Host Discrimination by the Green Peach Aphid (*Myzus persicae* Sulzer) Complex and Relative Importance of Non-Colonizing Aphid Species in the Transmission of Bushy-top and PVY in Tobacco

By

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ABSTRACT

This study focused on host preference by the tobacco-adapted form of *Myzus persicae*, suitability of different host plants to settling and reproduction of the aphid (*M. persicae*) and on monitoring aphid flight patterns using water traps. Focus was also on the ability to transmit PVY and Bushy-top virus of some non-colonising aphids observed on tobacco (*Nicotiana tabacum*) plants. Transmission efficiency of the non-colonising aphids was established through the real-time Polymerase Chain Reaction procedure on leaves of indicator plants. The non-colonising aphids evaluated were *Brevicoryne brassicae*, *Aphis fabae* and *Aphis gossypii*. Suitability tests were conducted on tobacco, *Solanum tuberosum*, *Brassica rapa*, *Raphanus sativus*, *Nicandra physalodes*, *Solanum lycopersicum*, *Solanum nigrum*, *Solanum melongena*, *Galinsoga parviflora*, *Tagetes minuta* and *Prunus persicae*. The tobacco-adapted *M. persicae* subspecies, *Myzus persicae nicotianae*, had a high preference for tobacco (which acted as the control), followed by *S. tuberosum* and *B. rapa*. No aphids were recorded on *T. minuta*, and a mean number of 0.33 aphid landings was recorded for *N. physalodes*. Tobacco also recorded the highest suitability index (2000). *N. physalodes* had a higher suitability (1666.7) compared to *S. tuberosum* (1466.7). *Tagetes minuta* was observed to be highly unsuitable for the development of *M. persicae nicotianae*. Aphids placed on *T. minuta* leaf discs failed to survive, and therefore no progeny were produced. *Prunus persicae* (peach tree), the primary host for *M. persicae*, was less preferred by the aphids, and was very unsuitable for their growth and reproduction. All the aphids placed on *P. persicae* discs did not survive. Aphid flight patterns were monitored at Kutsaga Research Station for a period of 14 months. The highest peak in *M. persicae* flight was observed in February 2011, and the lowest numbers were recorded in April 2011. All three non-colonising aphids that were tested for their ability to transmit PVY and bushy-top viruses to tobacco failed to do so. In conclusion, more replicated experiments using a large sample of plants as well as different aphid populations (*Myzus* and non-*Myzus* species) from

the different tobacco-growing regions of the country are recommended before we can make concrete conclusions. Of particular importance would be to thoroughly investigate the role of residual tobacco plants in the seasonal carryover of *M. persicae nicotianae* and the associated virus diseases it transmits. A very important study would be aimed at exploring ways of analysing the gut contents of aphids so that the host plant origins of *M. persicae* alates caught in water traps during the tobacco off-season are known. If such a study exonerates residual tobacco plants, this may necessitate revisiting the legislation on tobacco 'dead periods'. From a farmer's perspective, such a result would point to the need to come up with an integrated pest management strategy which controls *M. persicae* on non-tobacco hosts during the tobacco off-season.

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CHAPTER 1: INTRODUCTION

1.1 General Introduction

The Green Peach Aphid, *Myzus persicae* (Sulzer), is cosmopolitan and polyphagous on over four hundred plant species in more than fifty families (Weber, 1985). Three taxa previously identified as *M. persicae* have been described: *M. persicae, M. nicotianae* Blackman (or *M. persicae nicotianae*) and *M. antirrhinii* Macchiati according to their colour, biology, karyotypes and morphometric components. Green or pink (red) forms of *M. persicae nicotianae* were first reported on tobacco from four continents, and also proved to be able to reproduce also on potato and transmit potato viruses (Loebenstein *et al.*, 2001). Research by Clements *et al*. (2000) using RAPDs, mtDNA (COII) and nuclear EF-1α sequencing, failed to find either host-related or geographical differences in either red or green individuals taken from tobacco and non-tobacco hosts. *Myzus persicae nicotianae* shares the same number of chromosomes as *M. persicae* (2n = 12) from which it evolves (Van Emden and Harrington, 2007). Genetic markers and previous studies done showed that this species should therefore just be considered as a tobacco-adapted form of *M. persicae* and not a distinct species. *Myzus antirrhinii* was, however, shown to be a distinct species (Loebenstein *et al.*, 2001).

Myzus persicae is highly effective as a virus vector, and with a great range of genetically-based variability in properties such as colour, life cycle, host-plant relationships and methods of resisting insecticides. Phenotypic plasticity and genetic variation in the population apparently contributes to this polyphagy. It has a complex life cycle, which can vary according to the different environments in which it occurs. *Myzus persicae* has herbaceous summer (secondary) hosts, which include many annual crops such as potatoes, sugar beet, chrysanthemums, tobacco and various brassicas, on which it reproduces by parthenogenesis. Parthenogenetic populations develop as a mixture of clones, with the most favoured ones as potential dominators of the population.

Myzus persicae is a major vector in the transmission of Tobacco Bushy-top virus and potato virus Y (PVY) (DiFonzo *et al.*, 1997). PVY is a non-persistent virus, and does not circulate within the aphid's body. It is carried in the aphid vector's foregut, and transmission occurs rapidly when contaminated aphids probe host plants. Because the virus particles are quickly lost from the foregut, aphids can transmit them only once or a few times, rather than repeatedly over a long period of time. Because of rapid acquisition and transmission times for these non-persistent viruses, a few aphids, through probing, can transmit these viruses to many plants in a short time, and greatly reduce the effectiveness of chemical control in limiting the spread of viruses (Western Regional IPM Project, 2006).

In most of the high-value crop hosts attacked by *M. persicae* and in which yield depression is mainly due to virus diseases transmitted by the aphid, there is heavy reliance on chemical control. However, control of *M. persicae* has not resulted in the control of PVY and other aphid-transmitted viral diseases. This failure to control PVY when there is excellent control of *M. persicae* has been reported to be due to the presence of non-colonizing aphids which pick up the virus during probing (DiFonzo *et al.*, 1997; Halbert *et al*., 2003).

1.2 Justification and Research Questions

As phloem feeders and major vectors of plant viruses, aphids are important pests of agricultural and horticultural crops worldwide. The processes of aphid settling, reproduction and virus transmission on plants therefore have a direct economic impact, and a better understanding of these events may lead to improved management strategies. Aphids are also important model organisms in the analysis of population differentiation and speciation in animals, and new ideas on plant utilization influence our understanding of the mechanisms generating biological diversity.

Myzus persicae is a vector of many plant viral diseases, namely; Tobacco Bushy-top virus, Potato virus Y (PVY), Potato Leaf Roll virus (PLRV), Beet Yellows virus (BYV) and Beet

Mild Yellowing virus (BMYV). It is also a vector of tomato, lettuce, dahlia, canna and bee mosaics, tuber spindle and rugose mosaics. The fact that it is a vector of many viral diseases of crops and flowers makes it a pest of economic importance.

One of the many hosts of *M. persicae* is tobacco. The tobacco-adapted form, *M. persicae nicotianae*, is known to vector Tobacco Bushy-top and PVY in tobacco. Some tobacco farmers leave the fields uncleared after harvesting, allowing residual tobacco plants that might be infected with Tobacco Bushy-top to grow unmonitored. The study therefore focused on investigating whether these plants are the source of the virus diseases which soon become evident in newly-planted tobacco fields.

The aphid species, *Capitophorus elaeagni*, was not regarded as a vector of PVY until it was recorded for the first time by Halbert *et al*. (2003). It was found to be abundant in potato fields. In tobacco fields, it has been observed that even under very low *M. persicae nicotianae* infestation pressure, symptoms of Tobacco Bushy-top are observed on plants. Some abundant, non-colonising aphid species might actually play a significant role in its transmission, as is the case with *C. alaeagni*. These non-colonizing aphids probe the plant before determining that it is unsuitable for colony establishment and fly away. Since PVY and Tobacco Bushy-top are transmitted in a non-persistent manner, there is a great possibility that these aphids spread the viruses as they probe one plant after the other.

Better knowledge of virus-vector-plant interactions can undoubtedly result in improved strategies for plant resistance to viruses through host gene- and/or transgene-mediated resistance. For instance, non-persistent virus spread could be limited by using cultivars that decrease the chance for a vector to penetrate into the epidermal cells during probing (Loebenstein *et al.*, 2001).

The information gaps with regards to aphids and virus transmission in tobacco can be summarised in the form of the following research questions:

- Which plant species are more preferred and more suitable for the development of the tobacco aphid, *M. persicae nicotianae*?
- To what extent do diseased tobacco plants left undestroyed after the May 15th deadline contribute to the carryover of PVY and Bushy-top diseases in tobacco?
- To what extent does probing on tobacco by non-colonizing aphids contribute in the horizontal transmission of PVY and Bushy-top virus?
- Is the initial incidence of PVY and Bushy-top virus correlated in any way to the *M. persicae* flight patterns?

1.3 Objectives

The main objective of this study was to determine the possible host plant reservoirs of tobacco virus diseases (PVY and Bushy-top) during the non-tobacco growing season, and the role played by non-colonizing aphids in the transmission of these diseases in tobacco.

Specific objectives were:

(i) to determine host preference by *M. persicae nicotianae*,

(ii) to determine suitability of different plant species for the development of *M. persicae nicotianae*,

(iii) to investigate the relative importance of undestroyed tobacco plants in the seasonal carryover of PVY and bushy-top virus diseases,

(iv) to determine PVY and bushy-top virus pick up and subsequent horizontal transmission by non-*Myzus* species which land to probe on tobacco during their migration, and

(v) to determine aphid flight patterns and proportion of *Myzus* to non-*Myzus* species in trap catches.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is a major economic pest in many major crops. It is assumed to be of Asian origin because its primary host, peach (*Prunus persicae* L.) on which sexual reproduction occurs, originated there (Blackman and Eastop, 2000). *Myzus persicae* is known to develop populations specifically adapted to certain host plants (Magaritopoulos *et al*., 2000). The tobacco aphid *M. persicae nicotianae* (Blackman) is reported as a subspecies of *M. persicae*, particularly well adapted to tobacco, *Nicotiana tabacum* L. (Solanaceae), and exhibiting morphometric (Blackman, 1987; Blackman and Eastop, 2007; Magaritopoulos *et al*., 2000) and genetic differences (Blackman and Spence, 1992; Margaritopoulos *et al.*, 1998; Magaritopoulos *et al*., 2007) with respect to *M. persicae*. Relative preference for tobacco by *M. persicae nicotianae* has been demonstrated experimentally (Margaritopoulos *et al.*, 2005; Troncoso *et al.*, 2005), suggesting that its capacity to detoxify and thus overcome allelochemicals from tobacco plants has been key to its specialisation (Cabrera-Brandt *et al*., 2010).

2.2 Aphid Biology and Ecology

2.2.1 Distinguishing features of aphids

Aphids are small (1-10 mm), soft-bodied plant-sucking insects. These insects have an intricate life cycle. Several or all generations comprise parthenogenetic females which reproduce without egg fertilization and are viviparous (i.e. produce live young). Some species of aphids undergo cyclical parthenogenesis, whereby periods of asexual reproduction alternate with sexual reproduction (Dixon, 1998). Embryos developing in parthenogenetic females also have embryos developing within them. This parthenogenesis and telescoping of generations enables aphids to achieve very high rates of increase (Dixon, 1998).

Polyphenism, or the occurrence within a species of different forms or morphs, is also a characteristic of aphids (Dixon, 1998). *Myzus persicae* exhibits a wide range of phenotypic plasticity, whereby the same genotype can respond to different environments by producing alternate different phenotypes in a way that maximises its fitness (Halkett *et al*., 2006). Aphids also exhibit different reproductive modes, ranging from cyclical to obligate parthenogenesis. In cyclical parthenogenetic populations, aphids alternate several asexual generations each year, with a single sexual generation produced in response to short photoperiod in the autumn (Simon *et al*., 2002). The sexual generation produces genetically recombined, frost-resistant eggs. On the other hand, obligately (apomictic) parthenogenetic populations exhibit permanent all-female parthenogenesis that produce individuals unable to survive freezing temperature (Moran, 1992; Simon *et al*., 2002, 2003).

Aphids can also produce alternately winged and wingless forms in response to host plant quality, crowding and day length (Watt and Dixon, 1981; Loxdale *et al.*, 1993; Llewellyn *et al.*, 2003).The winged aphids are called alatae, and the wingless aphids are called apterae (Dixon, 1998).

2.2.2 *Myzus persicae* life cycle

The green peach aphid has a complex life cycle with 10 to 25 generations a year. Most generations consist of parthenogenetic females that produce live nymphs without mating. Sexual reproduction is absent altogether in areas with mild winters. The population spreads from one host to another all year round as new hosts become available, while others dry out or are destroyed by frost (Western Regional IPM project, 2006). In areas with cold winters, a generation of sexual (holocyclic) forms appears in the fall, and eggs are laid on a winter host, e.g. peach, apricot and certain plums (Western Regional IPM project, 2006). The holocyclic forms have host alternation between *Prunus* species, primarily *P. persicae*, and numerous herbaceous plants of many different families, for example, *Tulipa*, *Brassica* and *Solanum*

species (including potatoes) (Heie, 1994). Overwintering of eggs takes place on the primary host, *P. persicae*, but the aphids on the secondary hosts can also survive in winter and reproduce parthenogenetically in glass houses, beet clamps and other sheltered places (Heie, 1994).

Overwintering eggs hatch in the spring, producing a generation of wingless females called stem mothers (Figure 2.1). The stem mothers feed on the buds and young leaves of the winter host, each one producing 100 to 200 wingless females, beginning a series of generations on the winter host (Western Regional IPM Project, 2006).

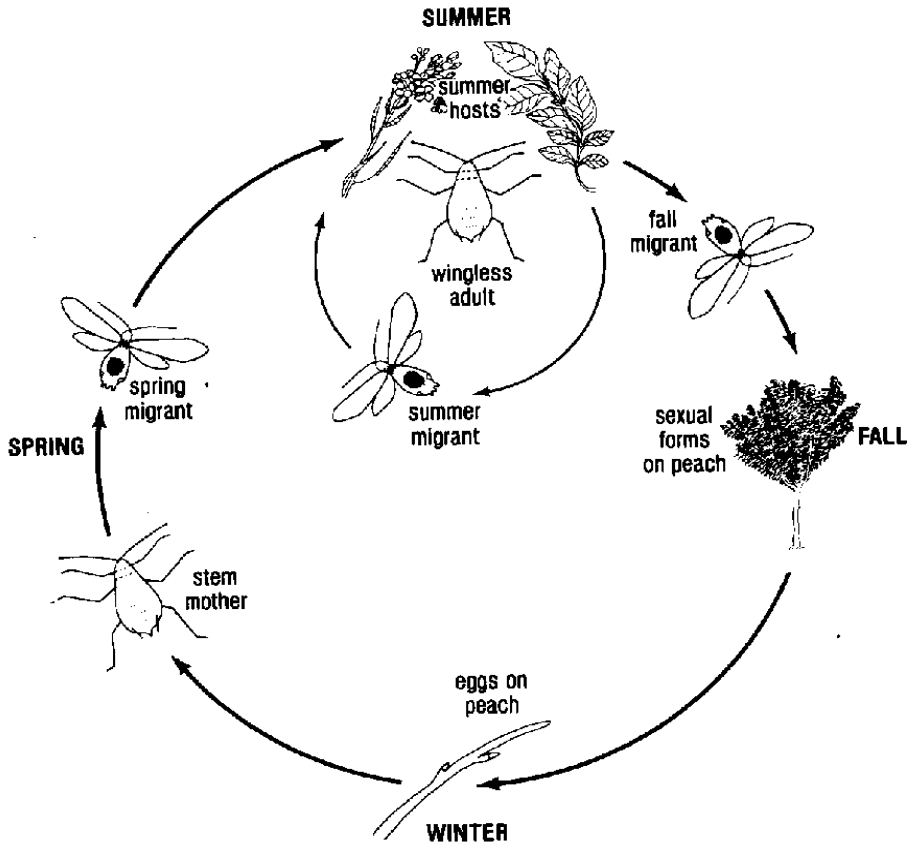

Figure 2.1. Life cycle of *Myzus persicae* in areas with cold winters

Starting in the third spring generation, some nymphs develop into winged migrants and fly to other hosts to begin a series of summer generations. When a migrant reaches a new host, it usually remains only long enough to produce a few nymphs, then moves to yet another plant.

Most flights by migrants are short, thus infestations usually spread gradually from the winter host. Migrants can, however, be carried over long distances by wind, thus even plants far downwind can be infested. Early spring hosts include mustards and related weeds in and around orchards, but as the season progresses, many plants become infested (Western Regional IPM Project, 2006).

Each set of nymphs deposited by a spring migrant can start, by asexual reproduction, a new colony of non-migrant, non-winged aphids. After one or more generations, the colony begins to produce a proportion of winged individuals (summer migrants) in each generation. The proportion of migrants to non-winged adults increases in each generation as colonies become crowded and hosts dry out or otherwise become less suitable for the aphids. A nymph on a summer host develops to maturity and begins producing a new generation of nymphs in as few as six days. In cooler weather, development may take two weeks or more (Western Regional IPM Project, 2006).

Eggs require a certain period of chilling and exposure to water to develop. After the chilling requirement is reached, eggs hatch in response to warm weather. In areas with mild winters, *M. persicae* has no sexual phase thus neither males nor eggs are produced. The population continues moving from one host to another during winter in a series of asexual generations (Western Regional IPM Project, 2006). Even in colder regions, *M. persicae* may continue development in green houses or in other situations where host plants are available and where temperatures remain favourable. Plants such as vegetables and annual flowers, produced in greenhouses and set out in spring, are often infested, and can be an important source of *M. persicae* that can later move on to potatoes, tobacco or other preferred hosts. Populations may also overwinter in protected places around heated buildings, along canals and around springs (Western Regional IPM Project, 2006).

2.2.3 Host preference

Host plant preference and discrimination has proved to be different between specialist and generalist aphids in earlier studies. The first example was shown by Bernays and Funk (1999) and Funk and Bernays (2001) in two races of *Uroleucon ambrosiae* (Thomas) (Hemiptera: Aphididae) which differed in their host range, one being specialised on giant ragweed, *Ambrosia trifia* L. (specialist race) and the other using several genera of Asteraceae (generalist race). These studies revealed behavioural differences in the detection of plant stimuli between races. The specialist race found its host plant faster, and reached the phloem sooner, and spent more time feeding than the generalist one. Furthermore, a study that compared host selection behaviours between the generalist *M. persicae* and its specialised tobacco-adapted form also found that the specialist form performed more direct searching and acceptance behaviours than the generalist (Vargas *et al.*, 2005).

2.2.4 Host plant colonisation and viral transmission

The success of *M. persicae* in colonizing different host plants has been related to the presence of aphid enzymatic mechanisms of detoxification, which are responsible for the metabolism of host plant allelochemicals (Francis *et al*., 2006). The performance of *M. persicae nicotianae* on tobacco should therefore have a genetic/ biochemical base, which could be related to the ability of the subspecies to colonize a well-defended host plant (i.e. tobacco with glandular trichomes and cuticular sucrose esters) (Cabrera-Brandt *et al*., 2010).

A virus in an infected plant may be more readily available to a vector at one time than at another. Young plants are usually best sources of viruses because the concentration of many viruses decreases as the plant ceases to grow (Gupta, 2004). The distribution of viruses within the plant can determine when the insects acquire the virus and on which parts of the plant they need to feed to do so. Many viruses can be acquired by some insects some days before a newly infected plant shows symptoms, e.g. cauliflower mosaic (Gupta, 2004). Different plant

species also differ in their effectiveness as the source of the same virus. For example, although pepper is a better host than chard for *M. persicae* and is more susceptible to southern cucumber mosaic virus, aphids acquire viruses more readily from chard than from pepper. Not only does the host affect insect transmissibility, but the virulence of a virus may be changed by passage through different hosts (Gupta, 2004). A host plant needs to be susceptible to infestation by the vector to be susceptible to infection by a virus; nevertheless, colonizing insects are usually more prevalent in crops than transient visitors. Thus, it is not unreasonable to consider the colonizers first when seeking vectors (Gupta, 2004).

It is true that insects are more active within a crop when they vainly seek a suitable host plant, but colonizers have to be active at some period if they are to find new hosts, and their potential as vectors often depends on the readiness with which they move again after landing on a host plant. Although *M. persicae* was identified early as the principal vector of PVY, experiments showed that *Macrosiphum solanifolli* (Ashmead) and *Aphis nasturtii* (Kaltenbach) (Buckthorn aphid) are also efficient vectors of PVY (Gupta, 2004).

Some viruses, usually non-persistent ones, are spread mainly by non-colonizing insects which bring viruses with them from the plants they have just left, or acquire it from infected plants within the crop as they move from plant to plant seeking suitable hosts (Gupta, 2004). The principal vector may be the least prevalent insect pest as is the case in the citrus gloves of California where the main vector of tristeza virus, *Aphis gossypii* (Glover) forms only about 3% of the aphids visiting trees. Even among vector species, it cannot be assumed that all the insects that feed on a diseased plant will be infective (Gupta, 2004). Obviously, the more the plants that are infected in the crop, the greater will be the proportion of potential vectors that become infective, although almost nothing is known about the proportions or numbers of infective aphids in crops. The proportion differs with different viruses and vectors, depending on the time insects take to become infective and the time they remain so (Gupta, 2004).

2.2.5 Feeding behaviour and food quality

Most species of aphids feed on phloem sap which they obtain by tapping the phloem elements with their stylets. Phloem cells are living cells which are located at some depth within a plant. The contents of these elements (phloem sap) are rich in sugars and relatively poor in amino acids, especially those that are essential for growth (Dixon, 1998).

Aphid antennae bear many sensilla amongst which are some whose structure and electrophysical response indicate that they are used in chemoreception or gestation and perception of the leaf surface (Dixon, 1998). In the laboratory, aphids respond to plant odours both when walking and when flying. Although there is little doubt the antennal olfactory sensilla are receptive to both positive signals associated with host plant volatiles and negative signals associated with the odour blend of non-host plants, their role in the location of plants from a distance is debatable (Dixon, 1998).

During their dispersal activities in search of new hosts, both apterous and alate aphids visit any plant species indiscriminately as a result of complex behavioural procedures, which appear to be predominantly based on responses to visual stimuli (Loebenstein *et al.*, 2001). By that time, however, they are still unable to discriminate between suitable and unsuitable hosts. Once they have come in contact with a new plant substrate, they display a further series of behavioural sequences in response to a variety of physical and physiological stimuli received from the plant. Only then will the insect sense whether it is a suitable host or not. During these steps, particular behavioural patterns may have important implications in both virus acquisition and inoculation processes (Loebenstein *et al.*, 2001).

Upon landing on a plant, the aphid shows an intense wandering activity, interrupted by short pauses of less than one minute, during which it inserts its stylet to probe into the superficial cell layers of the plant tissues (epidermis, parenchyma) before resuming walking or flying (Loebenstein *et al.*, 2001). Aphids scan the surface of a plant accepted as a potential host with

the tip of their proboscis. The tactile receptor on the tip of the proboscis responds to contact and surface texture, and enables aphids to detect contours of veins, their preferred feeding site (Dixon, 1998). Probing is a reflex activity elicited by any solid surface encountered on the plant, and is antagonistic to locomotion. Short probes act as test-feeding punctures for a first step recognition of the host by the aphid (Loebenstein *et al.*, 2001). Starved individuals tend to initiate penetration earlier and make shorter probes than larger and non-fasted ones. Transient aphids, which are not prone to accept the plant as a host, make a number of successive probes each becoming shorter and shorter while walking time increases, until they eventually leave the plant. Conversely, resident ones are more likely to settle soon for feeding and reproduction, depending on the level of resistance of the plant (Loebenstein *et al.*, 2001).

After finding a suitable host, resident aphids then probe into the plant with their mandibular and maxillary stylets, which together form a hollow needle-like structure (Dixon, 1998). Most probes are initiated in intercellular grooves that are located by the apex of the rostrum tapping the surface of the substrate. Prior to a probe, a drop of gelling saliva is secreted and deposited on the plant surface, forming a kind of plug with an external flange or collar (Loebenstein *et al.*, 2001). During stylet penetration into the tissues, secretion of gelling saliva continues, resulting in a continuous sheath around the stylet bundles. The sheath material encases the stylets, and is thought to be mainly lipoprotein, possibly containing some 10% phospholipid. The viscous precursor secretion begins to gel immediately after it leaves the tips of the stylets, possibly by enzymic oxidation of sulphydryl groups to form disulphide bonding (Dixon, 1998). Once formed, the sheath is relatively impermeable. This salivary sheath gives rigidity to the very flexible stylets, and enables the control of the direction of the probe by restricting bending except at the apex of the stylets. The stylet sheath usually ends in the phloem, indicating that aphids feed on the contents of the sieve elements (Dixon, 1998). Stylet penetration has been described as almost always intercellular, reaching the

epidermis in less than one minute on average. Epidermal cells can also be penetrated by stylets (Loebenstein *et al*., 2001).

2.3 Aphid-Transmitted Diseases

2.3.1 Potato virus Y (PVY)

Potato virus Y belongs to the *Potyvirus* genus. The genus is currently known to be the largest of all the plant virus genera and is thought to constitute the most destructive families of plant viruses affecting potato crops (Ward and Shukla, 1991) and many other economically important plant species. These plants include tobacco, tomato and pepper (McDonald and Singh, 1996). The level of damage to a crop is determined by the strain of PVY infecting the plants, the viral load, the time at which infection occurs, as well as the tolerance the host possesses toward the virus (Warren *et al*., 2005). Resistance to PVY infection by hosts is low in many cases. Infection of a potato field with PVY may ultimately result in 10-100% loss in yield (Warren *et al*., 2005).

PVY may be transmitted to potato plants through grafting, plant sap inoculation and through aphid transmission. The most common manner of PVY infection of plant material in the field is through the aphid. Although aphids on their own can directly damage host plants, it is their role as viral vectors which has the greatest economic impact (Radcliffe and Ragsdale, 2002). In cold climates, aphids spend the winter either as wingless aphids giving birth to live young (viviparae) or as eggs. Hosts such as weeds and other crops, serve as breeding grounds for these aphids, and form a temporary area of colonization before the aphids migrate to the tobacco fields (Radcliffe and Ragsdale, 2002). In moderate climates, such as in South Africa, aphids are thought to reproduce asexually on weeds, other crops, indigenous plants and garden plants. This means that there are a number of aphids present year-round. The importance of effective and stringent monitoring of aphid populations is stressed in a review

by Radcliffe and Ragsdale (2002) as PVY virions are introduced to potato fields almost solely by winged aphids from a virus source outside known fields.

Myzus persicae has been found to be most effective in its role as a virus vector, but other species, such as *Aphis fabae*, *Aphis gossypii*, *Aphis nasturtii*, *Macrosiphum euphorbiae*, *Myzus* (= *Nectarosiphon*) *certus*, *Myzus* (= *Phorodon*) *humuli* and *Rhopalosiphum insertum*, are also strongly associated with viral transmission (Halbert *et al*., 2003; Warren *et al*., 2005). In South Africa, *A. fabae*, *A. gossypii* and *A. nasturtii* are the most common and efficient PVY vectors found in the field (Warren *et al*., 2005).

Apart from being classed according to efficiency as vectors, aphids can also be divided into two subgroups, namely colonizing and non-colonizing species. Colonizing aphids are those which reproduce and establish themselves on the host plant in question, while non-colonizing aphids neither reproduce nor establish colonies on the host. Colonizing aphids are better adapted to life on the host plant, and are thus generally considered as better virus vectors than non-colonizing aphids. In the case of tobacco and PVY, non-colonizing aphids do not primarily feed on tobacco plants, but do occasionally feed on them while searching for a more suitable host. Their lower efficiency as PVY vectors is cancelled out by the sheer numbers in which they occur (Radcliffe, 1982; Thompson, 1997). Because of this, all aphids present in and around tobacco fields must be considered as possible vectors, and their numbers carefully monitored.

Transmission of PVY by aphids occurs in a non-persistent, non-circulative manner which suggests a less intimate interaction between virion and vector than is the case of circulative viruses (Gray, 1996). The fact that the viruses are transmitted in a non-persistent fashion means that viral replication does not occur within the aphid vector and that, unless the aphid feeds on infected plants, it loses its ability to infect plants after two to three feedings (Bradley and Rideout, 1953; Warren *et al*., 2005). The virions attach to the aphid stylet in a matter of

seconds, and may remain infectious for four to seventeen hours (Kostiw, 1975). The distance over which the virions can be transmitted is limited due to the short period during which they remain infectious (Robert *et al*., 2000). Although the short life span outside plants inhibits long-distance viral transmission, it does not reduce the transmission efficiency bestowed by the quick rate of viral acquisition and inoculation within a field.

The general symptoms of PVY include leaf mottling or yellowing, leaf deformation, necrotic leaf spots or rings, veinal necrosis, necrotic stem-streaking, leaf drop and premature death of stems (Jones *et al*., 2003). Plants infected with PVY strains may have bushy growth at the top, with few leaves at the bottom of the stem. However, plants infected with mild strains and tolerant cultivars may develop much milder foliage symptoms without any necrosis, leaf drop or premature death of shoots (Jones *et al*., 2003).

2.3.2 Tobacco Bushy-top disease

Tobacco Bushy-top virus was first reported in Zimbabwe in 1958 (Gates, 1962). The virus usually occurs as a complex with other viruses, for example PVY. The virus is readily transmitted by mechanical inoculation from plants that are infected with another virus (e.g. Tobacco Vein Distorting Virus), but not from plants infected with it alone (Gates, 1962). Tobacco Bushy-top disease is caused by a complex of the Tobacco Bushy-top virus, a member of the genus *Umbravirus* and Tobacco Vein Distorting Virus, a member of the genus *Poleovirus*, which acts as a vector encapsidating the Tobacco Bushy-top genomic RNA (Mo *et al*., 2011).

Tobacco Bushy-top virus causes stunted growth in plants, and leaves show symptoms of vein distortion, vein clearing and mottling, and rounding (Mo *et al*., 2002). It also stimulates the sprouting of axillary shoots from the main stem (Gates, 1962). These early sprouts form lateral shoots on which other shoots are produced, resulting in a ‘bushy’ appearance (Mo *et al*., 2002).

In a study done in Malawi in which plant species closely related to tobacco or associated with its cultivation were inoculated with the virus via *M. persicae,* severe symptoms were observed on Barnet special tobacco, *Capsicum annum*, and long purple eggplant (Chapola, 1980). Symptoms were mild on *Solanum nigrum*, *Datura stramonium* and *Amaranthus spinosus,* and were not observed on *Bidens pilosa* or marketer cucumber. No symptoms developed on mechanically-inoculated plants.

The main vector in the transmission of Tobacco Bushy-top is *M. persicae.* Broadbent and Tinsley (1951) suggested that the spread of viral diseases from field to field must necessarily be by alatae, but the transmission is negligible compared to that from infected plants within the crop. Much spread took place early in the season before an apterous population developed, which makes it clear that apterae are probably not the principal vectors.

In a study done by Duan *et al.* (2003), it was observed that the minimum inoculation access period of *M. persicae* was two minutes, and the maximum acquisition access period was an hour. The aphids retained the ability to transmit the virus over a nine-day period. Both alatae and apterae were seen to have the ability to transmit Tobacco Bushy-top. Young aphids could not transmit the virus. The aphids became increasingly infectious when the inoculation access time was increased up to 24 hours after virus acquisition feeding time of 24-48 hours, and they remained infectious for several days (Gates 1962).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Site

The study was carried out at Kutsaga Research Station (1 479 m above sea level, 17°55′S, 31°08′E). It is about 18km South of Harare, between the city and the dominating town of Chitungwiza. Mean annual rainfall varies between 800 and 1000 mm and normally falls from November to March.

Kutsaga Research Station has light, well-drained, sandy soils of granite origin which resemble those found in most tobacco growing areas of Zimbabwe. The soils are very low in clay content and have low water-holding capacity. They are slightly acidic (pH 5.2). The land was previously under fallow.

3.2 Choice Tests for Host Preference

Test plants

(a) Cultivated herbaceous**:** tobacco (fresh and undestroyed regrowths), *S. tuberosum* (potato), *B. rapa* (Chinese cabbage) and *R. sativus* (raddish).

(b) Weeds: *N. physalodes* (apple of Peru), *D. stramonium*, *S. nigrum* (Black nightshade), *S. melongena* (all belonging to family Solanaceae); *G. parviflora* (Asteraceae), *T. minuta* (Mexican marigold) (Asteraceae).

(c) Primary host: *P. persicae* (peach).

Plants were grown from seed in pots until two months old. These were arranged within a screened cage in a circle of 1 metre in diameter, with the distance between two adjacent pots being such that leaves did not touch each other during the experiment (Fig 3.1). One individual of each plant species was used.

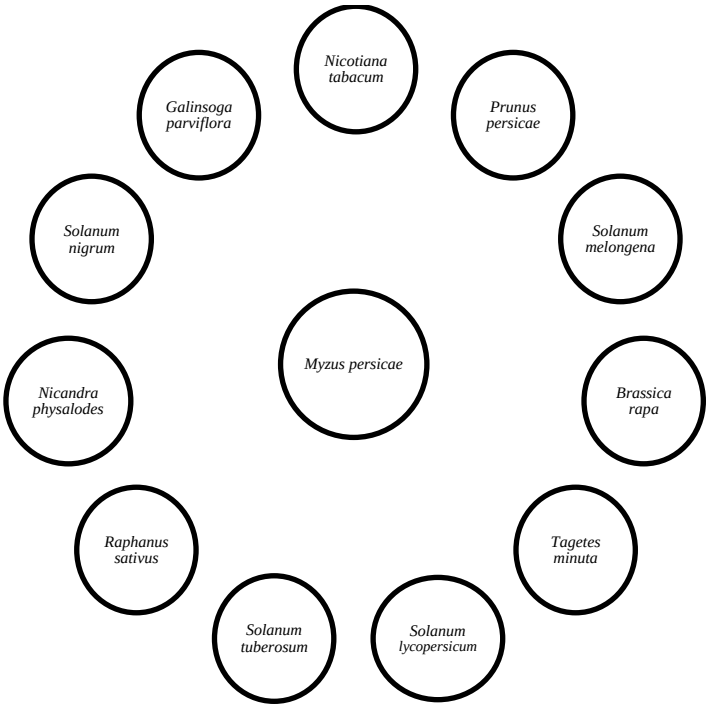

Figure 3.1. Schematic diagram representing the circular arrangement of plants for *M. Persicae* choice test.

A total of 11 plants were evaluated as hosts of *M. persicae nicotianae,* and three replications of each were set up, all of which were grown from seed under similar environmental conditions. Two hundred alatae *M. persicae* were used in each run. The alatae in a petri dish were placed at the centre of the circle. After each of five successive periods of 30 minutes, each plant was checked for aphid landings and the cumulative number recorded. Aphids were removed from plants at each assessment.

3.3 Host Suitability for Aphid Development

A total of 10 host plants (*N. tabacum, S. tuberosum, S. lycopersicum, T. minuta, P. persicae, R. sativus, N. physalodes, G. parviflora, B. rapa, S. nigrum*) were grown in pots until two months of age. *Solanum melongena* plants died before use in host suitability tests. Leaf discs were then cut from these plants, and each one was placed in a petri dish with the upper surface down, on moist filter paper. *Myzus persicae* apterae were individually placed in petri dishes each with a tobacco leaf disc, and left for 24 hours until the first set of nymphs was

produced. These wingless aphid nymphs were removed from the leaf disc and placed in a separate petri dish. They were then starved overnight, and placed individually on leaf discs in separate petri dishes previously prepared from different host plants. Three replicates for each of the ten host plants were set up. The number of progeny on each host plant was counted daily and recorded. This was done for seven days. The filter paper with leaf disc was moistened once daily and replenished as necessary.

3.4 Virus Detection on Field-Sampled Residual Tobacco Plants

Residual (undestroyed) tobacco plants showing symptoms of PVY were collected at Kutsaga and taken to the laboratory. The presence of PVY in the residual plants was confirmed by symptom observation. Virus-free *M. persicae* wingless nymphs previously reared on Chinese cabbage and fed on radish were starved for an hour (Halbert *et. al.*, 2003) then placed on a field-sampled diseased plant. After feeding for five minutes, ten nymphs were transferred to a healthy indicator plant grown in a pot. After feeding overnight, the aphids were killed by squashing, and the plant transferred to an-aphid free greenhouse for observation of disease symptoms. Real-Time Polymerase Chain Reaction (rt-PCR) tests were conducted on each plant showing symptoms to determine the presence of PVY. Symptom development was also checked after five weeks and scored using the CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco) scale.

CORESTA scale

B1: Mosaic symptoms

B2: Necrotic PVY

The scale ranges from 1 to 3 (mild to severe). 0 signifies no symptoms.

3.5 Real time- Polymerase Chain Reaction (rt-PCR)

Amplification was performed with 2.5 ml cDNA in a final volume of 25 ml containing 10 mm Tris (pH 8.3), 50 mm KCl, 1.5 mm $MgCl_2$, 0.15 mm of each dNTP, 1 mm down-stream primer (R5), 1 mm upstream primer (D1) and 0.5 units of Taq DNA polymerase.

Following an initial denaturation at 94.8^{o}C for 2 minutes, PCR conditions were as follows: 35 cycles at 94.8^{o}C, 30 seconds; 70.8^{o}C, 15 seconds and 72.8^{o} C, 15 seconds, and a final extension for 5 minutes at 72.8^{o}C. PCR products were analysed by electrophoresis through a 1.5% agarose gel followed by staining with ethidium bromide and visualization of DNA bands using a UV transilluminator.

3.6 Assessing Viral (PVY and Bushy-top) Pick up and Transmission by Various Aphid Species

A survey was done at Kutsaga research station for collection of aphid species that survive in the area. Three aphid species were collected and identified using the Rothamsted key (Harrington and Taylor, 1985). The aphids were identified as the vegetable aphid (*B. brassicae*), the bean aphid (*A. fabae*) and the cotton aphid (*A. gossypii).* These aphid species were then reared on their host plants separately in the laboratory until a colony of more than 50 individuals was established. Prior to transfer to tobacco plants, the aphids were fed on raddish (1 hour) for 'viral cleaning' and starved for an hour (Halbert *et. al*, 2003). A group of 10 aphid species was then placed on a diseased tobacco plant.

Aphids were allowed timed probes of five minutes on a diseased plant (inoculation access period), after which they were placed on healthy indicator plants. These were left overnight. The aphids on indicator plants were killed by squashing and the indicator plants kept in an aphid-free greenhouse for five weeks before being checked for symptom development. Additionally, leaf samples of plants showing symptoms were collected for PCR analysis.

3.7 Monitoring Aphid Flight Patterns and Sampling Virus-Vector Aphids on Plants

This study was conducted in two ways: (i) monitoring aphid flight patterns through yellow water traps, and (ii) monitoring plant landings by *M. persicae* and *non-Myzus* transient alatae species. Data were collected for the period March to November 2011 at Kutsaga Research Station. Data for the period October 2010 to February 2011 were obtained from Kutsaga Research Station where water traps are already operational. These traps were checked daily and trapped aphids taken to the laboratory for identification. Rothamsted keys (Harrington and Taylor, 1985) were used to separate *M. persicae* from non-*Myzus* species. The traps were located at different areas within the research station grounds. A total of 16 traps were used to capture aphids. Some samples of non-*Myzus* species were identified using the Rothamsted key and other identification keys (Liu and Sparks, 2001; Tharp *et al.*, 2005).

3.8 Data Analysis

The statistical package, SPSS Version 16 for Windows (SPSS Inc. 2007) and Genstat Release 9.2 (2007) were used to analyse host preference and suitability data.

3.8.1 Normal Q-Q Plot

Data on host preference and host suitability were tested for normality using the Q-Q Plot run in SPSS Version 16.

3.8.2 Analysis of Variance (ANOVA)

SPSS version 16 was used to obtain the ANOVA table for host preference. A test for homogeneity of variance (Levene's test) was carried out to test the hypothesis that variances of the aphid landings on each plant species were equal. Analysis of variance ($\alpha = 0.05$) was then used to test the hypothesis that there were significant differences among the numbers of aphids landing on each plant species.

Levene's test for homogeneity of variances was also carried out on host suitability data. The hypothesis being tested was that the variances of the number of progeny obtained on each plant host were the same. The Kruskal-Wallis test was carried out using Genstat Release 9.2 to determine if there were significant differences in suitability between hosts.

3.8.3 Multiple comparison test

A Post Hoc multiple comparison test (Least Significant Difference) was carried out to determine which host plants differed significantly in aphid landings. The Bonferroni multiple comparison test was carried out to compare suitability of the host plants.

3.8.4 Index of suitability

The index of suitability was used to determine which plant host was more suitable to aphid colonisation. It was calculated using data obtained in the experiment for the determination of host suitability to aphid development.

$$Index\ of\ suitability = \frac{100T}{2n},$$

Where *T* is the total score for all the plants of the species and *n* is the number of replicates in the sample.

CHAPTER 4: RESULTS

4.1 Host Preference

Nicotiana tabacum had the highest number of aphid landings in all runs and a mean of 50.3 aphid landings (Table 4.1). *Brassica rapa* had the second highest number of aphid landings after *N. tabacum*. No aphids were observed on *T. minuta* with *N. physalodes* having a very low mean aphid count (0.3). *Prunus persicae*, the primary host for *M. persicae* had a low mean number of aphid landings (2.3).

Table 4.1. Host preference by *Myzus persicae*

Host	Run			Mean number of aphid landings±SD
	1	2	3	
Tobacco (*N. tabacum*)	61	30	60	50.3±17.6
Chinese cabbage (*B. rapa*)	45	43	37	41.7±4.2
Potato (*S. tuberosum*)	20	38	15	24.3±12.1
Eggplant (*S. melongena*)	8	12	16	12±4.0
Raddish (*R. sativus*)	3	10	3	5.3±4.0
Potato weed (*G. parviflora*)	4	5	6	5.0±1.0
Peach (*P. persicae*)	3	2	2	2.3±0.6
Tomato (*S. lycopersicum*)	2	2	0	1.3±1.2
Black nightshade (*S. nigrum*)	1	1	1	1.0±0.0
Apple of Peru (*N. physalodes*)	0	0	1	0.3±0.6
Marigold (*T. minuta*)	0	0	0	0.0±0.0

There were significant differences ($P < 0.05$) in the aphid landings among the different host plants. Mean separation by LSD showed no significant difference in the number of aphid landings between *N. tabacum* and *B. rapa* ($P > 0.05$). On the other hand, aphid landings on *S. tuberosum* were significantly lower than those on *N. tabacum* and *B. rapa*. *Prunus persicae* also had significantly lower aphid landings than *N. tabacum*, *S. tuberosum* and *B.* rapa. The most preferred hosts were *N. tabacum* and *B. rapa* followed by *S. tuberosum. Prunus persicae* was among the least preferred hosts such as *T. minuta* and *N. physalodes*. Although

S. melongena had a relatively higher mean number of aphid landings than *P. persicae*, there were no significant differences in preference between them.

4.2 Host Suitability

There were significant differences ($P < 0.05$) in host suitability among the test plants (Table 4.2). *Nicotiana tabacum, B. rapa, R. sativus* and *N. physalodes* had higher suitability indices than the other hosts. *Nicotiana tabacum* (which acted as the control) was the most suitable host, and had the highest number of aphid progeny being produced in seven days. The second and third most suitable hosts were *B. rapa* and *N. physalodes*, respectively. *Solanum tuberosum* showed a lower suitability than expected. The least suitable were the hosts that did not allow *M. persicae* to survive and reproduce. *Prunus persicae* was not at all suitable for the survival of *M. persicae. Myzus persicae* placed on *T. minuta* died on the first day. *Solanum lycopersicum, G parviflora, P. persicae* and *T. mi*nuta had no progeny and, therefore, were not suitable hosts.

Table 4.2. Total number of progeny produced by *M. persicae* over a period of seven days and the index of suitability for each host

Number of progeny									
Host	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**	**Total**	**Index of suitability**
N. tabacum	0	0	4.3	8.7	10	7.7	9.3	**40**	2000
B. rapa	0	0	7	6.7	8.3	6.3	9.7	**38**	1900
N. physalodes	0	0	3.7	6	9.3	6.7	7.7	**33.3**	1666.7
R. sativus	0	0	6	6	5.7	7.3	5.7	**30.7**	1533.3
S. tuberosum	0	0	4.3	5	6.3	6.3	7.3	**29.3**	1466.7
S.nigrum	0	0	0	4.3	0	1.7	0.7	**6.7**	333.3
T. minuta	0	0	0	0	0	0	0	**0**	*
S. lycopersicum	0	0	0	0	0	0	0	**0**	*
G. parviflora	0	0	0	0	0	0	0	**0**	*
P. persicae	0	0	0	0	0	0	0	**0**	*

* No development occurred at all.

4.3 Viral Detection on Field-Sampled Residual Tobacco Plants

PCR failed to detect the presence of PVY in both residual and infected plants. However, symptom development was used to confirm the presence of PVY and bushy top virus (which occurs in a complex with PVY). The residual plant was rated B2-3 (severe necrotic PVY symptoms) on the CORESTA scale. Symptoms observed included leaf yellowing, leaf deformation, necrotic leaf spots or rings, veinal necrosis and necrotic stem-streaking.

The indicator tobacco plants used showed variable levels of symptom development, five weeks after PVY-infected *M. persicae* were allowed to feed on them. Four tobacco plants were rated using the CORESTA scale. One was rated at B2-2 (moderate necrotic PVY symptoms), two at B2-1 (mild necrotic PVY symptoms), and the remainder at B2-0 (no symptoms).

4.4 Virus Transmission by non-*Myzus* species

Three aphid species were used in this test: *B. brassicae, A. fabae* and *A. gossypii*. Three plants were also set up for each aphid species. PCR analysis could not detect the presence of

PVY in the tobacco plants infested with the aphids previously fed on infected plants. Symptom observation also gave negative results.

4.5 Flight Patterns

Myzus persicae population increased significantly in January and February 2011 with high numbers of non-*Myzus* species being recorded in the same months (Figure 4.1). The increase came after a drop in *M. persicae* in the last quarter of 2010.

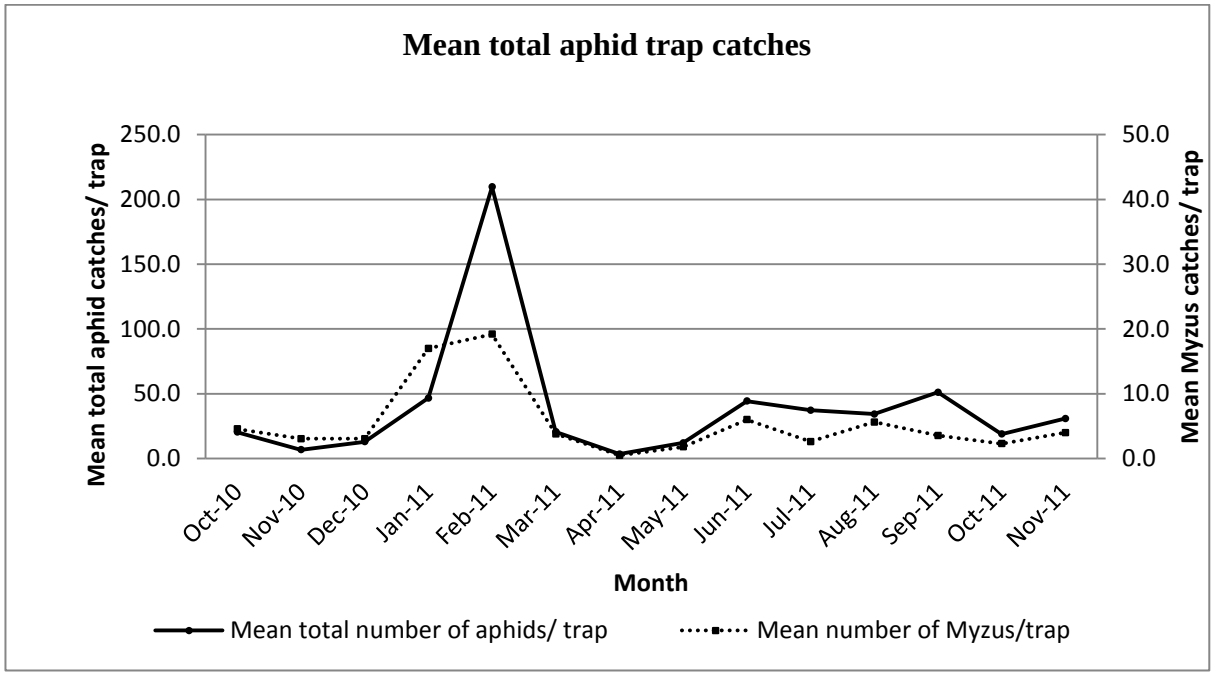

Figure 4.1. Monthly total aphid catches and *Myzus* catches for the period October 2010 to November 2011.

Several aphid species other than *M. persicae* were caught in traps. Those that were positively identified included Brevicoryne *brassicae* (cabbage aphid), *Rhopalosiphum maidis* (Corn leaf aphid), *Aphis gossypii* (Cotton aphid) and *Aphis craccivora* (Cow pea aphid).

On a weekly basis and considering *M. persicae* alone, there were fluctuations in the number of aphids caught throughout the 14- month trapping period (Figure 4.2). A peak for the *Myzus* population was observed in the 18th week, (February 2011). This peak was followed by a sharp fall in numbers, and the least catches were recorded during week 7, 24 and 57. Overall, the highest trap catches were recorded from week 11 to week 19 (between January and February 2011).

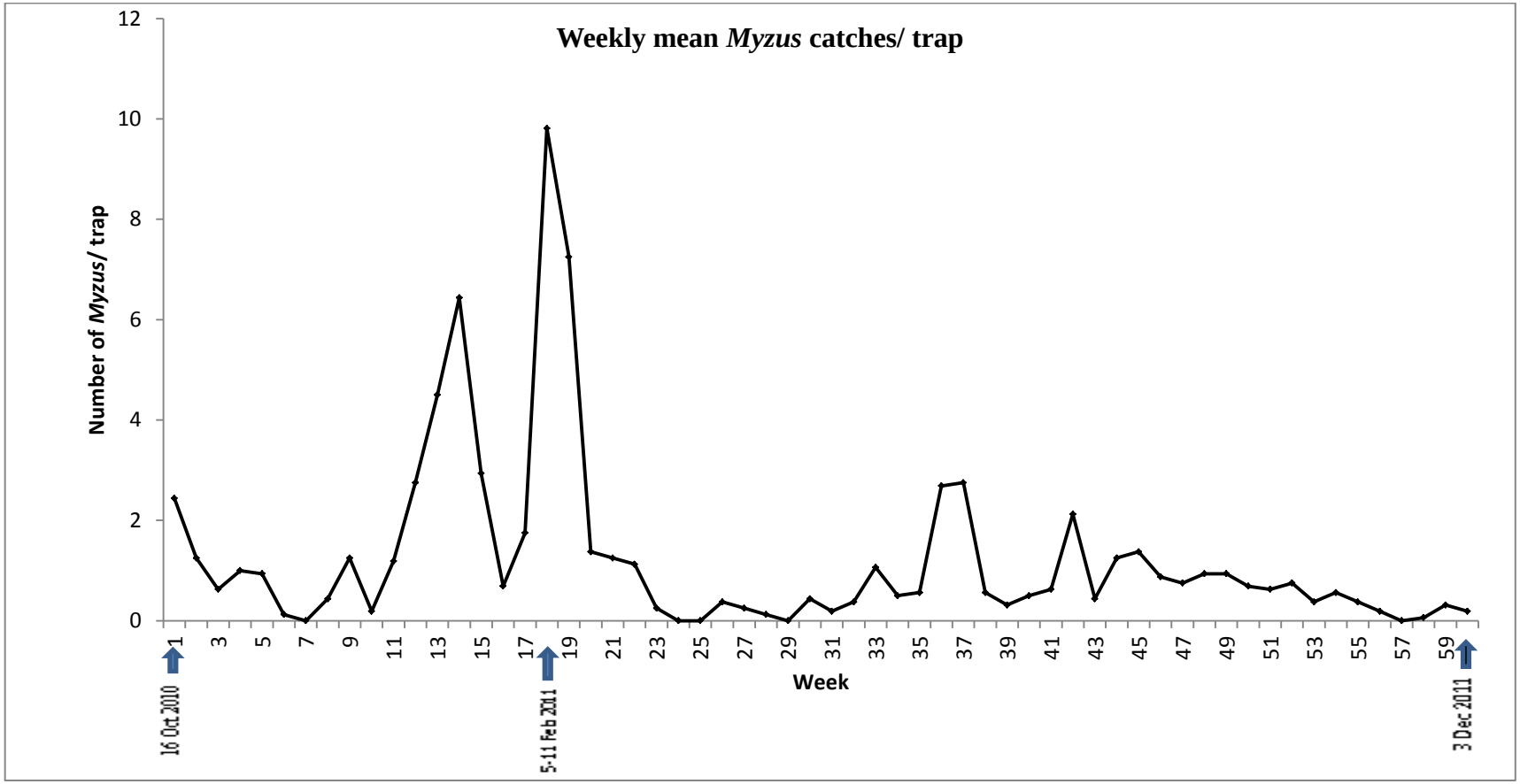

Figure 4.2. *Myzus persicae* weekly catches for the period October 2010 to early December 2011.

CHAPTER 5: DISCUSSION

The specialist tobacco-adapted form of *M. persicae* used in the experiment showed greater preference towards tobacco, its secondary host. The primary host, *P. persicae*, was not preferred at all. In an experiment carried out by Annis *et al.* (1981), *M. persicae* showed greater preference for raddish than *P. persicae*. The same result was observed in this experiment although raddish was less preferred to *S. tuberosum* and *B. rapa*. During a survey carried out prior to the tobacco growing season, it was observed that *P. persicae* had no aphids or just a few individual aphids. In Britain, the migrants of *M. persicae* from *P. persicae* were reported to be less important in initiating infestations of commercial crops than those that were overwintering in the parthogenic viviparous state on secondary (herbaceous) hosts (Broadbent and Heathcote, 1955). In the presence of alternative hosts, *M. persicae* would prefer these hosts to *P. persicae*. Therefore, *P. persicae* is less preferred as a host than *S. tuberosum* and *B. rapa* as shown by results of the present study.

In earlier studies (Bernays and Funk, 1999; Funk and Bernays, 2001; Vargas *et al*., 2005), aphid host preference was tested on a range of host plant species, including the one on which the aphid was reared, while Tosh *et al.* (2003) tested host preference on a range of plant species excluding the one on which the aphid was reared. Troncoso *et al.* (2005) observed that faster pre-alighting behaviour by *M. persicae* was absent on tobacco than on sugar beet when not reared on tobacco. All these studies show that integration of visual and alfactory stimuli for host finding depends on the aphid's prior experience (Troncoso *et al*., 2005).*Myzus persicae* was reared on tobacco, thus it is possible that the patterns of host preference observed (presumably even those found in nature) could be a result of previous experience.

Myzus persicae, which infests tobacco, favoured potato after tobacco. *Myzus persicae* has been seen to transmit PVY in potato (Eastop, 1977; Ragsdale *et al*., 2001; Radcliffe and

Ragsdale, 2002). This can be used as evidence that the same aphid which transmits PVY in tobacco is the one which transmits PVY in potato. In Zimbabwe, potatoes are grown throughout the year and may be the reservoir for the aphid during the non-tobacco growing season. When tobacco is in season, the aphids fly to tobacco farms where they infest tobacco.

Tagetes minuta had no aphid landings recorded. Aphids placed on leaf discs of *T. minuta* died on the first day. Tomova *et al*. (2005) tested the biological activity of essential oil volatiles obtained from *T. minuta* against *M. persicae*. They demonstrated that *T. minuta* oil volatiles significantly reduced the reproduction potential of the tested species. Essential oils assimilated by the aphids during probing or feeding on *T. minuta* have an adverse effect on the survival of *M. persicae*.

Secondary hosts differ considerably in the way they affect *M. persicae*. Kennedy *et al*. (1950) suggested that the degree of adaptation of an aphid to a given plant can be gauged by the extent to which an aphid can colonise the plant's leaves, not only when they are growing and senescing, but also when they are mature and fully functional. In this study, the leaves used were from immature and growing plants. *Brassica rapa* was highly suitable for the development of *M. persicae*. In a study by Heathcote (1962), it was seen that *Brassica* species were suitable for the development of *M. persicae*, especially those which grow rapidly.

Prunus persicae was highly unsuitable for the survival and reproduction of *M. persicae* nymphs. Though *P. persicae* is the primary host for *M. persicae*, it is not suitable for the survival of nymphs or adult aphids as shown in this study. In temperate climates, *M. persicae* has a strict requirement for *P. persicae* where it overwinters as sexually reproduced eggs. At the onset of summer, the eggs hatch and migrate to secondary hosts where asexual reproduction occurs (van Emden *et al.*, 1969; Devonshire *et al.*, 1998).

Tomato was also particularly unsuitable for the development of *M. persicae*. Although a few individual aphids preferred tomato, no progeny were produced and the aphids died. In a study by Johnson (1956), a rather unusual reaction of tomato to aphid probing was observed. It was noted that when glandular hairs were broken down by *M. persicae*, they exuded a liquid which adhered to the legs of aphids and impaired their grip or cemented the aphids to the leaf surface. This phenomenon led to death of the aphids.

Two distinct aphid flight peaks were observed in January and February 2011. This period was during the mid to late summer season when temperatures and rainfall were high. The finding is in line with the study done by Thomas *et al.* (1997) where aphid catches were low between spring and summer, but a large number was recorded between summer and fall.

In the study by Thomas *et al.* (1997), it was shown that three distinct and highly predictable *M. persicae* flights occur seasonally in late winter, summer and late summer. The late winter flight overwintered on *P. persicae*, but did not introduce viruses onto potato plants. The summer flight, which originated from volunteer potatoes and spring herbs originally colonized by the late winter flight, did introduce viruses into virus-free potatoes. The late summer flight was too late to affect potato production. Although *M. persicae* apterae and alatae were present on winter annual weed and crop hosts in the fall, none survived winters on these species. In this study, the peaks observed were in summer, and the summer flight is the one that initiates infestation and affects crop production. The period in which the peak was observed is the time when dead weeds and herbs spring to life after the rains, and are capable of harbouring the summer population of aphids. During this period, tobacco plants are still young and vulnerable to infection.

Myzus persicae flight patterns are directly linked to virus dissemination to cultivated crops and plants. Potatoes are grown throughout the year around Harare. At Kutsaga Research Station, peach trees were observed and most probably, *M. persicae* overwinters there but as

parthenogenetically-reproducing individuals. The small late winter flight in August from peach trees probably distributes the aphids to herbaceous, winter annual and late winter hosts throughout the tobacco-growing areas. A summer flight begins when late winter hosts begin to mature and senesce. This flight distributes aphids to summer hosts (Thomas *et al.*, 1990). Populations of *M. persicae* are therefore most probably maintained by potatoes in farms around Harare in late winter and then fly to tobacco farms in summer. PVY and Tobacco Bushy-top virus can be picked up by the late winter and summer flight from potatoes before the tobacco season and transmit it to tobacco.

PVY-infected residual tobacco plants in the field when tobacco is off season showed no signs of colonisation by aphids. In a study by Shaw (1968), it was concluded that tobacco regrowths are a source of aphids which infest tobacco in the following season. In earlier studies (Heie 1954; Baker, 1960), it was a common observation that sugar beet and potato plants showing yellows virus symptoms were more heavily colonised by *M. persicae* than healthy plants. Such plants were more attractive by their yellow colour for arriving alatae, but also the virus appeared to alter the plant so as to increase the rate of reproduction of the aphid. With potato leaf roll virus, no difference in suitability for *M. persicae* was found between diseased and healthy plants (van Emden *et al*., 1969). When field-collected residual tobacco plants were transferred to the laboratory, watered and rejuvenated, some aphid landings were observed on them while other aphids preferred fresh plants. During the tobacco off season, *M. persicae* populations may therefore not prefer residual tobacco plants in the field. When the rainy season starts and the residual plants rejuvenate, they tend to attract aphids.

The study by Davis and Radcliffe (2008) suggests that green peach aphids can effectively use winter wheat as a host, and can successfully colonize barley and rye, providing the potential to rapidly increase early in the season and subsequently colonize potato. The utilisation of

other hosts by *M. persicae* in the tobacco off season enables perpetuation of the aphid generation. The presence of other suitable hosts (weeds and cultivated crops) explains the absence of aphids on the residual tobacco plants before the rainy season. In January and February 2011, *M. persicae* populations began to rise, and there was a simultaneous increase in rejuvenated residual plants and young freshly grown tobacco plants. Aphids began to disperse from less preferred hosts towards tobacco fields. Because aphid dispersal is important to non-persistent virus spread (Kennedy and Booth, 1951), and because green peach aphid disperses more frequently from less preferred hosts (Annis *et al*., 1981), there is the potential for increased virus spread as non-preferred host acreage increases.

Myzus persicae was reported to be most effective in its role as PVY and bushy-top vector (Halbert *et al*., 2003, Warren *et al*., 2005). In South Africa, *A. fabae* and *A. gossypii* are among the most common and efficient PVY vectors found in potato fields (Warren *et al*., 2005). However, these non-colonising aphids have been seen to be unable to transmit PVY to tobacco. From previous studies, information can be gathered that PVY can be transmitted indirectly to tobacco by non-colonising aphids. From the current study as well as by Davis and Radcliffe (2008) and Van Hoof (1980), it was seen that potato and Chinese cabbage are the preferred hosts of *M. persicae*, and the aphid is able to pick up PVY from potatoes. Potatoes are grown all year round, and Chinese cabbage is also grown in farms and greenhouses around Harare. *Myzus persicae*, therefore, seems to have common hosts which enable easy virus transmission and acquisition between infected and uninfected aphids. The non-colonising aphids were seen to be unable to transmit PVY and bushy-top directly, but there is a possibility for indirect transmission.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Myzus persicae nicotianae preferred tobacco over other alternative host plants. Tobacco is also the most suitable for development of the aphid. During the rainy season, the aphid population attained its highest peak in February 2011. On the basis of symptoms, residual plants were seen to harbour PVY and bushy-top, and had a high potential of being transferred to next season tobacco. The non-colonising aphid species tested — *A. gossypii*, *A. fabae* and *B. brassicae* — were not able to transmit PVY and bushy-top diseases to tobacco. However, this is not conclusive proof as a limited number of plants were tested.

Myzus persicae has a very wide host range although it prefers tobacco, potato and Chinese cabbage more than other weed hosts. To circumvent infestation early in the tobacco season, Integrated Pest Management has to be practiced in large areas of land simultaneously and continuously. Residual tobacco plants have to be eliminated before the rainy season as they might be a source of infestation.

Tomato and *T. minuta* were highly unsuitable hosts for the establishment of *M. persicae*. Intercropping with such plant species or growing tobacco in close proximity to such plants would deter the aphids or help reduce their numbers. Intercropping of compatible plants also encourages biodiversity, by providing a habitat for a variety of insects and soil organisms that would not be present in a single-crop environment. This biodiversity can in turn help to limit outbreaks of crop pests (Altieri, 1994) like *M. persicae*, by increasing the diversity or abundance of natural enemies, such as spiders or parasitic wasps. Increasing the complexity of the crop environment through intercropping also limits the places where pests can find optimal foraging or reproductive conditions. Intercropping does not only reduce the aphid population, but can also improve tobacco quality and yield (Shikai *et al*., 2009).

The non-colonising aphids tested in this study failed to directly transmit PVY and Tobacco Bushy-top viruses. However, a study needs to be done to determine if the non-colonising aphids can indirectly transmit the viruses through intermediate hosts, which could also be favourable hosts for *M. persicae*. It is also recommended that as many 'populations'of *M. persicae nicotianae* as possible be tested as the results of the current study cannot be extrapolated to all parts of Zimbabwe. In addition, the importance of residual tobacco plants in the carryover of *M. persicae nicotianae* and consequently, PVY and Tobacco Bushy-top virus, needs to be studied more thoroughly.

The exoneration of residual tobacco plants from being of major importance in the carryover of *M. persicae nicotianae* and the transmission of PVY and Tobacco Bushy-top can only come about if studies to analyse the gut contents of aphids (i.e. *M. persicae* complex) caught in water traps during the dry season are carried out. If such tests show that aphids caught in traps would have originated from non-tobacco plants, then the legislation on the tobacco 'dead period' may need to be revisited. For farmers, this might mean taking deliberate measures to control aphids on non-tobacco hosts during the off-season.

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