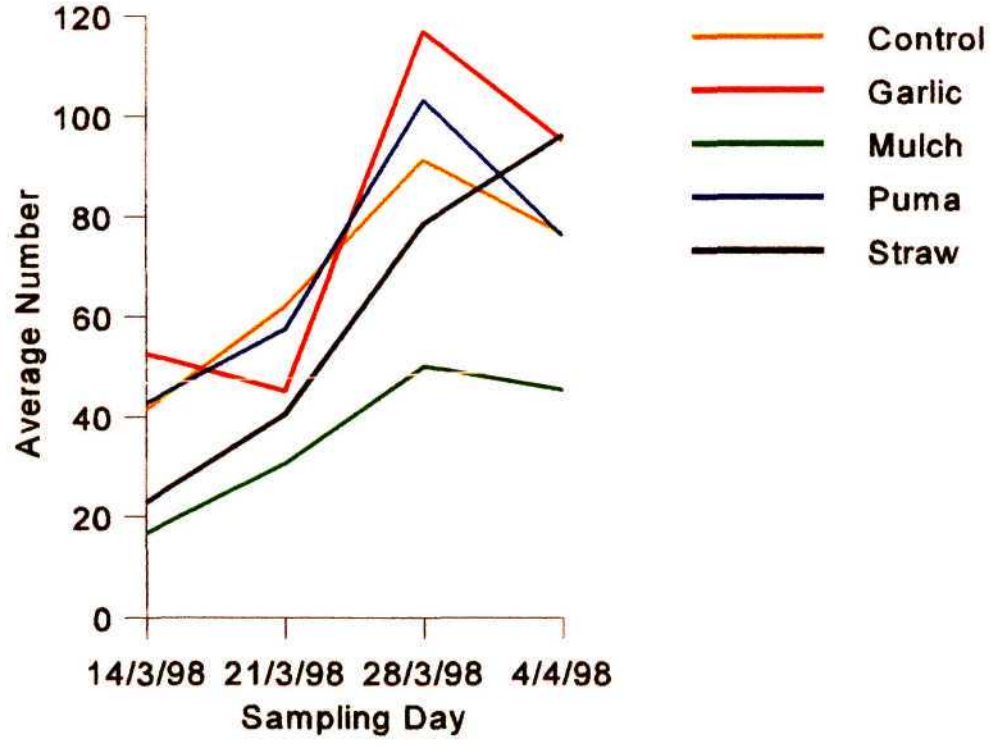

Figure 3.8. The pest progress curves of aphids in response to five treatments (Trial 3) at the Bayne's Drift site (7 March 1998 - 4 April 1998).

The statistical differences between the treatments used in Trial 2 and Trial 3 are shown in Tables 3.1 to 3.2. In Table 3.1 the effects on aphid numbers are assessed, while in Table in 3.2 the effect the differences in the area under the pest progress curve (AUPPC) are assessed.

Table 3.1. The effects of different aphid repellents and companion planting on aphids: Average numbers and rank of the treatments in Trial 2 and Trial 3.

	Trial 2		Trial 3	
Treatment	Number	Rank	Number	Rank
Control	179.50b[1]	4	271.25b	3
Garlic Repellent	159.50b	3	309.50b	5
Reflective Mulch	85.00a	2	142.50a	1
Fennel	185.00b	5	N/A	N/A
Puma	N/A[2]	N/A	279.50b	4
Straw Mulch	74.50a	1	238.00b	2
F-ratio	6.156 **		4.894 *	
P	0.0062 **		0.0142 *	
CV%	31.24%		23.43%	

[1] Treatments with no letters in common are significantly different.

[2] N/A = Not Applied NS = Not Significant * = Significant ** = very significant

*** = highly significant

There was no significant difference between the replicates in either trial (Trial 2 F = 0.119 NS; P = 0.9469 NS; Trial 3 F = 0.390 NS; P = 0.7625 NS) showing that there was no blocks effect.

In both Trial 2 and Trial 3 the reflective mulch and straw mulch were the two best treatments, although in Trial 3 only the reflective mulch was significantly different from the other treatments.

Table 3.2. The AUPPC values and rank of the treatments in Trial 2 and Trial 3.

	Trial 2		Trial 3	
Treatment	AUPPC	Rank	AUPPC	Rank
Control	628.25b[1]	4	1485.75b	3
Garlic Repellent	558.25b	3	1649.38b	5
Reflective Mulch	297.50a	2	780.50a	1
Fennel	647.50b	5	N/A	N/A
Puma	N/A[2]	N/A	1540.88b	4
Straw Mulch	260.75a	1	1249.50ab	2
F-ratio	6.156 **		4.511 **	
P	0.0062 **		0.0187 **	
CV%	31.24%		24.28%	

[1] Treatments with no letters in common are significantly different.

[2] N/A = Not Applied NS = Not Significant * = Significant ** = very significant

*** = highly significant

There were no significant differences between the replicates in either of the trials (Trial 2 F = 0.119 NS, P = 0.9469 NS; Trial 3 F = 0.353 NS, P = 0.7877 NS).

3.4 DISCUSSION

Trial 1 was a pilot trial to test the feasibility of implementing various monitoring and control techniques. Data obtained from this trial were pooled, and therefore only trends and no statistical analysis can be shown. Aphid numbers during this part of the season were low compared to later counts (Figure 3.6). The area where the trials were conducted (Bayne's Drift) has frost during the winter season which kills a large number of aphids. Thus local aphid populations are probably largely dependent on individuals recolonizing the area from the warmer coastal regions at the start of each new growing season, although some individuals or colonies may survive on hosts other than cucurbits. As the population build-up is gradual in the early part of the season, the recolonization is probably due to a progressive and continuous movement over a number of generations, as suggested by Taylor (1986).

The yellow sticky traps used in the trials were effective for monitoring aphid numbers. As yellow acts as an attractant, a better reflection on the effectiveness of the treatments may have been gained by using green tile traps as used by (Webb *et al.*, 1994). The overall effectiveness of the control measures in the second and third trials can be seen in figures 3.4 to 3.5 and tables 3.1 to 3.2. The performance of the treatments during the course of the trials is shown in figures 3.7 to 3.8. At this time aphid numbers had increased considerably from the start of the season (Figure 3.6). Trial 2 was prematurely terminated when the entire field was washed away during a severe storm. In both trials the straw mulch and the white reflective mulch were the best treatments, having the lowest aphid counts. This was particularly noticeable in Trial 2 where aphid numbers reached their season peak (Figure 3.6), and pest (vector) pressure was high. In both trials the reflective mulch was significantly better than all other treatments, except the straw mulch, in repelling aphids. The performance of the reflective mulch is believed to be due to two factors. The first of these is that the white surface reflected short-wave radiation which is unattractive to flying aphids. This effect has been reported on by Wyman *et al.* (1979), Zitter and Simons (1980), Prokopy and Owens (1983), Gibson and Rice (1989) and Jones (1991). The second reason is that the presence of the mulch altered the appearance of the crop by changing the crop background from brown to white. This would not match the search pattern of immigrating aphids, and therefore fewer insects would enter the plots (Prokopy & Owens, 1983). The action of the straw mulch is probably similar to that of the reflective mulch but at a lower intensity.

The garlic repellent appeared to offer some level of control in the first and second trials (Figure 3.3-3.4), although this control was not significant when compared with the control (Table 3.1-3.2). In the third trial no level of control was given (Figure 3.5; Table 3.1 -3.2). Its failure to offer any benefit in this trial may be due to the high rainfall during this time, as well as the use of sprinkler irrigation. These two factors would have resulted in the repellent being washed off the leaves. The situation may have been improved if an adjuvant which increased the adhesion of the repellent to the leaves had been added. The true efficacy of the repellent may not be apparent when aphid numbers are monitored with sticky traps. This is due to the repellent probably only being active when the insects are in close proximity to the plants. Thus the insects are still present in the plots and will be attracted to the traps, even though they are not actually landing and/or feeding on the crop plants. This possibility was demonstrated in Trial 3 (Table 4.1) where this

treatment ranked second in effectiveness in reducing disease severity.

In Trial 1 when aphid numbers were low the use of fennel as a companion plant gave some measure of aphid control (Figure 3.3). In Trial 2 when aphid numbers were high, this treatment performed worse than the control. During this trial the temperature was higher than earlier in the season, and the fennel plants suffered from heat stress. Fennel is a slower grower than *C. pepo* and did not reach the flowering stage before the fruiting stage of the crop. As the motivation for using fennel was the attractiveness of its flowers to beneficial insects (parasitoid wasps, syrphid flies, etc.) (Salto *et al.*, 1991) its slow development makes it unsuitable as a companion plant in this crop. Some benefit may be derived from planting stands of fennel between or near fields a few weeks earlier than the crop to be protected. This would allow the fennel to reach the flowering stage at a time when the presence of beneficial insects could increase the productivity of the crop (i.e. from planting until the start of fruiting). In both trials where it was used, a high number of aphids were recorded from the fennel plots (Figure 3.3 - 3.4). This was probably due to fennel having aphid species which use it, but not *C. pepo*, as a host. Therefore a greater number of individuals would be attracted to the plots. Due to its poor performance this treatment was discontinued from the study and replaced by the use of the virus-resistant zucchini cultivar 'Puma'.

The use of the cultivar 'Puma' had a poor effect on aphid numbers (Figure 3.5; Tables 3.1 - 3.2) as this treatment performed worse than the control too, although this was not significant. The reason for this is probably due to some characteristic of the plants making them more attractive to aphids than the cultivar 'Elite' which was used in the other treatments.

For controlling aphid numbers in zucchini fields, the use of either a white reflective mulch or a straw mulch is recommended. The synthetic reflective mulch could be better used by large-scale producers, whilst the straw mulch could be used by small-scale or resource-poor farmers. The recommendation of use of the materials by different spheres of farming is largely due to the initial cost of the material, and the subsequent labour involved in applying it. The reflective mulch is expensive, but gives good aphid control and can be laid down with the aid of mechanical implements. The straw mulch is cheaper and offers a comparable level of aphid control, but is

labour intensive to apply. The garlic repellent may offer some benefit in controlling aphid numbers, especially if it were to be applied in conjunction with either the reflective mulch or the straw mulch.

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CHAPTER 4: CONTROL OF THE APHID VECTORS AND THE VIRUSES

4.1 INTRODUCTION

Plant virus diseases are difficult to control in the tropics for a number of reasons: ① winter temperatures are not severe enough to break the disease cycles; ② reservoirs of insect vectors are present at all times; ③ resistant and tolerant cultivars are not generally available; and ④ the crops are usually grown in many small, scattered plots making isolation difficult if not impossible (Gonsalves & Garnsey, 1989). These problems are exacerbated under extreme epidemic conditions (Raccah, 1985).

Since the realization that chemical control of pests and pathogens has detrimental side effects and is not always effective, other avenues of control have been explored and attitudes have changed. It is now acceptable to keep pathogen populations below the economic threshold rather than eradicate them. Cultural methods of crop protection, such as biological control and crop rotation, were developed or improved; and it has been realized that the use of more than one control method is better than total reliance on one, usually chemical, control measure (Rabbinge & van Oijen, 1997).

In the epidemiology of plant viruses the date that the virus is first introduced to a field or area is extremely important, as the earlier (and therefore the longer) that the virus is present, the greater the damage to the crop (Thresh, 1974). There are farming practices which ultimately result in a better crop as well as reduce the impact of virus diseases by affecting the efficiency of virus vectors and crop management strategies (Zitter & Simons, 1980). The most common management practices are: ① virus source elimination (see Duffus (1971), Zitter (1977), Al-Musa (1989), Sylvester (1989), and Altieri (1994)), ② crop isolation from virus sources (see Maelzer (1986), Sylvester (1989), Altieri (1991; 1994), and Hokkanen (1991)), ③ crop manipulation (see Zitter & Simons (1980), Klingauf (1987), Sylvester (1989), Jones (1990), Namba *et al.* (1992), Gilbert-Albertini *et al.* (1993), Altieri (1994), Desbiez & Lecoq (1997), and Robinson & Decker-Walters (1997)), and ④ a variety of cultural methods which aim to reduce the number of active vectors either regionally or locally (Sylvester, 1989).

Insect vector control is important in the control of plant virus diseases. This aspect of disease management has been examined by a range of authors and topics. Chemical control has been covered by Zitter & Simons (1980), Maelzer (1986), Clough & Hamm (1992), and Desbiez & Lecoq (1997). The biological control aspect of vector control has been discussed by Nentwig (1988), Brown & Stephenson (1990), Altieri (1994), and Jervis & Kidd (1996). The effectiveness of reflective mulches has been discussed by Zitter (1977), Zitter & Simons (1980), Jones (1991), Pinese *et al.* (1994), and Desbiez & Lecoq (1997). De Oliveira *et al.* (1990) and Rummel *et al.* (1995) discussed the use of straw mulches. Mild strain protection was covered by Gonsalves & Garnsey (1989) and Desbiez & Lecoq (1997). A range of other cultural or non-chemical methods of control were covered by Zitter & Simons (1980), Yang & Tang (1988), Wiles *et al.* (1989), Grossman (1993), Pinese *et al.* (1994), and Briggs *et al.* (1996). Hori (1996) discusses the use of garlic oil in repelling aphids in *in vitro* studies.

Due to the many options available to effectively control insects, the use of integrated pest management (IPM) is becoming more widespread by necessity (Edwards & Stinner, 1990). IPM is essential in the management of virus diseases (Zitter & Simons, 1980). This study was undertaken to investigate the effectiveness of some of these strategies under the environmental conditions experienced in KwaZulu-Natal.

4.2 MATERIALS AND METHODS

All the trials mentioned here were conducted at Bayne's Drift (Gilmorehill Farm). The soil-type at the farm was a Tukulu Form (identified by Prof. J. Hughes, Dept of Agronomy, University of Natal, Pietermaritzburg). The trials were laid out in a randomised complete block design. The rows were spaced at 1.5 m, while plants were spaced at intervals of 0.5 m. Sprinkler irrigation was used to water the crop. *C. pepo* cv 'Elite' (zucchini) (Stark Ayres) was used as the host plant.

4.2.1 Weed Monitoring

Weeds which were common in the fields where studies were conducted were collected and identified using the guide by Bromilow (1995). These plants were also tested for the presence of viruses.

4.2.2 Control Methods

4.2.2.1 Companion Planting

Fennel (*Foeniculum vulgare* L.) seedlings were planted after every two zucchini plants. This meant that each zucchini plant had a fennel plant adjacent to it. The motivation behind this was to encourage natural enemies into the plots which would move from the fennel plants onto the zucchini plants.

4.2.2.2 Garlic Repellant

This substance was an experimental garlic extract (Kombat Chemicals, Greytown) which was diluted 1:10 in water and sprayed to the point of runoff onto the zucchini plants at seven day intervals. The odour is reportedly unattractive to many insects, and would thus discourage them from landing on the plants treated with the repellant. In the second and third trial a fish oil extract was added to the garlic repellant at a dilution of 1:3 in garlic repellant.

4.2.2.3 Straw Mulch

Straw can be used as an alternative to plastic mulches, and has the added advantage of creating a habitat for terrestrial predators within the otherwise hostile field environment. The reflectance of the straw and the presence of more predators was expected to influence the number of pests visiting the plants. The mulch was spread to a depth of approximately 12 cm and extended approximately 0.5 m on either side of the plants.

4.2.2.4 Reflective Mulch

White Knittex Shade Net® (Knittex, Durban, South Africa) giving 60% shade was used as the reflective mulch. Strips of this material (8 m x 0.5 m) were laid down on both sides of each row of zucchini plants. The reflectance of this material was expected to repel pest insects as well as change the visual appearance of the plants by giving a green against white matrix, as opposed to a green against brown matrix as is normal in crop fields.

4.2.2.5 Resistant/Tolerant Cultivars

In the third trial the fennel treatment was replaced by the use of the tolerant cultivar 'Puma' (ZYMV, WMV2) (Harris Moran Seed Company, California supplied by Hygrotech). The

tolerance information was obtained from the company catalogue.

The effect of the treatments on beneficial arthropods (spiders (Aranae), ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae)) was determined by placing two pit traps in each of the plots. The traps were emptied once a week.

4.2.2.6 Cultivar Trial

A cultivar trial was started at the same time in a different field as the third trial. The cultivars used were 'House Zucchini' (Premier Seeds), 'Season Opener' (Premier Seeds) , 'Gemma' (MacDonald's/Mayfords), 'Consul' (MacDonald's/Mayfords), 'Elite' (Stark Ayres), 'Verde', 'SQ 197' (Stark Ayres), 'SQ 228' (tolerant to ZYMV, WMV2)(Stark Ayres), 'SQ 229' (tolerant to ZYMV, WMV2)(Stark Ayres), 'Puma' (tolerant to ZYMV, WMV2)(Harris Moran Seed Company, California supplied by Hygrotech), and 'Jaguar' (tolerant to ZYMV, WMV2)(Harris Moran Seed Company, California supplied by Hygrotech). The information on the Stark Ayres cultivars was supplied by M. Burnett (pers. comm., 1999). The information on the Harris Moran Seed Company cultivars was obtained from the company catalogue.

4.2.2.7 Rating Scale

In order to judge the effectiveness of the different treatments, a rating scale was developed which gave each plant a score depending on the severity of the symptoms (Figure 4.1 - 4.5):

9: Plant severely stunted; fruit with distinct symptoms/or no fruit produced; severe mosaic; severe leaf distortion.

7: Plant stunted; distinct fruit symptoms; severe mosaic, severe distortion.

5: Plant slightly stunted; some fruit symptoms; mosaic; distortion.

3: No stunting; no fruit symptoms; mosaic; distortion.

1: No stunting; no fruit symptoms; mosaic OR distortion.

0: No symptoms.

Yield improvements were determined by picking and weighing the fruit from within the plots, and comparing the total number of fruit, individual fruit weight, and total fruit weight. Only marketable fruit was collected.

The area under the disease progress curve (AUDPC) (Berger, 1981) and two-way analysis of variance (ANOVA) were used to analyse the data obtained in the above trials. "Statsgraphics" was used to conduct the ANOVAs.

4.3 RESULTS

4.3.1 Weed Monitoring

The most common weeds found in the field in the late summer and autumn of 1997 were *Amaranthus deflexus* L. (perennial pigweed), *A. hybridus* L. subspecies *hybridus* (= *A. paniculatus*) (common pigweed), *Galinsoga parviflora* Cav. (gallant soldier; small-flowered quick weed), and *Portulaca oleracea* L. (purslane).

Weeds which were common during the spring and early summer of 1997 were *G. parviflora*, *Ipomoea purpurea* (common morning glory), and *P. oleracea* (Figure 4.6 - 4.8). Some *Amaranthus* plants were present. *Cyperus esculentus* (yellow nutsedge) (Figure 4.9) was present in some numbers. *I. purpurea* was particularly common in the second trial. In the third trial *C. esculentus* was by far the most common weed, and may have had some negative competitive effect on the crop. The volunteer cucurbits first appeared in December 1997. The viruses found to be present in these weeds is displayed in Table 2.2).

Figure 4.1. *Galinsoga parviflora* from the Bayne's Drift field.

Figure 4.2. *Ipomoea purpurea* from the Bayne's Drift field.

Figure 4.3. *Portulaca oleracea* from the Bayne's Drift field.

Figure 4.4. *Cyperus esculentus* from the Bayne's Drift field.

Some of these weeds were tested for viruses and found to be infected (See Chapter 2). In addition, a rubbish dump on the farm provided a site for volunteer cucurbits to grow (Figure 4.5). These volunteers appeared in November and December. Some of these were infected with ZYMV and WMV2 (Figure 4.6).

Figure 4.5. A rubbish dump (Bayne's Drift farm) with volunteer cucurbits growing on it.

Figure 4.6. An infected cucurbit volunteer on the rubbish dump.

Figure 4.7. The disruption of the reflective mulch by weeds in Trial 3, particularly *Cyperus esculentus*.

4.3.2 Control Methods

4.3.2.1 Companion Planting

The fennel was unable to perform adequately to be effective in the way anticipated before the trials commenced. It could not withstand the heat of summer despite the presence of sprinkler irrigation, and did not flower as it grew too slowly, thereby reducing its attractiveness to beneficial insects.

4.3.2.2 Garlic Repellant

The garlic repellant was found to be unattractive to a wide range of insects (aphids (Homoptera: Aphididae); whitefly (Homoptera: Aleyrodidae), but had little or no effect on some beneficials (assassin bugs (Heteroptera: Reduviidae); lady bird beetles (Coleoptera: Coccinellidae); robber flies (Diptera: Asilidae)), despite the insects being sprayed directly with the repellant. The fish oil-garlic combination was repellant to those insects repelled by the garlic alone, and was unattractive to crickets (Orthoptera: Gryllidae). When the fish oil was added during the second trial it was repellant to honey bees (Hymenoptera: Apidae, *Apis mellifera*) . This could reduce pollination rates within the field. Some spiders were also repelled by this combination. If an insect was disturbed by the presence of the fresh spray, it was considered to have been repelled. Neither treatment had any effect on cucumber beetles (Coleoptera: Coccinellidae) or lacebugs (Heteroptera: Tingidae). These observations were made during spraying and need further investigation.

4.3.2.3 Straw Mulch

The straw mulch was effective in reducing virus infection and increasing yield. This was particularly noticeable in the second trial. It was also effective in reducing the number of weeds present in these plots, and aided in moisture retention by the soil. The only weeds which penetrated this mulch to any great degree were *I. purpurea* and *C. esculentus*.

4.3.2.4 Reflective Mulch

This treatment was expected to be the best treatment. It was effective in reducing weed populations, although some weeds did grow underneath the mulch, particularly *I. purpurea* and *C. esculentus*. Figure 4.12 shows the effect of uncontrolled *C. esculentus* population growth can

have on this treatment.

The second trial was terminated prematurely after three weeks due to a severe hail storm which destroyed all the plants in the field. The third trial was terminated after five weeks when, due to a misunderstanding, farm workers harvested the fruit in the experimental plots. The results of the trials are shown in Tables 4.1. Disease progress curves were plotted by taking an average reading for all plots from a treatment for each rating day. Viruses identified in plants used in these trials were ZYMV and WMV2 (Trial 2) and ZYMV, WMV-M and CMV (Trial 3).

Table 4.1. The AUDPC values and rank of the treatments in Trial 2 and Trial 3.

	Trial 2		Trial 3	
Treatment	AUDPC	Rank	AUDPC	Rank
Control	32.34c[1]	5	29.67a	5
Straw Mulch	25.26a	1	28.42a	4
Reflective Mulch	25.62ab	2	24.79a	1
Garlic Repellant	28.97bc	4	25.58a	2
Fennel	27.21ab	3	N/A	N/A
Puma	N/A[2]	N/A	25.95a	3
F-ratio	6.206 **		1.331 NS	
P	0.006 **		0.3141 NS	
CV%	8.34%		13.33%	

[1] Treatments with no letters in common are significantly different.

[2] N/A = Not Applied NS = Not Significant * = Significant ** = very significant

*** = highly significant

There was no significant difference between the replicates in any of the trials (Trial 2: F = 1.416 NS, P = 0.2863 NS; Trial 3: F = 1.123 NS, P = 0.3786 NS). This shows that there was no blocks effect.

4.3.2.5 Cultivar Trial

The cultivar trial was terminated after six weeks. The results of this trial are shown in Tables 4.2.

The cultivar 'Verde' had very poor germination and was not included in the further analysis of the cultivar trial. The following viruses were identified in plants from the trial site: ZYMV (Season Opener, SQ197, SQ228), WMV2 (Consul, Gemma, Puma, Season Opener, SQ228) and WMV-M (House Zucchini, SQ197).

Table 4.2. The AUDPC means and rank of the treatments used in the Cultivar Trial.

Cultivar	AUDPC	Rank
House Zucchini	66.24cde[1]	7
Season Opener	91.19f	10
Gemma	58.25abcd	4
Consul	77.93ef	9
Elite	62.74bcd	6
SQ 197	59.60abcd	5
SQ 229	47.62a	1
SQ 228	52.14abc	3
Puma	51.24ab	2
Jaguar	68.76de	8
F-ratio	6.713 ***	
P	0.0001 ***	
CV	16.11%	

[1] Values which do not share a common letter are significantly different.

NS = Not Significant * = Significant ** = very significant *** = highly significant

There was no significant difference between the replicates (F = 0.961 NS; P = 0.4252 NS), showing that there was no blocks effect. Graphic comparison based on the AUDPC values of the different treatments in the three trials is shown in Figures 4.8 to 4.10.

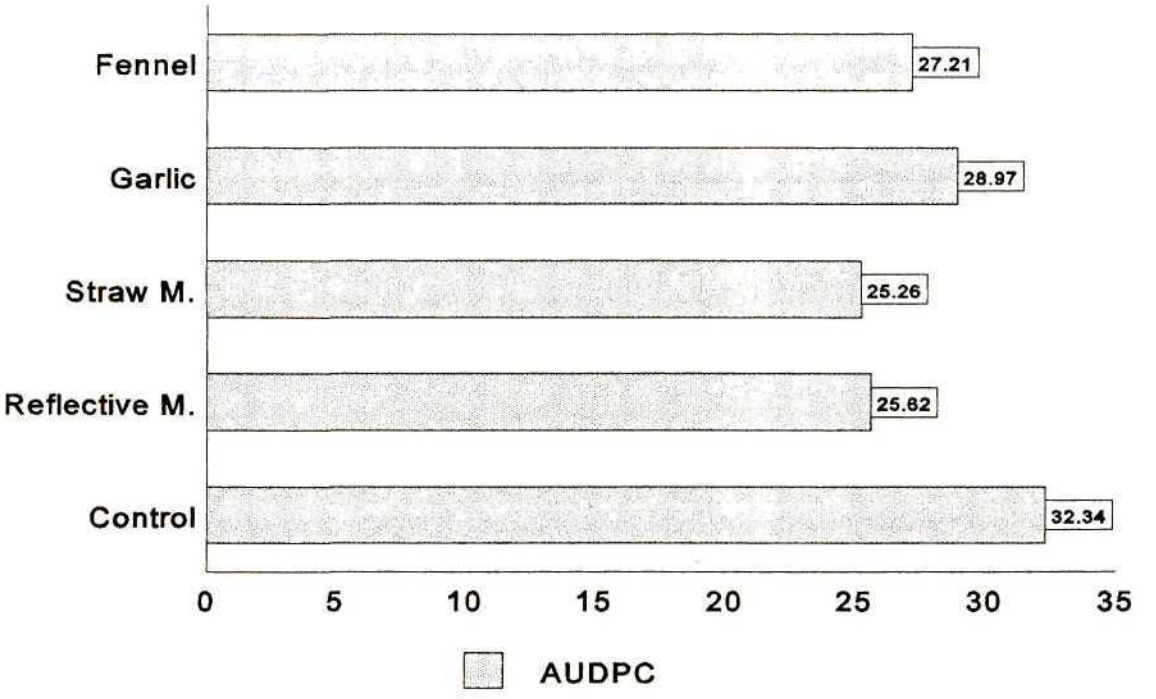

Figure 4.8. The AUDPC values of the treatments used in Trial 2.

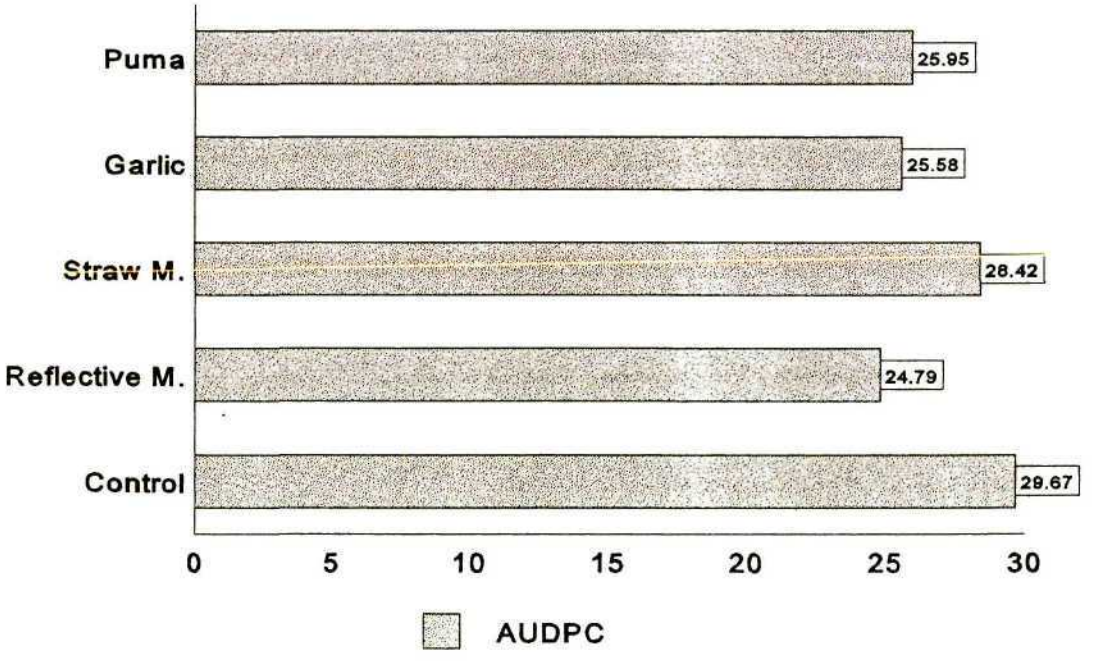

Figure 4.9. The AUDPC values of the treatments used in Trial 3.

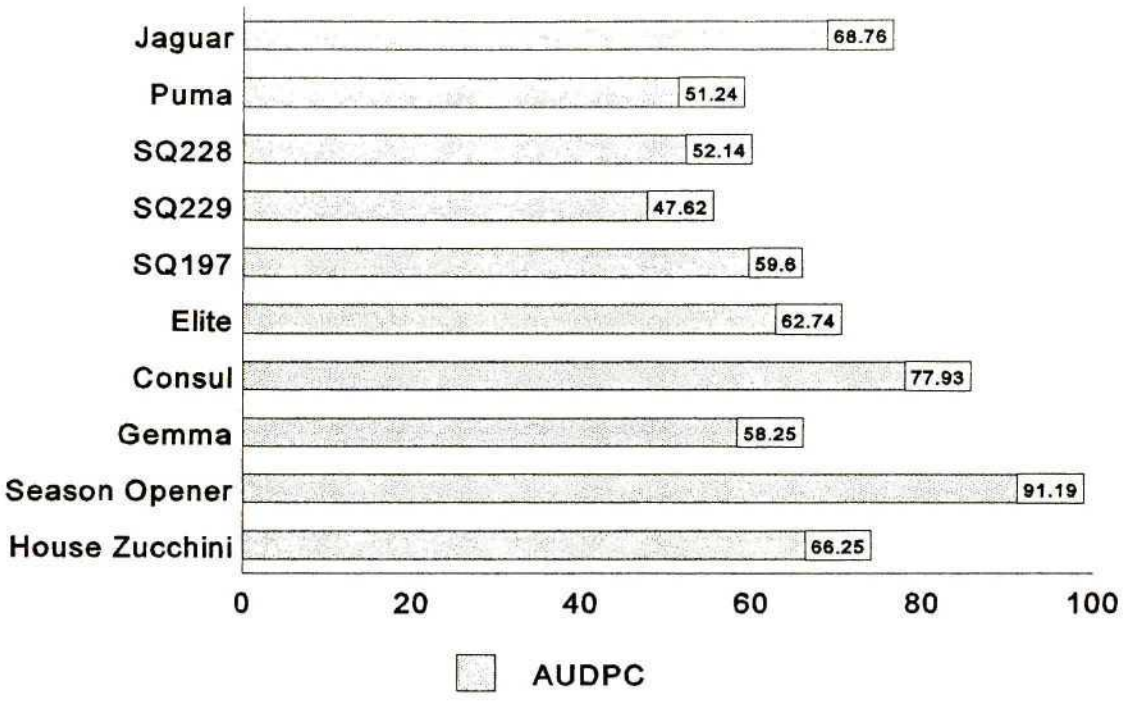

Figure 4.10. The AUDPC values of the cultivars used in the Cultivar Trial.

Although very little yield data could be obtained due to the reasons mentioned above, some trends were visible from what data were obtained. These trends are shown in Figures 4.11 to 4.13.

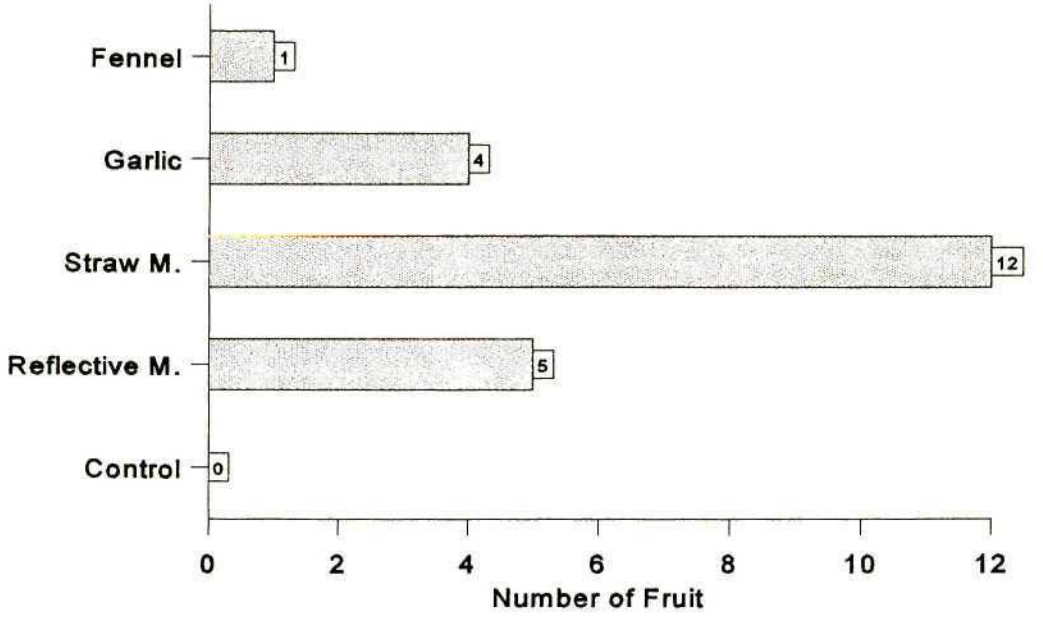

Figure 4.11. The number of fruit produced in the different treatments in Trial 2.

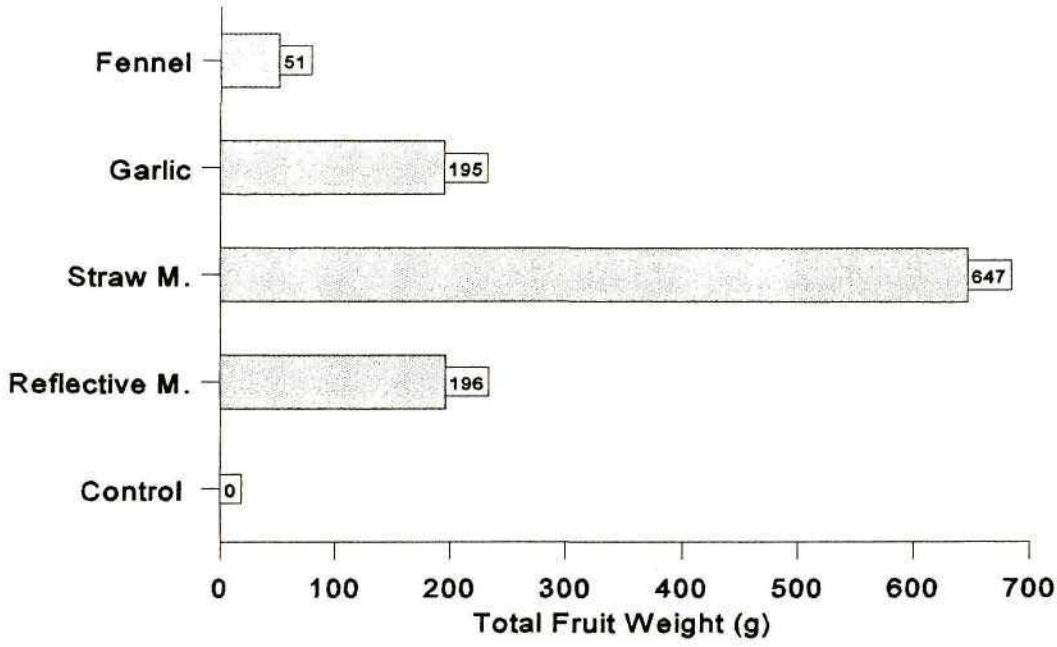

Figure 4.12. The total weight of the fruit produced in the different treatments in Trial 2.

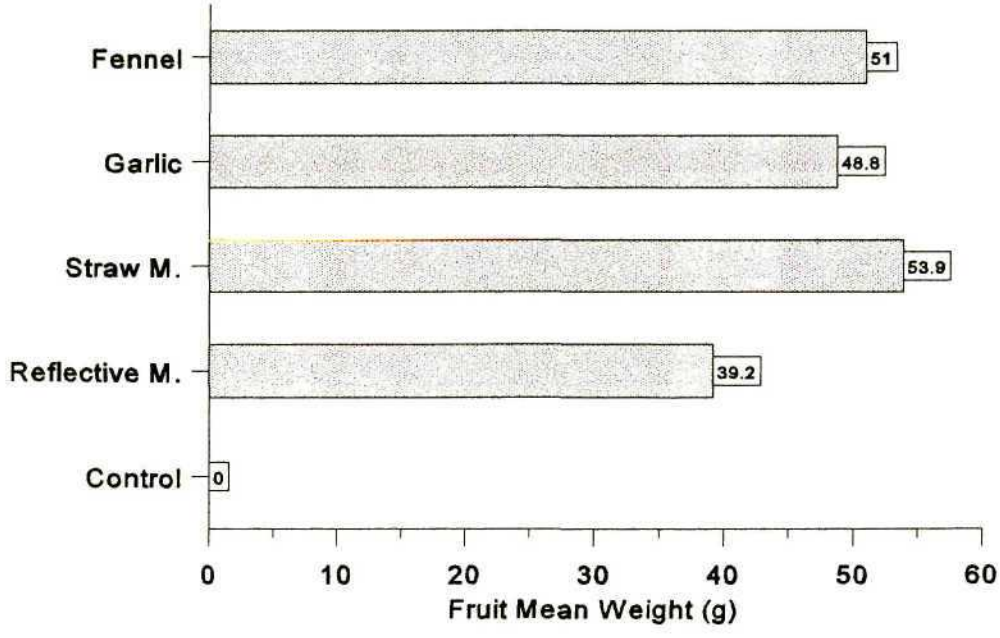

Figure 4.13. The mean weight of the individual fruit produced in each treatment in Trial 2.

The effect of the different treatments on the number of predators present in the plots in Trial 1 is shown in Figures 4.14 to 4.16.

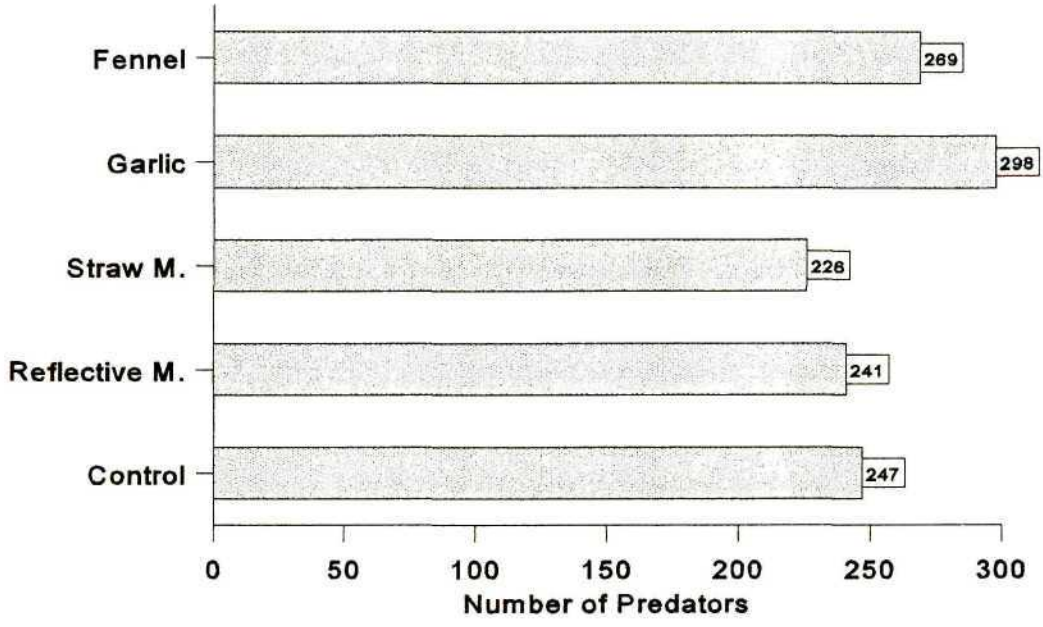

Figure 4.14. The total number of predators caught in the plots during the course of Trial 1.

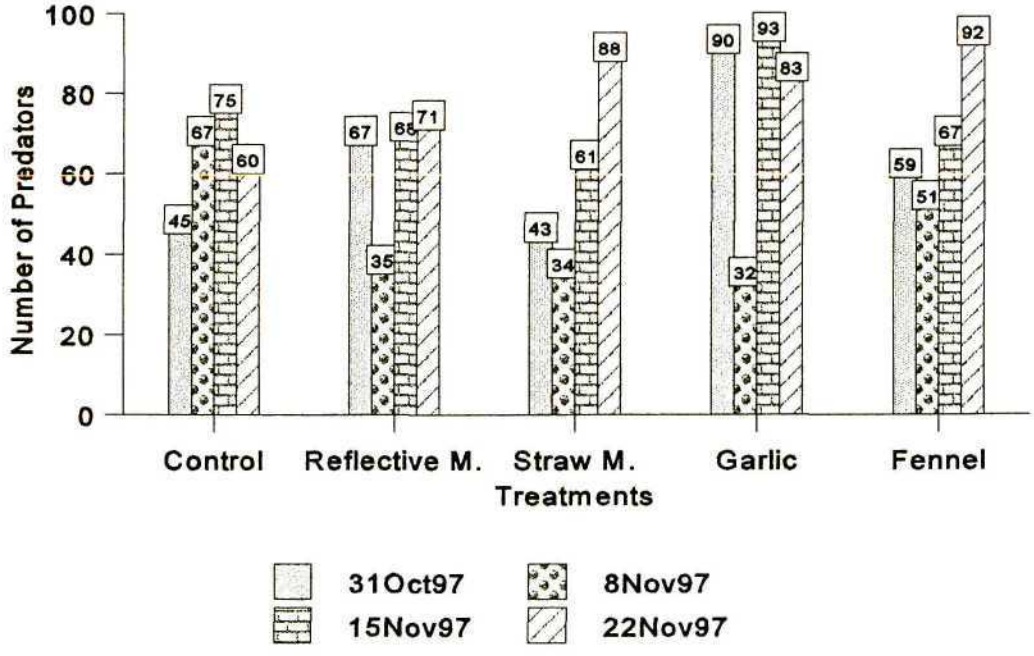

Figure 4.15. The change in predator density with time in the different treatments in Trial 1.

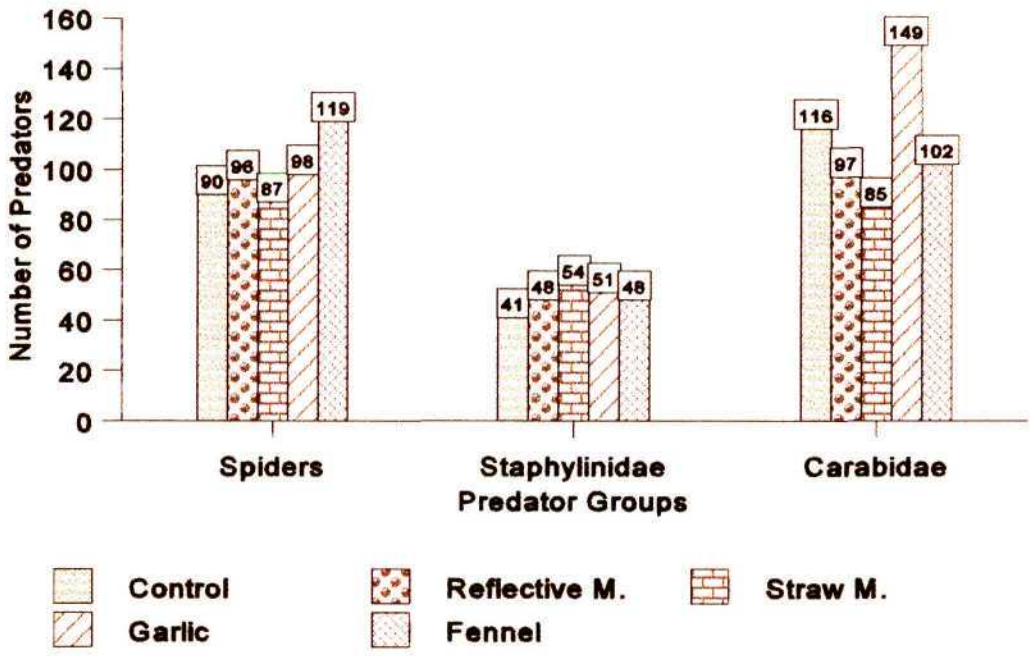

Figure 4.16. The effect of the treatments on the predator groups investigated (M = Mulch).

The progress of disease in Trial 2 and Trial 3 is shown in Figures 4.17 - 4.18. The progress of disease in the cultivar trial is shown in Figure 4.19.

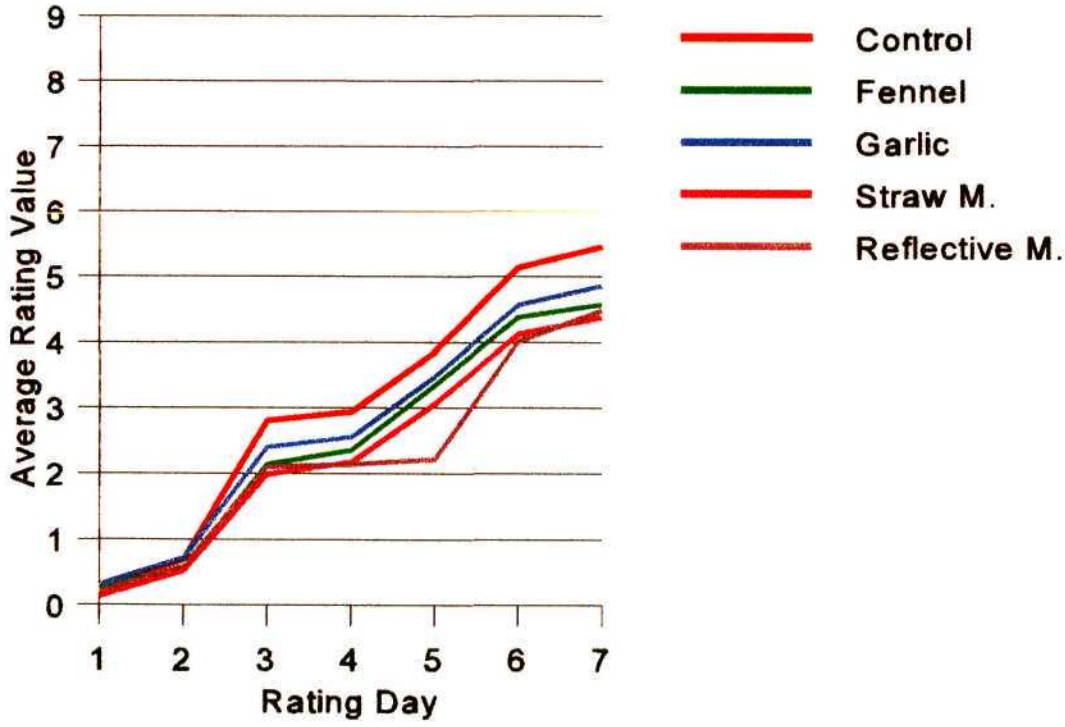

Figure 4.17. The progress of disease in Trial 2 (Bayne's Drift farm) (M = Mulch).

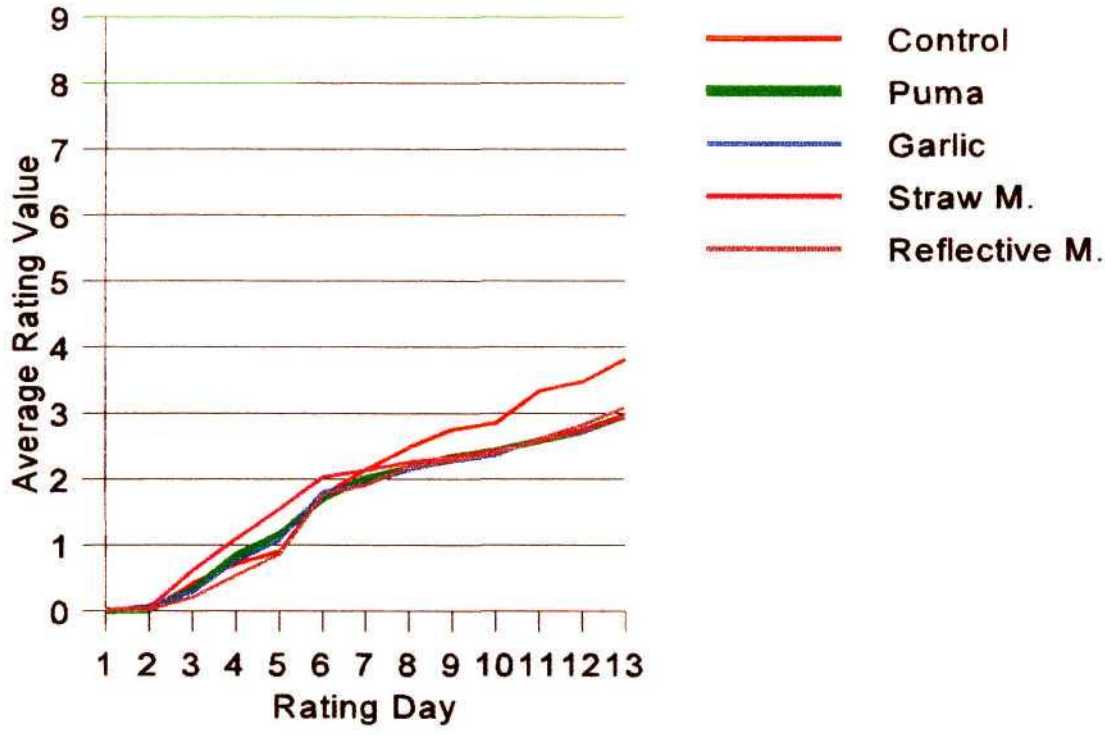

Figure 4.18. The progress of disease in Trial 3 (Bayne's Drift farm) (M = Mulch).

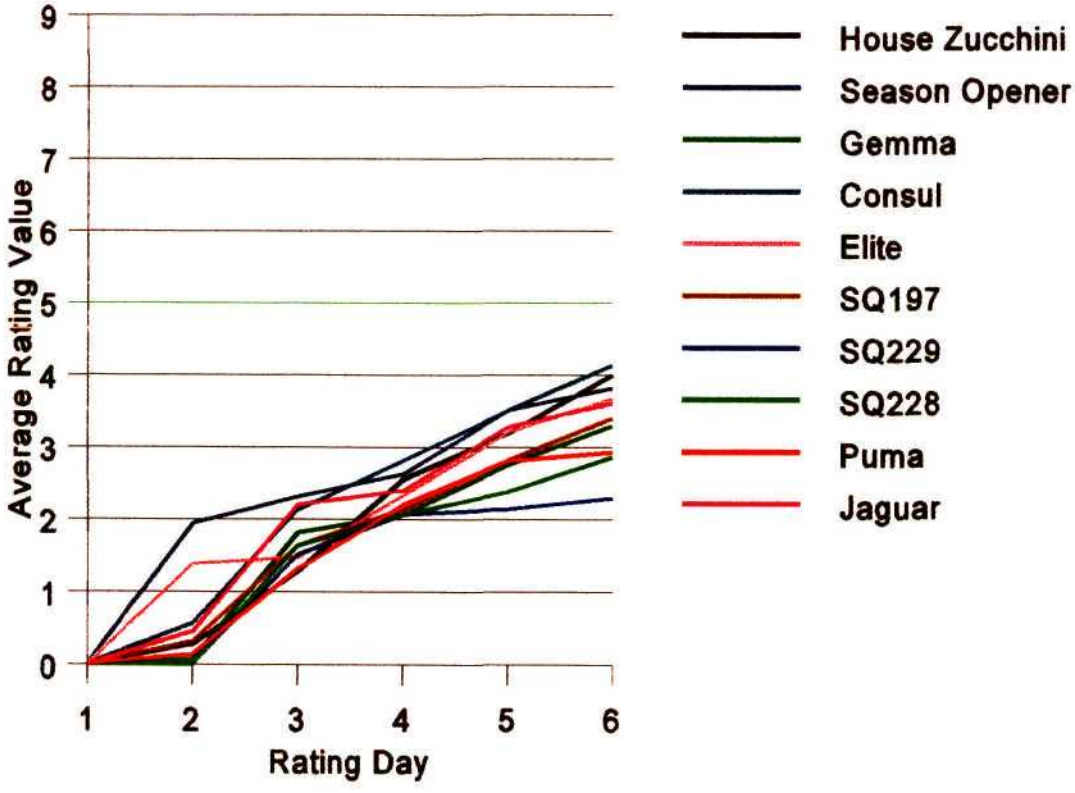

Figure 4.19. The progress of disease in the cultivar trial (Bayne's Drift farm).

4.4 DISCUSSION

The presence of weeds in the fields can have a number of different effects on the crop. In the first place they can compete directly with the crop plants for light, water and nutrients, as well as releasing chemicals into the soil which inhibit the growth of other plants. Of the weeds commonly recorded from the research plots, *Cyperus esculentus* (Figure 4.4) was the most important species in this regard. Secondly, weed species can act as reservoirs for aphids and other pests, and as local sources of virus diseases (Figure 4.6). See Chapter 2 for discussion on the importance of weeds in this context. Weed species were responsible for disrupting the reflective mulch treatment (Figure 4.7) in Trial 3, thus reducing its effectiveness. Only vigourous species such as *C. esculentus* and *Ipomoea purpurea* were a serious problem. A potentially positive aspect of having weeds in the field is that they mask the crop plants from passing aphids, especially when the plants are young. A similar effect can be achieved by increasing the planting density of the crop(Zitter & Simons, 1980). In general, the negative aspects of weeds out-weigh the potential benefits of their presence.

There was a rubbish dump on the farm where unwanted plant material was placed. Volunteer cucurbits were growing on this dump (Figure 4.5). Some of these exhibited symptoms of virus infection (Figure 4.6). Plants such as these act as sources of virus throughout the season unless they are removed. These plants are killed by frost each winter. The presence of infected volunteers can negate the potential benefits derived from virus control measures practised in the fields (Duffus, 1971; Zitter, 1977; Zitter & Simons, 1980; Maelzer, 1986). Roguing of infected plants from within a field would probably be of little use in reducing virus spread within the field, as the plants will have been infectious for a few days prior to symptom expression. The ineffectiveness of this technique when dealing with non-persistent virus transmission is covered by Thresh (1976), Zitter (1977), Sylvester (1989) and Jones (1991).

In Trial 2 all treatments performed better than the control, although only the fennel, white reflective mulch and the straw mulch were significantly different from the control (Table 4.1, Figure 4.8, 4.17). As the fennel did not perform in the manner expected (i.e. attracting beneficial arthropods), the improved performance of the zucchini plants could have been due to a commensal relationship between the two plant species. Despite this effect, the use of fennel as

a companion plant in zucchini crops cannot be recommended due to its slow rate of development compared to that of *C. pepo*, and its intolerance of field conditions during the growing season. Ideally, a companion plant should have a similar rate of development to that of the main crop. This would make farm management easier. A further potential negative aspect is that the presence of *F. vulgare* in a field may serve as a source of one of the potyviruses. Recently a strain of potato potyvirus Y (PVY-0) was found in fennel plants growing as weeds (Espino de Paz *et al.*, 1997). No reference was found which indicated that fennel could act as a host for any of the viruses involved in this study.

The failure of the garlic repellant in reducing disease incidence is probably due to the use of sprinkler irrigation rather than drip irrigation. The crop had to be irrigated regularly due to the high temperatures (>35°C) experienced at this time of year. There was also a great deal of convectional rainfall at this time. A combination of the rainfall and irrigation would have washed the repellant from the leaves of the crop, reducing or eliminating its beneficial effect. The use of an adjuvant which increases adhesion of the repellant to the leaves may correct this problem. An advantage of using the garlic repellant is that it appears to have little or no effect on beneficial insects. Indeed, with ground beetles (Coleoptera: Carabidae) there seems to be an attractant effect (Figure 4.16). The addition of the fish oil to the garlic extract, while being more repellant to homopterans (aphids and whiteflies), seems to make it repellant to some beneficial arthropods such as spiders and honey bees. This could lead to other pest problems and reduced pollination of flowers which would ultimately reduce the yield. Further studies on the effect of the garlic repellant on various arthropod groups need to be conducted, as the effects reported here are from casual observations only.

Although the straw mulch ranked higher than the white reflective mulch, the two treatments were very similar in their performance in reducing disease (Table 4.1, Figure 4.17). This action is due to the reduced number of aphids in these plots (see Chapter 3). By reducing the number of aphids in an area the chance of infection of the crop with one or more of the viruses investigated is reduced. The reduced weed growth and water loss from these plots reduced both competition and water stress, thus resulting in healthier plants. The disruption of the white reflective mulch by *C. esculentus* (Figure 4.7) could be reduced or eliminated by the use of herbicides before

planting. These two treatments could be successfully implemented to reduce virus disease. The white reflective mulch could be better used by large scale producers (due to the cost of the mulch), while the straw mulch could be used by the resource poor farmer (due to its ease of availability). An advantage of the Knittex Shadenet® as a reflective mulch is that it is durable and can be used for more than one crop, unlike many of the conventional plastic reflective mulches. The net structure as opposed to the solid plastic allows some evaporation of water which could reduce potential waterlogging, although it is this structure which allows for the growth of weeds. The use of a greater shade factor may reduce this problem. A potential negative aspect of the straw mulch is that it can encourage infection by *Rhizoctonia* (H.A.J. Hoitink, 1998, *Opportunities for control of plant diseases with composts*, presentation at International Congress of Plant Pathology, Edinburgh). The effectiveness of the reflective materials confirms the findings of Daiber and Donaldson (1976), Eulitz (1977), and other studies that have found reflective mulches to be effective in reducing aphid-borne virus disease in cucurbit crops.

In the third trial there was no significant difference between the treatments, although all treatments performed better than the control (Table 4.1, Figure 4.9, 4.18). The failure of the straw mulch to produce quality plants was probably due to the high degree of water retention in these plots which stressed the plants. It may also have led to root diseases which would have reduced the vigour of the plants. The performance of the cultivar 'Puma' is encouraging as this virus resistant cultivar is available to farmers and appears to offer some benefit in virus disease control.

No insecticide treatments were used in these trials as their use has previously been shown to have no benefit where stylet-borne viruses are involved in the pathosystem (Budnik, 1995). The use of these chemicals can increase the rate of virus spread within a field (Swenson, 1968; Zitter & Simons, 1980; Maelzer, 1986; Gibson & Rice, 1989; Pinese et al., 1994; Budnik, 1995; Yuan & Ullman, 1996; Desbiez & Lecoq, 1997) and eliminate beneficial insects (Brown & Stephenson, 1990)

The cultivar trial was conducted concurrently with Trial 3, a time of high disease pressure. The performance of the cultivars is shown in Table 4.2 and Figures 4.10 and 4.19. The cultivar 'Elite'

can be regarded as the control treatment, as this cultivar is widely used in the province. 'Puma' is being recommended as a virus tolerant cultivar. This trial indicates that it performs well under pressure. 'Gemma', which is not known to possess any virus tolerance or resistance qualities, also performed well. 'SQ 229', which performed the best, was withdrawn as an available cultivar by the seed company for unknown reasons. 'SQ 228', which ranked third has similarly been withdrawn. As 'Puma' performed as an intermediate between these two cultivars, a cultivar with improved performance over 'Elite' is available to farmers. 'Jaguar' is reported to have resistance to virus diseases, but failed to perform well in this trial, ranking eighth over all. The use of a virus resistant or tolerant cultivar in the latter half of the growing season (December - April) is essential if a crop of any size is to be obtained. This is due to the large population of aphids (see Chapter 3) and the presence of potentially infected cucurbits occurring in close proximity to the zucchini fields. These potential sources of virus may be other cucurbit crops, old zucchini fields or volunteers. There is also the possibility of cucurbit crops with transgenic resistance being the source of infection for other crops, especially where transcapsidation (Bourdin & Lecoq, 1991) is a possibility. It is interesting to note that 'Puma' and 'SQ228' still performed well, despite being infected by these viruses. This indicates that the tolerance is durable under high disease pressure. 'Jaguar' and 'SQ229' were infected by a virus, but not one that was involved in this study. The performance of 'Jaguar' indicates that it is susceptible to the virus responsible, while 'SQ229' appears to have some level of tolerance to it. It must always be remembered that resistance or tolerance that has been developed in one area may not hold in other areas.

Misunderstandings with the farm labourers led to the fruit from the trial plots being harvested and included with the fruit from the rest of the field in Trials 1 and 3. The collection of yield data in Trial 2 was terminated when the field was destroyed by a severe hail storm. The data collected is shown in figures 4.11 to 4.13. This data could not be statistically analysed, and only gives an indication as to the effectiveness of the treatments in improving yield during high virus disease pressure. The straw mulch appears to out perform all the other treatments, but there were a number of fruit in the mulch and garlic plots nearing harvestable size when the trial was destroyed. The straw mulch did, however, produce the first harvestable fruit. There was some fruit in the control plots, but these were so distorted and discoloured due to virus infection that they were unmarketable, and as such were not harvested. Very few fruit would probably have been

harvested from the fennel plots due to a high level of symptom expression in the zucchini plants. From these results, the use of either a straw mulch or a reflective mulch and/or the garlic repellant would improve the yield from zucchini fields. More research is required, however, to confirm these results. Although not specifically investigated in this study, the spread of viruses within the field may be reduced if the pickers clean their knife blades with a 1:9 solution of sodium hypochlorite between plants. This could be an important means of mid to late crop spread as all the viruses considered in this study can be mechanically transmitted (Purcifull et al., 1984; Francki & Habili, 1990; Purcifull, 1990; Desbiez & Lecoq, 1987).

The effects of the treatments on beneficial arthropods (predators only) provided some interesting results. No one treatment was preferred over another by the rove beetles (Coleoptera: Staphylinidae), although slightly more specimens were captured in the straw plots. The straw mulch was expected to have the highest numbers of predatory arthropods. This was found not to be the case (Figure 4.14). The reasons for this are varied. Firstly, pieces of straw were constantly falling into the pit traps, providing escape routes for specimens falling into the traps. Secondly, the straw mulch reduced the rate of evaporation of water from the ground. The water moved into the holes made for the traps and forced the traps out of the ground. This prevented specimens from falling into the traps.

The reflective mulch performed similarly to the control as far as total numbers of predators are concerned (Figure 4.14). This is probably due to the reflective mulch and control treatments offering similar habitats and opportunities for predators. Some insects may use the mulch to shelter from unfavourable environmental conditions (e.g. high temperatures). As the reflective mulch repels insects (i.e. potential prey), this treatment could be expected to have a much reduced predator population, although this was not the case in this study.

The plots where fennel was used as a companion plant had the second highest number of predatory arthropods (Figure 4.14). This may be due to a number of factors. The addition of plants to the plots increased the structural diversity which would increase the attractiveness of these areas to beneficial arthropods (Coll & Bottrell, 1996). Fennel has its own pests, thus increasing the diversity of available prey in these plots. It is possible that a chemical is released

from the fennel plants which attracts predators to the area. Spiders were especially attracted to the fennel plots (Figure 4.16).

The plots where the garlic repellent was used had the greatest number of predatory arthropods (Figure 4.14). This was due to the large number of ground beetles (Carabidae) (Figure 4.16) captured in these plots. It is possible that some component in the garlic repellent was attractive to these insects. As the garlic repellent was also found to reduce aphid numbers (Chapter 3), its attractiveness to beneficial arthropods enhances its value as a crop treatment. Its appeal to some predators may play a role in its effectiveness as an insect control method.

During the course of the trial, predator numbers were found to remain essentially static in the garlic repellent and reflective mulch plots. In the plots where companion planting and the straw mulch were used, the number of predators steadily increased during the trial. In these four treatments there was a decline between the first predator count (31 October 1997) and the second count (8 November 1997) (Figure 4.15). At the same time there was an increase in the number of predators in the control plots. The reasons for this are not known. The numbers of predators in the control plots increased during the early and middle part of the trial, and then declined slightly towards the end. The slight decline in the control and garlic repellent plots could be due to mechanical weed control during the week prior to the final count, which would have destroyed the available habitats within the field. This had no effect on the reflective mulch and straw mulch plots as no weed control was required in these plots. It is not known why there was no effect on the fennel plots.

From these results the following recommendations can be made: all cucurbit crops should be grown over a white reflective mulch; drip irrigation should be used instead of sprinkler irrigation as the latter may increase aphid movement within the field; a virus resistant or tolerant cultivar should be planted, especially in the latter part of the growing season. Old cucurbit crops and volunteers should be ploughed in or pulled out and disposed of well away from fresh plantings.

4.5 REFERENCES

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CHAPTER 5: CONCLUSION

This study has shown that there is a variation in the distribution of the viruses considered. This information could be used by seed companies when breeding for resistance to specific viruses. For example, a cultivar with resistance to ZYMV and WMV2 would be more useful than a cultivar with resistance to WMV-M and CMV.

Aphid (vector) numbers can be effectively monitored using coloured sticky traps, although the specimens are generally in a poor condition and are useless for taxonomic and morphological studies. By monitoring the number of aphids in a plot, the effectiveness of the treatment in that plot in reducing vector numbers can be assessed. By reducing the number of vectors in a plot, there is a reduced chance or a delay in the introduction of viruses into the plot. This reduces the overall severity of disease in the plot, thus improving the crop yield and quality. Although no yield data was obtained from this study, an improvement in overall plant quality was noticed in some treatments. The plots with the best plants were generally also the plots with the lowest aphid numbers. Thus the potential of a treatment in improving plant quality by reducing the rate of infection by viruses can be shown by the number of aphids (vectors) trapped relative to the number of aphids caught in the control plots.

The two best treatments which showed potential for application on farms were the straw mulch and the reflective mulch. The straw mulch would be most easily applied by small scale resource poor farmers, whilst the reflective mulch could best be applied by large scale producers. The reasons for this division are the cost, availability and labour requirements of the materials. Due to the expense and effort required to implement the mulch treatments, it is likely that it is only useful to use mulches when the chance of infection is high, i.e. December to April.

As the weed species found to act as hosts were widespread in the areas surrounding the fields it would be difficult to control them completely. Volunteer cucurbits, however, tend to occur on the edges of fields and on rubbish dumps. These could be easily controlled either by herbicides or mechanical removal.

Further study is required to determine which aphid species are present in cucurbit crops, and which of these are acting as vectors. Other control techniques which require investigation are companion planting with a grain such as maize, which is commonly practised by resource poor farmers; the use of floating row covers during the early part of crop growth; the use of different plug sizes when growing seedlings in a nursery in preparation for planting in the field. The effectiveness and potential problems of the use of transgenic cultivars also needs to be investigated.

APPENDIX I

Table 1. A list of cucurbits with both the common names and botanical equivalents taken from Robinson & Decker-Walters (1997) and Rubatzky & Yamaguchi (1997).

SCIENTIFIC NAME	COMMON NAME
Acanthosicyos horridus	!nara
Actinostemma tenerum	He-zi-cao
Benincasa hispida	Wax gourd ; Winter melon
Bolbostemma paniculatum	Pseudo-fritillary
Bryonia spp.	Bryony
Citrullus colocynthis	Colocynth ; Egusi
Citrullus lanatus	Watermelon
Citrullus lanatus var. *citroides*	Citron ; Egusi ; Preserving melon
Coccinia grandis	Ivy gourd
Cucumeropsis mannii	White-seeded melon ; Egusi
Cucumis anguria	Bur gherkin ; West Indian gherkin
Cucumis dipsaceus	Teasel gourd
Cucumis melo	Melon
Cucumis metuliferous	African horned cucumber
Cucumis sativus	Cucumber
Cucurbita argyrosperma	Squash ; Pumpkin
Cucurbita ficifolia	Malabar gourd ; Fig leaf gourd
Cucurbita foetidissima	Buffalo gourd
Cucurbita maxima	Squash; Pumpkin
Cucurbita moschata	Squash ; Pumpkin; Butternut
Cucurbita pepo	Squash ; Pumpkin ; Gourd
Cyclanthera pedata	Caihua ; Stuffing cucumber
Diplocyclos palmatus	Lollipop climber
Ecballium elaterium	Squirting cucumber
Echinocystis lobata	Wild cucumber
Fevillea cordifolia	Antidote vine
Gynostemma pentaphyllum	Jiao-gu-lan
Hemsleya amabilis	Luo-guo-di
Hodgsonia macrocarpa	Lard plant
Lagenaria siceraria	Bottle gourd

Table 1. Cont.

SCIENTIFIC NAME	COMMON NAME
Luffa acutangula	Angled loofah
Luffa aegyptiaca (L. cylindrica)	Smooth loofah
Momordica angustisepala	Sponge plant
Momordica charantia	Bitter melon ; Balsam pear
Momordica cochinchinensis	Sweet gourd
Momordica dioica	Kaksa
Praecitrullus fistulosus	Round melon ; Tinda
Sechium edule	Chayote
Sicana odorifera	Casabanana
Siraitia grosvenorii	Luo-han-guo
Telfairia occidentalis	Fluted pumpkin
Telfairia pedata	Oyster nut
Thladiantha dubia	Red hail stone
Trichosanthes cucumerina	Snake gourd
Trichosanthes dioica	Pointed gourd
Trichosanthes kirilowii	Chinese snake gourd
Trichosanthes lepiniana	Indreni
Trichosanthes ovigera	Japanese snake gourd
Trichosanthes villosa	Mi-mao-gua-lou

APPENDIX II

Table 1. Bacterial diseases of cucurbits (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

DISEASE	CAUSAL ORGANISM	SPREAD/SYMPTOMS	CROP	CONTROL
Bacterial Wilt	*Erwinia tracheiphila*	Spread by and overwinters in cucumber beetles. Infected leaves develop a dull green area at the site of infection, & a leaf/branch/whole plant suddenly wilts & dies.	Cucumber; Melon; occasionally Watermelon	Control the beetles. Genetic control in cucumbers with the non-bitter allele (*bi*).
Bacterial Rind Necrosis	*Erwinia carnegieana*	The rind of melon & watermelon develop brown necrotic areas.	Melon; Watermelon	Genetic resistance.
Soft Rot	*Erwinia carotovora*	The fruit has water-soaked blotches which become a wet soft rot & the fruit collapses.	Cucumber; Melon	Avoid fruit injury. Chlorinated fruit sprays/dips.
Brown Spot	*Erwinia ananas*	Yellow-brown spots measuring up to 4cm in diameter on the fruit.	Melon ('Honey Dew' & related cultivars)	Avoid fruit injury.
Bacterial Leaf Spot	*Xanthomonas campestris* pv. *cucurbitae*	Leaves have small (2-4mm) brown lesions surrounded by a yellow halo. Diseased fruit rots.	Squash; Cucumber; occasionally other species.	Prevent splash. Seed treatments. Remove infected plants from the field. Genetic resistance in *C. moschata* & *C. okeechobeensis*.
Angular Leaf Spot	*Pseudomonas syringae* pv. *lachrymans*	Water-soaked lesions on all plant parts - those on the leaf drop out ; those on the fruit are white to tan & may crack.	Cucumber; Melon; Squash; Watermelon	Disease-free seeds. Seed treatments. Crop rotation control *Aulacophora femoralis* (cucumber leaf beetle). Keep plants dry. Prevent injury. Polygenic resistance in cucumber.
Bacterial Fruit Blotch	*Acidovorax avenae* ssp. *citrulli*	Lesions on the foliage & stems are water-soaked, become necrotic with yellow margins. Lesions on the fruit crack & have exudation.	Watermelon (only in the USA)	Copper-based foliar sprays. Clean seed. Testing of seed lots before planting. Avoid rotations with alternate hosts (citron, melon, eggplant). Triploid cultivars are more resistant than diploids.

Table 2. Fungal diseases of cucurbits (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

DISEASE	CAUSAL ORGANISM	SPREAD/SYMPTOMS	CROP	CONTROL
Damping Off	*Pythium* spp. *Phytophthora* spp. *Rhizoctonia solani* *Fusarium* *Acremonium* spp. *Thielaviopsis*	Poor germination. Seedling death. Root rot in older plants. Roots are water-soaked & flaccid. Foliage wilts.	All species.	Seed treatment with fungicide. Sterilization of growing media & plant containers. Keep foliage dry. Shallow planting. Rotation.
Target Leaf Spot	*Corynespora cassicola*	Leaves have yellow & necrotic lesions & spots. These lesions may drop out. Young fruits which are infected shrivel.	Cucumber & others.	Remove infected material. Application of fungicides. Genetic resistance in cucumber.
Alternaria Leaf Blight	*Alternaria cucumerina*	The leaves have light brown spots which enlarge leading to defoliation. If the fruit becomes infected, round, sunken, black/brown lesions form.	Winter squash; Melon; Cucumber; Watermelon; & others.	Crop rotation/deep ploughing/Removal of infected plant debris. Avoid overhead irrigation. Fungicides. Some melon & Watermelon cultivars are resistant.
Anthracnose	*Colletotrichum orbicularae*	Leaves have water-soaked areas which develop into tan cankers. Stem lesions may result in wilting & plant death. Fruits develop sunken black spots.	Watermelon; Cucumber; Melon; Squash; Bottle Gourd; Fluted Pumpkin; Pointed Gourd; Ivy Gourd; Chayote; Snake Gourd.	Sanitation. Rotation. Clean seed. Seed treatment (hot water). Fungicides. Low temperature storage of fruit. Genetic resistance.
Cercospora Leaf Spot	*Cercospora citrullina*	Leaf lesions which may lead to defoliation.	Watermelon; Melon; Cucumber; Fluted Pumpkin; & others.	Sanitation. A good fungicide programme.
Powdery Mildew	*Sphaerotheca fuliginea* *Erysiphe cichoracearum*	Circular white spots on the leaf surface which expand & coalesce. Both leaf surfaces are affected. The leaves die prematurely.	All species.	Sulfur treatment. Protectant fungicides. The pathogen is resistant to benomyl & triadilmefon (systemic fungicides). Genetic resistance.
Downy Mildew	*Pseodoperonospora cubensis*	Produces a 'checkerboard' effect on the upper leaf surface. There is black sporulation from the lower leaf surface. Defoliation.	Most species.	Fungicides (variation is important to avoid resistance). Keep foliage dry. Genetic resistance.
Phytophthora Root & Crown Rots	*Phytophthora capsici* & other species	Rapid wilt & plant death. The crown may have black lesions. Roots are water-soaked & have a black/brown rot.	All species.	Good drainage. Keep the foliage dry. Fungicides.
Belly Rot	*Rhizoctonia solani*	Water-soaked lesions on fruit where they touch the soil.	Cucumber; Melon.	Roguing. Avoid excessive irrigation. Soil fumigation. Genetic resistance.
Gummy Stem Blight & Black Rot	*Didymella bryoniae* (sexual stage) *Phoma cucurbitacearum* (asexual stage)	The leaves exhibit a brown, blotchy blight with black spots. Fruits become discoloured & may rot.	Watermelon; Cucumber; Melon; Squash; Bitter Melon; Wax Gourd; Chayote; & others.	Prevent fruit injury. Disease-free seed. Roguing. Rotation. Keep foliage dry. Fungicides (resistance to benzimidazoles). Genetic resistance.

Table 2 cont.

DISEASE	CAUSAL ORGANISM	SPREAD/SYMPTOMS	CROP	CONTROL
Fusarium Wilt	*Fusarium oxysporum* f.sp. *melonis*, *niveum*, *cucumerinum*	Sudden severe wilt.	Melon; Watermelon; Cucumber.	Long rotations (4-10yrs). Raise soil pH to 6.5. Use disease-free seed.
Verticillium Wilt	*Verticillium dahliae* *V. albo-atrum*	Wilting in nearly mature plants. Chlorosis & necrosis of crown leaves.	Melon; Squash; Cucumber; & others.	Do not plant in fields with a history of this disease.
Sudden Wilt	*Pythium ultimatum* & related species, & CMV	Abrupt plant collapse. Frequently occurs after cold nights.	Melon.	
Cottony Soft Rot	*Sclerotinia sclerotiorum*	White, cottony growth on the stem, leaves & fruit. Black sclerotia may be embedded in the cottony growth.	Many species.	Sanitation. Fungicides. Careful irrigating & harvesting. Good storage conditions (10-15°C ; 50-75% RH). Roguing infected fruit.
Southern Blight	*Sclerotium rolfsii*	Plants wilt & turn yellow. White fungal growth with brown sclerotia develop on the stem & fruit.	Melon; Squash; Watermelon.	Rotation. Sanitation. Raising soil pH to 7.0. fungicides.
Gray Mould	*Botrytis cineraria*	Mycelia & spores develop on the blossom end of the fruit causing rotting. Brown lesions develop on the leaves which become covered with grey spores.	Cucumber; Melon; Squash.	Good ventilation. Warm temperature. Avoid free moisture (in glasshouse).

Table 3. Pests of cucurbits (Robinson & Decker-Walters, 1997; Rubatzky & Yamaguchi, 1997).

PEST	NAME	DAMAGE	CROP	CONTROL
Cucumber Beetles	*Acalymma vittatum* *Diabrotica* spp.	Root damage. Eating holes in cotyledons & leaves. Spreading diseases (bacterial wilt ; SqMV).	All species	Insecticides. Cultivars with low concentrations of cucurbitacins.
Epilachna Beetles	*Epilachna* spp.	Defoliation & fruit & stem boring.	Watermelon; Ivy gourd; Summer squash; Cucumber;	Insecticides.
Squash Vine Borer	*Melittia cucurbitae*	Stem collapse, wilting, plant death.	Mainly Squash Also Melon; Cucumber; Watermelon	Insecticides. Rotation.
Melon Fruit Fly	*Dacus cucurbitae*	Fruit damage leading to rotting.	Cucumber; Melon; Squash; Watermelon & others.	Covering of developing fruit. Cultivars with thicker rinds & higher silica content of the rinds.
Oriental Fruit Fly	*Dacus dorsalis*	As above.	All species.	Poisoned baits. Roguing infested fruit. Sterilized males. Cultivation to destroy soilborne pupae.
Wireworms	Elateridae e.g *Limonius canus*	Root damage	All species.	
Thrips	*Heliothrips femoralis* *Frankliniella occidentalis* & others	Silvery areas on the leaves with black necrotic spots & downward curling margins. Vector tospoviruses.	Cucumber; Melon & others.	Weed control. Insecticides.
Leafminer	*Liriomyza* spp.	Physical leaf damage which increases susceptibility to fungal infection. May spread viruses.	All species.	Natural enemies. Resistance.
Leafhoppers	*Empoasca abrupta* *Empoasca fabae* & others	Vector aster yellows.	All species.	Insecticides. Light traps.
Cutworms	*Agrotis* spp. *Proxenus mindara* *Feltia subterranea*	Feed on plant stems.	All species.	Baits. Insecticides.
Glasshouse Whitefly	*Trialeurodes vaporariorum*	Stunting. Cause of sooty mould. Vectors viruses.	Cucumber; Melon.	Biological control (*Encarsia formosa*). Insecticides. Yellow sticky traps.
Sweet Potato Whitefly	*Bemisia tabaci*	Transmits viruses. Silvering of the leaves.	Squash; Melon.	Insecticides. Yellow sticky traps.
Leaf-footed Bug	*Leptoglossus australis*	Dark spots on the fruit. Premature fruit drop. Stem tip death.	Cucumber & others.	Insecticides. Sanitation.
Spider Mites	*Tetranychus urticae* & related species.	Leaf distortion. Leaf chlorosis. Yield reduction. Quality impairment.	All species.	Acaricides. Predators. Resistance.

APPENDIX III

Phosphate Buffer: For 100 ml - 30.5 ml di-sodium orthophosphate (Na_2HPO_4) and 19.5 ml NaH_2PO_4 were placed in an Erlenmeyer flask. This was made up to 100 ml by adding distilled water.

Extraction Buffer (indirect and DAS ELISA) *and Conjugate Buffer* (indirect ELISA): 0.5M trisodium citrate buffer, pH 6.5, with 0.1% thioglycollic acid added.

0.5M trisodium Citrate Buffer: 105.05 g citric acid monohydrate ($C_6H_8O_7$.H2O) and 147.05 g tri-sodium citrate ($C_6H_5O_7Na_3.2H_2O$) dissolved in 1 l distilled water.

Coating Buffer (0.05M sodium bicarbonate, pH 9.6): 1.59 g di-sodium carbonate (Na_2CO_3), 2.93 g $NaHCO_3$, 0.20 g sodium azide (NaN_3), dissolved in 1 l of distilled water.

Conjugate Buffer (DAS-ELISA): PBS-Tween, 0.2% ovalbumin.

Substrate Buffer (10% diethanolamine, pH 9.8): 19.4 ml diethanolamine, 160 ml distilled water, 0.032 g sodium azide (NaN_3); make up to pH 9.8 with concentrated HCl.

0.02M PBS, pH 7.4 (PBS): 0.20 g KH_2PO_4, 2.90 g di-sodium orthophosphate dodecahydrate ($Na_2HPO_4.12H_2O$) 8.76 g sodium chloride (NaCl), 0.20 g sodium azide (NaN_3), 0.20 potassium chloride (KCl), dissolved in 1 l of distilled water.

PBS-Tween: 0.5 ml Tween 20 per litre of PBS.

APPENDIX IV

Table 1. The effects of different aphid repellents and companion planting on aphids: The least squares means for the treatments and replicates in Trial 2.

Level	Count	Average	Standard Error	95% Confidence for mean	
Grand Mean	20	478.450	33.421	405.613	551.287
Treatment					
Control	4	628.250	74.732	465.381	791.119
Garlic Repellent	4	558.250	74.732	395.381	721.119
Reflective Mulch	4	297.500	74.732	134.631	460.369
Fennel	4	647.500	74.732	484.631	810.369
Straw Mulch	4	260.750	74.732	97.881	423.619
Replicate					
1	5	459.200	66.842	313.525	604.875
2	5	490.000	66.842	344.325	635.675
3	5	505.400	66.842	359.725	651.075
4	5	459.200	66.842	313.525	604.875

Table 2. The effects of different aphid repellents and companion planting on aphids: The least squares means for the treatments and replicates in Trial 3.

Level	Count	Average	Standard Error	95% Confidence for mean	
Grand Mean	20	1341.200	72.827	1182.483	1499.917
Treatment					
Control	4	1485.750	162.845	1130.848	1840.651
Reflective Mulch	4	780.500	162.845	425.599	1135.401
Puma	4	1540.875	162.845	1185.974	1895.776
Straw Mulch	4	1249.500	162.845	894.599	1604.401
Garlic Repellent	4	1649.375	162.845	1294.474	2004.276
Replicates					
1	5	1396.500	145.653	1079.067	1713.933
2	5	1401.400	145.653	1083.967	1718.833
3	5	1351.000	145.653	1033.567	1668.433
4	5	1215.900	145.653	898.467	1533.333

Table 3. The least squares means for the treatments and replicates in Trial 1.

Level	Count	Average	Standard Error	95%Confidence for mean	
Grand Mean	20	279.746	0.647	278.335	281.156
Treatment					
Control	4	275.883	1.447	272.730	279.035
Straw Mulch	4	280.180	1.447	277.027	283.333
Reflective Mulch	4	282.733	1.447	279.580	285.885
Garlic Repellant	4	279.688	1.447	276.535	282.840
Fennel	4	280.245	1.447	277.092	283.398
Replicate					
1	5	280.174	1.294	277.354	282.994
2	5	281.976	1.294	279.156	284.796
3	5	280.552	1.294	277.732	283.372
4	5	276.280	1.294	273.460	279.100

Table 4. The least squares means for the treatments and replicates in Trial 2.

Level	Count	Average	Standard Error	95% Confidence for mean	
Grand Mean	20	27.878	0.520	26.744	29.011
Treatment					
Control	4	32.340	1.163	29.806	34.874
Fennel	4	27.205	1.163	24.671	29.739
Garlic Repellant	4	28.973	1.163	26.439	31.506
Straw Mulch	4	25.255	1.163	22.721	27.789
Reflective Mulch	4	25.618	1.163	23.084	28.151
Replicate					
1	5	29.608	1.040	27.342	31.874
2	5	27.598	1.040	25.332	29.864
3	5	26.668	1.040	24.402	28.934
4	5	27.638	1.040	25.372	29.904

Table 5. The least squares means for the treatments and replicates in Trial 3.

Level	Count	Average	Standard Error	95% Confidence Mean	
Grand Mean	20	26.883	0.801	25.136	28.629
Treatment					
Puma	4	25.953	1.792	22.048	29.857
Reflective Mulch	4	24.788	1.792	20.883	28.692
Straw Mulch	4	28.423	1.792	24.518	32.327
Control	4	29.670	1.792	25.765	33.575
Garlic Repellant	4	25.580	1.792	21.675	29.485
Replicates					
1	5	25.936	1.603	22.444	29.428
2	5	26.778	1.603	23.286	30.270
3	5	29.304	1.603	25.812	32.796
4	5	25.512	1.063	22.020	29.004

Table 6. The least squares means for the treatments and replicates in the Cultivar Trial.

Level	Count	Average	Standard Error	95% Confidence for mean
Grand Mean	40	63.570	1.620	60.246 66.894
Cultivar				
House Zucchini	4	66.248	5.122	55.736 76.759
Season Opener	4	91.190	5.122	80.678 101.702
Gemma	4	58.25	5.122	47.738 68.762
Consul	4	77.926	5.122	67.414 88.438
Elite	4	62.740	5.122	52.228 73.252
SQ 197	4	59.595	5.122	49.083 70.107
SQ 229	4	47.618	5.122	37.106 58.129
SQ 228	4	52.136	5.122	41.624 62.648
Puma	4	51.240	5.122	40.728 61.752
Jaguar	4	68.760	5.122	58.248 79.272
Replicate				
1	10	61.865	3.239	55.217 68.513
2	10	62.185	3.239	55.537 68.833
3	10	61.902	3.239	55.253 68.550
4	10	68.330	3.239	61.681 74.978