The Relationship Of Time Of Year, Geographic Location, Insecticide Exposure And The Genotype Of Red And Green Morphs Of The Tobacco Aphid, Myzus nicotianae Blackman, In Virginia

by

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(ABSTRACT)

The relationship of geographic location, insecticide exposure, time of season and the genotype of red and green morphs of the tobacco aphid, Myzus nicotianae Blackman, on tobacco was investigated in Virginia in 1988 and 1989. Color morph and karyotype were found to be highly related. The translocated karyotype was associated with the red morphs and the normal karyotype was associated with the green morphs. The karyotype of red and green morphs did not change markedly from the beginning to the end of the tobacco growing season.

Studies were also conducted to determine if the red and green morphs of the tobacco aphid were developing resistance to acephate (Orthene Tobacco Insect Spray), the most commonly used aphicide on flue-cured tobacco in Virginia. Slight resistance was present in both red and green aphid populations from several counties.

Studies were also conducted to determine if males were present in tobacco aphid populations in Virginia. Cool temperatures and short

photoperiods which initiate male production in Myzus persicae (Sulzer) with holocyclic life cycles did not cause the production of males in M. nicotianae native to Virginia.

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Chapter 1
INTRODUCTION AND OBJECTIVES

Tobacco, Nicotiana tabacum (L.), is a subtropical plant whose history began in the New World on October 12, 1492 when Columbus landed in the West Indies (Hawks and Collins 1983). The commercial tobacco industry was started in Jamestown, Virginia in 1612 by John Rolfe. In 1989, 37,000 acres of flue-cured tobacco were grown in Virginia. Tobacco farmers marketed 95.6 million pounds of tobacco in Virginia and collected cash receipts of $162 million. In the United States in 1989, tobacco growers produced 916 million pounds of tobacco with a cash receipt of $1.5 billion (Stan Duffer, VA Dept. of Ag, personal communication).

Since 1946 aphids have been pests of tobacco in the United States (Dominick 1949; Chamberlin 1958). The aphid commonly found on tobacco in North America has been called the green peach aphid, Myzus persicae (Sulzer) (Chamberlin 1958). Blackman (1987) examined aphids from North America, the Mediterranean region, Middle East, Africa, and Sri Lanka, and described the aphid found on tobacco as a new species specially adapted for feeding on tobacco. He named it Myzus nicotianae Blackman and the official common name is the tobacco aphid. Myzus nicotianae differs morphologically and is reproductively isolated from M. persicae. It closely resembles M. persicae, but has a longer ultimate rostral segment relative to body size and a longer terminal process of the antennae. The tobacco aphid is larger than the green peach aphid and differs slightly in the shape of the cornicles (siphunculi).

The tobacco aphid is an economically important pest on tobacco in Virginia (Dominick 1949, Semtner 1983). It injures tobacco mainly by removing plant juice and depositing honeydew (Chamberlain 1958). There is a premature yellowing of infested leaves and necrotic tissue may develop along the leaf margins and at the base of the petiole (Dominick 1949). Sooty mold (Fumago vagans) can develop on leaves coated with honeydew and cause additional leaf discoloration (Dominick 1949). Myzus persicae can also damage tobacco by the transmission of viruses. Myzus persicae is the most important aphid species in plant virus transmission (Sylvester 1954)

Recent reports in Virginia of failure in controlling the red morph of the tobacco aphid has caused much concern because currently only three foliar insecticides and one soil insecticide are recommended for the control of aphids on tobacco (Jones et al. 1988). The foliar insecticides still effective against the tobacco aphid include: methomyl (Lannate), acephate (Orthene), and endosulfan (Thiodan). Aldicarb (Temik) applied as a pretransplant soil treatment is also effective. Studies in North Carolina by Lampert (pers. comm.) indicate that the red morph of the tobacco aphid is more resistant than the green morph to certain insecticides including monocrotophos (Azodrin) and acephate (Orthene). A chromosome translocation found in many of the red morphs causes some concern because the translocated karyotype found in M. persicae is often associated with increased tolerance to organophosphorous insecticides.

It is important to determine whether the translocated chromosomes occur at similar levels in the populations of red and green morphs of the tobacco aphid at various times of the year, at different geographic locations

in Virginia, and following exposure to various insecticides used for its control. Thus the objectives of the study were:

1. To determine karyotypic variation of M. nicotianae as influenced by time of year, geographic location in Virginia, and following exposure to various insecticides used for aphid control.

2. Investigate the level of tolerance of aphids from various counties in Virginia to acephate (Orthene Tobacco Insect Spray).

3. Determine the relationship between karyotype and insecticide tolerance to acephate (Orthene Tobacco Insect Spray).

4. Investigate the life cycle of M. nicotianae in Virginia.

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Chapter 2
LITERATURE REVIEW

Since Myzus nicotianae has been recently described as a new species (Blackman 1987a) separate from Myzus persicae (Sulzer), little information is available on this species. However, many papers have been published on M. persicae. Because literature on M. nicotianae is limited and because M. nicotianae is similar to and has been synonomous with M. persicae for many years, information about M. persicae will be considered in this literature review.

Myzus nicotianae Blackman, the tobacco aphid, and Myzus persicae, the green peach aphid, are hemimetabolous insects belonging to the order Homoptera. They have piercing-sucking mouthparts and feed on plant sap. Myzus persicae probably originated in southeast Asia, since its primary host plant is the peach, Prunus persicae which is a native of China (Blackman 1981). Blackman (1987a) believes M. persicae and M. nicotianae share a common origin because M. persicae was a pest of tobacco in East and South East Asia for many years before it was a pest in North America.

Although the primary host of M. persicae is peach, M. persicae may colonize other Prunus spp. and a wide variety of secondary hosts (Blackman and Eastop 1984). According to Tamaki (1981), M. persicae has a host range of over 875 plant species. Essig (1948) believed that man's development of modern agriculture is responsible for the wide range of secondary hosts. At present little is known about the host range of M. nicotianae. Myzus nicotianae is a form of M. persicae adapted for feeding on tobacco

(Blackman 1987a). The tobacco aphid is found on several other host plants but was not reported on potatoes (Blackman 1987a); however, Boiteau and Lowery (1989) found M. nicotianae on potatoes in the greenhouse.

Color Morphs

There are two color morphs associated with M. nicotianae -- a red morph and a green morph. In North America, M. nicotianae populations have been composed of the green morph. However, a red morph was infrequently observed from 1983-1985 (Reed and Semtner 1989) and became the predominant form found on tobacco in 1986 (Reed and Semtner1989, McPherson 1989).

In Japan, yellow, green, and red morphs of M. persicae are found on tobacco (Takada 1981). The green and yellow forms of M. persicae are much rarer on tobacco than the red morph (Takoaka 1958) (cited in Ueda and Takada 1977). Ueda and Takada (1977) attribute the relative abundance of red and green morphs of M. persicae infesting a plant to the family of host plant upon which the aphids are feeding. They believe cruciferous plants are more likely to be colonized by the green-yellow morphs, and solanaceous plants by the red morph.

According to Takada (1981), one pair of alleles with the red color characteristic dominant is responsible for the production of color. Environmental factors, nutritional conditions of the host, and population density may also modify color (Takada 1981). Studies in Japan by Takada (1981) demonstrated that under short day conditions yellow and green clones produce progeny of two different colors. The apterous progeny were the

same color as the parent; however, the winged progeny were red. Blackman (1974a) stated that color results from the presence of a series of glycosides (protaphin, aphinin, and a colorless fluorescent) in the aphid hemolymph. The aphid's color depends primarily on the proportion of these three substances in the hemolymph (Blackman 1974a).

Studies have shown that the red morph of the tobacco aphid is more tolerant of high temperatures than the green morph (Reed 1987). At 25 and 30^{o}C, the red morph can survive and reproduce at higher rates than the green morph (Reed 1987). Research by Ueda and Takada (1977) indicates that red anholocyclic populations of M. persicae are able to survive winter better than green anholocyclic strains.

Karyotype

Karyotypic variation within a population of a given aphid species is quite common. Blackman (1980) has suggested two mechanisms to explain this phenomenon. First, aphids have holocentric chromosomes. The centromeric activity of the holocentric chromosome is dispersed along the entire length of the chromosome. Therefore, if a chromosome breaks into two or more parts the fragments can all move independently into the daughter cells (Ris 1942). Second is the ability of aphids to reproduce by thelytoky. Thelytokous reproduction is the production of females from an unfertilized egg (Blackman 1980).

Blackman (1971a) and Sun and Robinson (1965) reported 2n = 12 as the normal karyotype for M. persicae. The 2n = 12 karyotype is present in M. nicotianae (Blackman 1987). Myzus persicae from Chile and California had

a 2n=11 chromosome complement (Blackman 1980). In Italy, Cognetti (1961) (cited in Blackman 1971a) reported 2n=14 to be the normal karyotype in M. persicae. Myzus persicae from tobacco in India normally has a 2n=8 karyotype (Chattopadhyay and Raychaudhui 1980).

Two chromosomal abnormalities have been found in M. persicae (Blackman and Takada 1977). One type is due to a translocation between autosome 1 and 3 resulting in a 2n=12 translocated karyotype (Blackman and Takada 1977). A translocation is also found in M. nicotianae (Blackman 1987). The other type of chromosomal abnormality is a dissociation of one of the autosomes producing a 2n=13 karyotype (Blackman and Takada 1977). Blackman and Takada (1977) speculate that both chromosome abnormalities arise from the same mutation of autosome 3. The dissociation could be the first step in the translocation (Blackman and Takada 1977). Some M. persicae have a second dissociation resulting in a 2n=14 karyotype (Blackman 1980).

The translocated and dissociated forms occur only in the heterozygous condition and can be inherited through the sexual phase of aphid reproduction (Blackman and Takada 1977). Translocated heterozygotes are usually not found in sexually reproducing populations (White 1973) (cited in Blackman and Takada 1977) because they will produce a large proportion of gametes with duplication or deficiencies of genetic material. Therefore, in aphid populations which can reproduce continuously by parthenogenesis, it is not suprising to find a high proportion of translocated individuals (Blackman and Takada 1977). In Japan, Blackman and Takada (1977) reported

translocations in field populations of holocyclic M. persicae which undergo sexual reproduction in autumn.

Life Cycle of Myzus persicae and Production of Sexuales

Myzus persicae has a worldwide distribution and its life cycle varies between different regions of the world (Blackman 1974b). Blackman (1974b) describes life cycle variation as a characteristic of M. persicae. Life cycle variation of an aphid can be explained by climatic differences and the effect of these differences on gene and genotype frequencies within the species (Blackman 1974b). Myzus persicae can overwinter on winter hosts as parthenogenetically reproducing females (anholocycly) or overwinter as eggs on peach trees (holocycly). In androcyclic populations, reproduction occurs throughout the year by parthenogenesis; however, some males are produced to contribute to the sexual phase. The success of holocyclic reproduction depends upon the ability of the oviparae to reach sexual maturity before leaf fall of the primary host, which in the case of M. persicae is the peach tree (Takada 1982b). Anholocycly occurs because environmental conditions are not suitable to cause a switch from parthenogenetic to sexual reproduction (Blackman 1974b). Therefore, there is a continuous production of aphids adapted for parthenogenetic reproduction (Blackman 1974b). In parthenogenetic reproduction the karyotype of the mother is passed on to her progeny. The parthenogenetic female also has an influence on her grandaughters (Blackman 1979). Anholocyclic reproduction of M. persicae on secondary hosts has been reported in South Carolina, Georgia, and Florida (Lawson and Chamberlin 1957). Anholocyclic reproduction is also

possible in Kentucky if the secondary hosts can survive the winter conditions (Fusco and Thurston 1968).

Studies by Blackman (1971b) show that there is considerable variation in photoperiodic response of aphids not only in different regions of the world; but also, between individuals within a natural population of M. persicae. Myzus persicae collected from summer populations in Southern England and reared in the laboratory under a 10h photoperiod and 20^{o}C showed various responses to the short photoperiod. Blackman (1971b) separated these responses into four categories: 1) Holocyclic -- a condition in which parthenogenesis is interrupted by the production of male and female sexual morphs; 2) Intermediate -- a mixed response involving an alate intermediate between gynoparae and virginoparae; 3) Androcyclic -- reproducing by parthenogenesis during the year, but producing some males to contribute to the sexual phase; and 4) Anholocyclic -- continuous parthenogenesis without the production of male and female sexual morphs.

Sexual morphs are produced in response to a certain photoperiod and temperature (Blackman 1971b). Under laboratory conditions, the host plants can have a temporary effect on the production of sexual morphs (Blackman 1974b). The production of males occurs parthenogenetically. This type of reproduction is termed arrhentokoy (Blackman 1987b). Male production results from the loss of half of the sex chromatin of the parent female in a single maturation division producing a XO individiual (Blackman 1980).

A study by Mittler and Matsuka (1985) found that only two successive long night (LN) exposures were necessary for the production of males. However, many LN exposures were necessary to produce males exclusively.

Mittler et al. (1979) states that males of M. persicae are produced as a result of a reduction in juvenile hormone produced by the corpora allata. A precocene analogue: 6-methoxy-7-ethoxy-2,2-dimethylchromene has been used successfully to produce males in M. persicae (Hales and Mittler 1983). Precocene causes male production by affecting juvenile hormone levels (Hales and Mittler 1983).

Color morphs of M. persicae can differ in their response to photoperiod and temperature. A laboratory study by Takada (1982a) compared the influence of photoperiod and temperature on sexual morph production of red and green morphs of M. persicae. Myzus persicae were reared at photoperiods ranging from 9-15 hours and temperatures ranging from 13 to 25°C. Takada (1982a) found the critical daylength at which 50% of the first generation apterous progeny became gynoparae of the red and green morphs of M. persicae to be 12h 30 min, and 12h 45 min respectively at 18°C. The suppression of alate viviparae was seen when temperatures were increased above 21°C. This phenomenon was more prominant in the red morphs than in the green. Appearance of males was also suppressed at long photoperiods and high temperatures. Male production in the red morph was suppressed more by these conditions than in the green morph.

Another study by Takada (1982b) demonstrated that temperature had more influence than photoperiod on production of sexual morphs of M. persicae reared under natural field conditions. If daylength was the controlling factor, then appearance of sexual morphs should occur at the same time each year (Takada 1982b). However, the appearance of sexual morphs in the two experiments performed by Takada in 1975 and 1976

occurred at different times. Under laboratory and natural conditions the green morphs began sexual morph production earlier than the red morphs.

Matsuka and Mittler (1979) found the critical daylength for production of sexuales to be 13h 45min - 14h, an hour longer than that found in Takada's study. Bonnemaison (1951) (cited in Takada 1982a) in France showed the critical daylength to be 12h 30min -14h. This demonstrates another example of the variation that can occur in populations of M. persicae in different regions of the world.

Insecticide Resistance In The Green Peach Aphid

Insecticide resistance has been a problem for growers of agricultural crops for many years. Insecticide resistance in aphids was first reported in 1928 for the cotton aphid, Aphis gossypii (Glover) (Boyce 1928). The effect of repeated insecticide application is responsible for the increase in insecticide resistance (Ffrench-Constant et al. 1988). Resistance problems seem inevitable when insect pressure and injury to the plant begin to increase, because higher dosages of the insecticide are used and more frequent sprays are necessary (Georghiou 1963). Thus aphids are becoming better fit to tolerate the insecticides.

Resistance in M. persicae was first reported by Anthon (1955). Myzus persicae on peach trees in north central Washington showed resistance to three organophosphorous insecticides (malathion, parathion, and tetraethyl pyrophosphate). Baunerfield and Chapman (1985) reported M. persicae resistance to parathion and endosulfan on potatoes. Koziol and Semtner (1984) found resistance to acephate in several field strains of aphids

on tobacco from Virginia. However, no resistance was shown to disulfoton, malathion, methyl parathion, and monocrotophos. Resistance to organophosphorous insecticides has been prevalent in Japan for more than ten years (Takada 1979). Aphid populations resistant to organophosphorous insecticides are common in greenhouses in Great Britain (Blackman et al. 1978). Dunn and Kempton (1966) reported resistance to demeton-methyl in a greenhouse strain.

Resistance in aphids to organophosphorous and other insecticides is caused by an insecticide hydrolyzing enzyme (Sawicki et al. 1980). Organophosphorous resistant M. persicae show an elevated level of carboxylesterase (E4) (Needham and Sawicki 1971, Devonshire and Moores 1982, Sudderuddin 1973)). Baraneck and Oppenoorth (1977) have demonstrated that carboxylesterase (E2) is the same as the organophosphorous hydrolyzing enzyme. These findings are based on the fact that both enzymes are present in the same starch fraction after electrophoresis. E4 can detoxify the insecticde by hydrolysis and sequestration of the insecticide (Devonshire and Moores 1982). Blackman et al. (1978) showed that E4 activity varied among individuals in the 12 translocated F_1 clones. However, research by Takada (1979) did not show intraclonal variation. It is possible that carboxylesterase is not the only resistance mechanism and that more than one mechanism is involved (Blackman et al. 1977, Sudderuddin 1973). However, at present no other mechanisms have been examined.

In M. persicae a chromosome translocation involving autosome 1 and 3 is often associated with aphids showing resistance to organophosphorous

insecticides (Blackman 1980). Translocations have been found in aphid populations in the U.S., Japan, and Great Britain. The first translocation was discovered in highly resistant androcyclic clones of M. persicae in greenhouses in Great Britain (Blackman and Takada 1975). Blackman (1980) postulates that the spread of the translocated resistant form throughout the world may have occurred predominantly through thelytokous reproduction but with some gene recombination introduced by an occasional holocycle.

In England, crosses between organophosphorous susceptible sexual females with a 12N karyotype and organophosphorous resistant males with 12T karyotype revealed that the translocated karyotype and resistance mechanism are linked when inheritance is through the male (Blackman et al., 1978). Studies by Blackman et al. (1978) correlate the 12T karyotype with an increase in carboxylesterase (E4) levels. On these assumptions, it seems probable that resistance is found in the translocated individuals in the population. However, aphid populations with organophosphorous resistance in sugar beet fields in England and from peach trees in France had the normal karyotype (Blackman et al., 1978). Takada (1979) states that the translocation may cause a rearrangement of the genes contributing to the resistance mechanism. Blackman et al. (1977) proposes a similar idea that the translocation may provide an advantageous rearrangement by bringing two genetic loci for resistance on the same chromosomal element.

In many instances the red morph of M. persicae has the translocated karyotype, which may be associated with an increased resistance to organophosphorous insecticides (Blackman et al., 1978). Lampert (pers.

comm.) found that the red morph of M. nicotianae is more tolerant to monocrotophos (Azodrin) and acephate (Orthene) than the green morph, but not to methomyl (Lannate). Takada (1979) has found no evidence to show that increased activity of E4, causing insecticide resistance, is linked with body color, life-cycle or karyotype.

Organophosphorous-resistant clones of M. persicae can grow and reproduce better on suitable hosts under favorable conditions than organophosphorous-susceptible clones. However, when reared on unsuitable hosts in unfavorable conditions the susceptible clones develop and reproduce better (Eggers-Schumacher, 1983). Baker (1977) showed that repeated insecticide sprays select for resistant aphids, but in the absence of insecticide pressure susceptible aphids are favored and resistant aphids disappear. A laboratory experiment by Gordon and McEwen (1984) indicated that in M. persicae populations treated with azinphosmethyl (Guthion) the adult aphids reproduce earlier, thus increasing the initial populations. Lowery and Sears (1986) found M. persicae reared in the laboratory on potato leaf disks treated with azinphosmethyl at 550ppm produced 28% more offspring than those on untreated leaf disks. Banks and Needham (1970) showed that apterous M. persicae with resistance to dimethoate began reproducing at the same age as susceptible aphids. The resistant aphids initially reproduced faster than the susceptible aphids; however, they began to slow down after reproducing for 10 days so that the susceptible aphids eventually produced the same number of progeny. Morphs of resistant aphids develop faster, are larger, and survive for a shorter period of time than susceptible morphs (Banks and Needham, 1970). Resistant aphids were as effective as

susceptible ones in transmitting disease and when resistant aphids were the disease vectors the plants showed symptoms earlier (Banks and Needham 1970).

Loss of resistance is associated with a decrease in carboxylesterase (E4) activity (Sawicki et al. 1980). When reared in insecticide-free environments the resistant aphids may revert to susceptible individuals. Bauenerfield and Chapman (1985) reported that endosulfan and parathion resistant M. persicae populations on potatoes reverted to susceptible aphids in 10 to 27 generations when reared in an insecticide-free environment. Dunn and Kempton (1966) have also demonstrated a decline in resistance in demeton-methyl resistant aphids with complete reversion to susceptibility in 30 generations. There is some variability in the rate of loss of resistance even between individuals from the same clone (Sawicki et al 1980). The rate of loss can range from 7 generations to 3 years (Sawicki et al., 1980). Ffrench-Constant et al. (1988) observed four different patterns of decline in resistance when aphids were reared in insecticide-free environments: 1) slight loss of resistance; 2) a stable intermediate with no susceptibility; 3) susceptibility within nine generations; and 4) fluctuation between susceptibility and resistance. In Japan, no loss of resistance was shown when M. persicae were reared without insecticide pressure (Takada, 1986). Ffrench-Constant et al. (1988) found that all aphid colonies that had lost resistance in their study had the translocation involving autosome 1 and 3. This suggests that there is some involvement between the translocated karyotype and instability of resistance (Ffrench-Constant et al., 1988). The translocated individuals also demonstrated recovery of resistance when

retreated with insecticides (Ffrench-Constant et al., 1988). According to Ffrench-Constant et al. (1988), loss and recovery of resistance only occurs in the translocated individuals.

Green Peach Aphid On Tobacco

The aphid commonly found on tobacco was called the green peach aphid, Myzus persicae (Sulzer), until Blackman (1987) described it as a specialized form of M. persicae adapted for feeding on tobacco. He named the aphid Myzus nicotianae. Blackman examined preserved specimens of aphids collected from tobacco in the 1920's from Southeast Asia and Europe, in the 1930's from Africa, and in the 1940's from North America and determined that these were M. nicotianae. Myzus nicotianae has been the aphid pest on tobacco in the United States for many years and is probably synonomous with M. persicae on tobacco. Since it is primarily a pest of tobacco it was given the common name the tobacco aphid. This aphid is one of the most important insect pests of tobacco in North America. It can injure all types of tobacco. Tobacco grown in fields that are shaded part of the day is the most susceptible to aphid infestation because it provides a favorable environment for aphid development. Burley tobacco is least susceptible to aphid attack (Chamberlin 1958). The aphid became an economic pest of tobacco in the United States in the mid 1940's (Dominick 1949, Chamberlin 1958, Kulash 1949). In 1946 aphid infestations in Virginia were confined to three fields in Pittsylvania County (Dominick 1949, Chamberlin 1958). Distribution of aphids from field to field and within field was sporadic (Dominick 1949). Moderate infestations occurred in 1974 and serious

damage developed in 1975 and 1976 in North America (Cheng and Court 1977). The green peach aphid is an economically important pest in Virginia (Semtner 1983).

Myzus persicae feeds in the tobacco plant phloem, thus avoiding the nicotine and other alkaloids present in the xylem (Gutherie et al. 1962). Aphids injure tobacco by removing plant sap and depositing honeydew (Chamberlin 1958, Cheng and Court 1977). Black sooty mold, Fumago vagans, can develop on leaves coated with honeydew and cause leaf discoloration (Chamberlin 1958, Cheng and Court 1977). There is premature yellowing of infested leaves and necrotic tissue may develop along leaf margins and at the base of the petiole (Dominick 1949). Heavy aphid infestations can stunt the growth of young plants in the field (Chamberlin 1958). Myzus persicae may also damage tobacco by the transmission of various plant diseases (Chamberlin 1958).

Heavy aphid infestations can alter the chemical composition of the tobacco leaves. A decrease in nicotine is seen in plants with high aphid populations (Feinstein and Hannan 1951, Chamberlin 1958). Reducing sugars are significantly higher in uninfested leaves and aphid infested leaves contained higher concentrations of total nitrogen and starch than the uninfested leaves (Cheng and Court 1977). These injuries to the tobacco plant result in reductions in both yield and quality of the cured tobacco leaf.

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Chapter 3

Comparisons of the Chromosomes of Red and Green Morphs of the Tobacco Aphid, Myzus nicotianae Blackman, as Affected By Time of Year and Geographic Location in Virginia.

The tobacco aphid, Myzus nicotianae Blackman, has been a serious pest of tobacco in the United States since the mid 1940's (Dominick 1949, Chamberlin 1958). There are two color morphs associated with M. nicotianae -- a red form and a green form. Traditionally tobacco aphid populations have been composed of the green morph. However, a red morph was infrequently observed from 1983-1985 and became the predominant morph found on tobacco in Virginia in 1986 (Reed and Semtner 1989).

There are three karyotypes characteristic of M. nicotianae: 1) 2n=12 normal chromosome complement; 2) 2n=12 translocated form (10 nomal sized, 1 long and 1 short chromosome); 3) 2n=13 dissociated form (11 normal chromosomes and 2 short chromosomes) (Blackman 1987). Karyotypic variation is quite common among aphids especially those which have an anholocyclic life cylce (Blackman 1980). Aphids have holocentric chromosomes and the centromeric activity of the holocentric chromosome is dispersed along its entire length. Therefore, if a chromosome breaks into two or more parts the fragments can all move independently into the daughter cells (Ris 1942).

Blackman (1987) found that all red morphs sampled from Virginia, North Carolina, and Maryland had a chromosome translocation. The translocation is a characteristic of insecticide resistant Myzus persicae.

Therfore, it is important to determine whether the translocated chromosomes occur at similar levels in the field populations of red and green morphs at various times of the year in different geographic locations in Virginia. Thus, this study was initiated to compare the chromosomes of red and green morphs of the tobacco aphid in various counties in Virginia and determine if there was a seasonal change in the karyotype of the aphid throughout the tobacco growing season.

Materials and Methods

In 1988 tobacco aphids were collected from tobacco grown on commercial farms in eight counties in Virginia. In 1989 tobacco aphids were collected once or twice monthly from tobacco grown on eleven farms in nine counties to determine if there was a seasonal change in the karyotype of the aphid during the tobacco growing season.

Aphids were collected from five locations within the field at each sampling. When it was possible, both red and green morphs of M. nicotianae were sampled. A camel's hair brush was used to remove the aphids from the infested tobacco leaf. The aphids were maintained alive on excised tobacco leaves from greenhouse plants. The leaves were inserted in water agar in the bottom of 473 ml styrofoam cups. A translucent plastic lid was placed on each cup and the cups were placed in growth chambers maintained at 22^{o}C and a 16h photophase. The colonies were allowed to become established on the leaves and reproduce before the karyotypes were determined. Ten aphids were karyotyped from each colony.

The aphid chromosome squash preparation developed by Blackman (pers. comm.) was used for the chromosome observation. This procedure involves placing a live aphid in 0.75% KCl and removing the embryos. The embryos are placed in aphid preservative (3 parts methanol:1 part glacial acetic acid) for 10-15 minutes. Finally 5-6 embryos without eye pigmentation are placed in a drop of 45% proponoic acid on a glass microscope slide. A coverslip is placed over the embryos and the slide is inverted on a piece of filter paper with pressure applied to the coverslip to squash the embryos. Clear fingernail polish is put around the perimeter of the coverslip to prevent the slide from drying up.

The chromosomes within the cells of the embryos were examined using a Baush and Lomb Balplan Phase Contrast Microscope. The slides were scanned at 350X magnification for cells with visible chromosomes in mid to late prophase. The chromosomes were observed at 1500X magnification. Chromosome number and length were determined and the presence of the translocation was also noted. Chromosome length was measured using a micrometer located in one of the microscope eyepieces.

Results and Discussion

The survey in 1988 was conducted to determine the variation in karyotypes of M. nicotianae from different locations in Virginia. Aphids were sampled from three counties in the burley tobacco producing region and from five counties in the flue-cured tobacco region (Fig 3.1). The numbers for each of the karyotypes from the eight counties in Virginia where aphid populations were collected are shown in Table 3.1.

Ninety red morphs of M. nicotianae were karyotyped. A large majority of the red aphids had a non-reciprocal translocation (90%) (Figure 3.2), while only 9% possessed the 2n=12 normal chromosome complement and 1% the 2n=13 karyotype (Table 3.1). Populations of green aphids were only present at 4 of the 11 farm locations. Among the green morphs examined 87.5% had the 2n=12 normal karyotype (Figure 3.3). The translocated karyotype was found in 7.5% of the populations tested and 5% contained the 2n=13 dissociated chromosome complement (Figure 3.4).

The occurrences of the 2n=12 normal karyotype between the green and red morphs of M. nicotianae was significantly different ($P < 0.001$). Likewise, the presence of the translocated chromosome complement was significantly different ($P < 0.001$) between the two color morphs. The total number of aphids with the 2n=13 karyotype was very small and there was no significant difference between the red and green morphs (Table 3.2).

The aphid survey in 1989 was conducted to determine the seasonal change in the aphid karyotype throughout the tobacco growing season. Aphids were sampled from 2 farms in the burley tobacco region and 9 farms in the flue-cured tobacco region (Fig 3.5). The non reciprocal translocation occurred in 99.2% of the 350 red morphs sampled (Table 3.3). There were no red morphs with the 2n=13 karyotype and only 0.8% showed the 2n=12 normal karyotype. Four percent of the green morphs sampled had the translocation. The most common chromosome complement found in the green morphs was the 2n=12 normal (96%). No green morphs contained the 2n=13 karyotype. The occurrence of the translocated karyotype in the red morphs was significantly different ($P < 0.0001$) from that in the green

morphs. The presence of the normal karyotype also differed between the two color morphs (Table 3.4).

The relative lengths of the chromosomes for each of the 3 karyotypes is shown in Table 3.4. Determining the differences in autosome 2 and 3 of the normal karyotype was difficult since the relative lengths are the same for these chromosomes. The non-reciprocal translocaton found in 1988 and 1989 is assumed to be the same as was found by Blackman (1987). This assumption is based upon Blackman's identification of the translocation in populations of the red morph of M. nicotianae from Virginia and the lengths of autosome 1 and 3 which indicate that a break has occurred in one chromosome and has become translocated on another chromosome.

The results from the 1988 and 1989 study indicated that karyotype and color morph are highly related. The translocated karyotype was related to the red morphs of M. nicotianae and the $2n=12$ normal karyotype was associated with the green morphs. The $2n=13$ chromosome complement was most prevalent in the green morphs. This seems somewhat surprising since the $2n=13$ dissociated karyotype is thought to be the first step in the translocated karyotype (Blackman and Takada 1977). According to this theory the $2n=13$ karyotype should be more closely associated with the red morphs.

There appears to be no relation between karyotype and geographic location in Virginia. M. nicotianae collected from all counties in 1988 and 1989 showed the translocation to be most prevalent in the red morphs, while the green morphs most frequently possessed the normal karyotype. The type of tobacco did not influence the karyotype of M. nicotianae. Red and green

morphs of M. nicotianae collected from burley and flue-cured tobacco showed no differences in karyotypes.

The 1989 study indicated that there was no apparent seasonal change in the karyotype of M. nicotianae. The 2n = 12 karyotype was only found in 3 of 100 red morphs examined the first week of June. All other red morphs sampled had the translocation (99.2%). The normal chromosome complement was most commonly found in the green morphs (96%). Only 6 of the 150 green morphs sampled had the translocation.

There were slightly more red morphs with the 2n = 12 normal karyotype in 1988 than in 1989. This may show a trend toward the occurrence of only translocated individuals in the population of red morphs of the tobacco aphid. In 1987 all red morphs examined by Lampert in North Carolina had the translocation. Blackman (1987) found that all red morphs sampled in North Carolina, Virginia, and Maryland had the translocated karyotype. However, green tobacco aphids from Maryland examined by Blackman in 1977 had the 2n = 12 normal chromosome complement (Blackman 1987).

In separate observations, red tobacco aphids from Canada and Connecticut were examined. All red morphs examined from Canada in 1989 had the translocation. The translocated karyotype was found in 70% of the population of red morphs examined from Connecticut in 1988. However in 1989, 90% of the red morphs from Connecticut had the translocation. This may also be an indication that in the future only translocated individuals will be present in the population of red morphs of the tobacco aphid.

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Table 3.1. Karyotype of red and green morphs of the tobacco aphid from eight tobacco producing counties in Virginia, 1988.

Farm	County	Collection Date	Color Morph	Karyotype[1] 12N	12T	13
Crews	Charlotte	14 Sep	green	9	0	1
Crews	Charlotte	14 Sep	red	0	9	1
Easley	Pittsylvania	4 Aug	red	2	8	0
SVAES[2]	Washington	14 Jul	red	1	9	0
Greene	Lee	9 Aug	green	7	2	1
Hawthorne	Brunswick	1 Aug	red	0	10	0
Jones	Smythe	1 Aug	red	1	9	0
Moore	Charlotte	14 Sep	red	1	9	0
Motley	Pittsylvania	14 Sep	red	1	9	0
SPAES[3]	Nottoway	6 Jul	green	10	0	0
SPAES	Nottoway	6 Jul	red	0	10	0
Townsend	Dinwiddie	15 Jun	green	9	1	0
Townsend	Dinwiddie	15 Jun	red	1	9	0
Tuck	Brunswick	16 Jun	red	1	9	0

[1] 12N: 2n = 12 karyotype; 12T: 2n = 12 translocated karyotype; 13: 2n = 13 karyotype.

[2] SVAES = Southwest Virginia Agricultural Experiment Station, Glade Springs, VA.

[3] SPAES = Southern Piedmont Agricultural Experiment Station, Blackstone, VA.

Table 3.2. Karyotypes(%) of the red and green morphs of the tobacco aphid in Virginia, 1988.

Color Morph	N*	Mean (%) Occurrence	Standard Error
		2N = 12 Karyotype	
Green	4	87.5**	6.3
Red	10	8.0**	2.0
		2N = 12 Translocated Karyotype	
Green	4	7.5**	4.8
Red	10	91.0**	1.8
		2N = 13 Karyotype	
Green	4	5.0	2.9
Red	10	1.0	1.0

* N = number of aphids karyotyped

** Red and green morphs significantly different ($P < 0.001$)

Table 3.3. Karyotype of red and green morphs of the tobacco aphid from nine tobacco producing counties in Virginia, 1989.

Farm	County	Collection Date	Color Morph	Karyotype[1] 12N	12T	13
Barnes	Dinwiddie	5 Jun	red	1	9	0
Barnes	Dinwiddie	23 Jun	red	0	10	0
Barnes	Dinwiddie	10 Jul	red	0	10	0
Barnes	Dinwiddie	26 Jul	red	0	10	0
Crews	Charlotte	26 Jun	green	10	0	0
Crews	Charlotte	20 Jul	green	10	0	0
Crews	Charlotte	7 Aug	green	10	0	0
Crews	Charlotte	26 Jun	red	0	10	0
Crews	Charlotte	20 Jul	red	0	10	0
Crews	Charlotte	7 Aug	red	0	10	0
Easley	Pittsylvania	15 Jul	red	0	10	0
Easley	Pittsylvania	8 Aug	red	0	10	0
SVAES[2]	Washington	26 Jul	green	10	0	0
SVAES	Washington	22 Aug	green	10	0	0
SVAES	Washington	26 Jul	red	0	10	0
SVAES	Washington	22 Aug	red	0	10	0
Greene	Lee	15 Jul	red	0	10	0
Hawthorne	Brunswick	28 Jul	green	7	3	0
Hawthorne	Brunswick	5 Sep	green	10	0	0

-- Continued --

Table 3.3 Continued.

Table 3.3 Karyotype of red and green morphs of the tobacco aphid from nine tobacco producing counties in Virginia, 1989.

Farm	County	Collection Date	Color Morph	Karyotype/1 12N	12T	13
Hawthorne	Brunswick	28 Jul	red	0	10	0
Hawthorne	Brunswick	5 Sep	red	0	10	0
Moore	Charlotte	26 Jun	green	10	0	0
Moore	Charlotte	25 Aug	green	10	0	0
Moore	Charlotte	26 Jun	red	0	10	0
Moore	Charlotte	12 Jul	red	0	10	0
Moore	Charlotte	27 Jul	red	0	10	0
Moore	Charlotte	7 Aug	red	0	10	0
Pittard	Mecklenburg	1 Sep	green	10	0	0
Pittard	Mecklenburg	14 Jun	red	0	10	0
Pittard	Mecklenburg	26 Jun	red	0	10	0
Pittard	Mecklenburg	12 Jul	red	0	10	0
Pittard	Mecklenburg	27 Jul	red	0	10	0
Pittard	Mecklenburg	7 Aug	red	0	10	0
Pittard	Mecklenburg	1 Sep	red	0	10	0
SPAES/3	Nottoway	2 Jun	green	9	1	0
SPAES	Nottoway	14 Jun	green	10	0	0

-- Continued --

Table 3.3 Continued.

Table 3.3. Karyotype of red and green morphs of the tobacco aphid from nine tobacco producing counties in Virginia, 1989.

Farm	County	Collection Date	Color Morph	Karyotype/1 12N	12T	13
SPAES	Nottoway	15 Jul	green	10	0	0
SPAES	Nottoway	1 Jun	red	2	8	0
SPAES	Nottoway	14 Jun	red	0	10	0
SPAES	Nottoway	5 Jul	red	0	10	0
SPAES	Nottoway	1 Aug	red	0	10	0
SPAES	Nottoway	13 Oct	red	0	10	0
Tuck	Dinwiddie	30 Jun	red	0	10	0
Tuck	Dinwiddie	25 Jul	red	0	10	0
Via	Patrick	15 Jul	green	10	0	0
Via	Patrick	27 Aug	green	8	2	0
Via	Patrick	18 Jun	red	0	10	0
Via	Patrick	15 Jul	red	0	10	0
Via	Patrick	31 Jul	red	0	10	0
Via	Patrick	27 Aug	red	0	10	0

/1 12N: 2n = 12 karyotype; 12T: 2n = 12 translocated karyotype; 13: 2n = 13 karyotype.

/2 SVAES = Southwest Virginia Agricultural Experiment Station, Glade Springs, VA.

/3 SPAES = Southern Piedmont Agricultural Experiment Station, Blackstone, VA.

Table 3.4. Karyotypes(%) of the red and green morphs of the tobacco aphid in Virginia, 1989.

Color Morph	N*	Mean (%) Occurrence	Standard Error
		2N = 12 Karyotype	
Green	15	96.0**	2.4
Red	35	1.0**	0.6
		2N = 12 Translocated Karyotype	
Green	15	4.0**	2.4
Red	35	99.0**	0.6

* N = number of aphids karyotyped

** Red and green morphs significantly different ($P < 0.0001$)

Table 3.5. Relative lengths of the chromosomes for the three karyotypes of Myzus nicotianae Blackman.

	Karyotype		
Chromosome*	2N = 12 Normal	2N = 12 Translocated	2N = 13
X	7.5	7.5	5.0
	7.5	7.5	5.0
A1**	5.0	7.5	5.0
	5.0	5.0	5.0
A2	4.0	4.0	3.5
	4.0	4.0	3.5
A3	4.0	5.0	5.0
	4.0	2.5	(2.5)(2.5)
A4	2.5	2.5	2.5
	2.5	2.5	2.5
A5	2.5	2.5	2.5
	2.5	2.5	2.5

*The assignment for the chromosomes is based on previous work by Blackman 1971.

**A1 = autosome 1

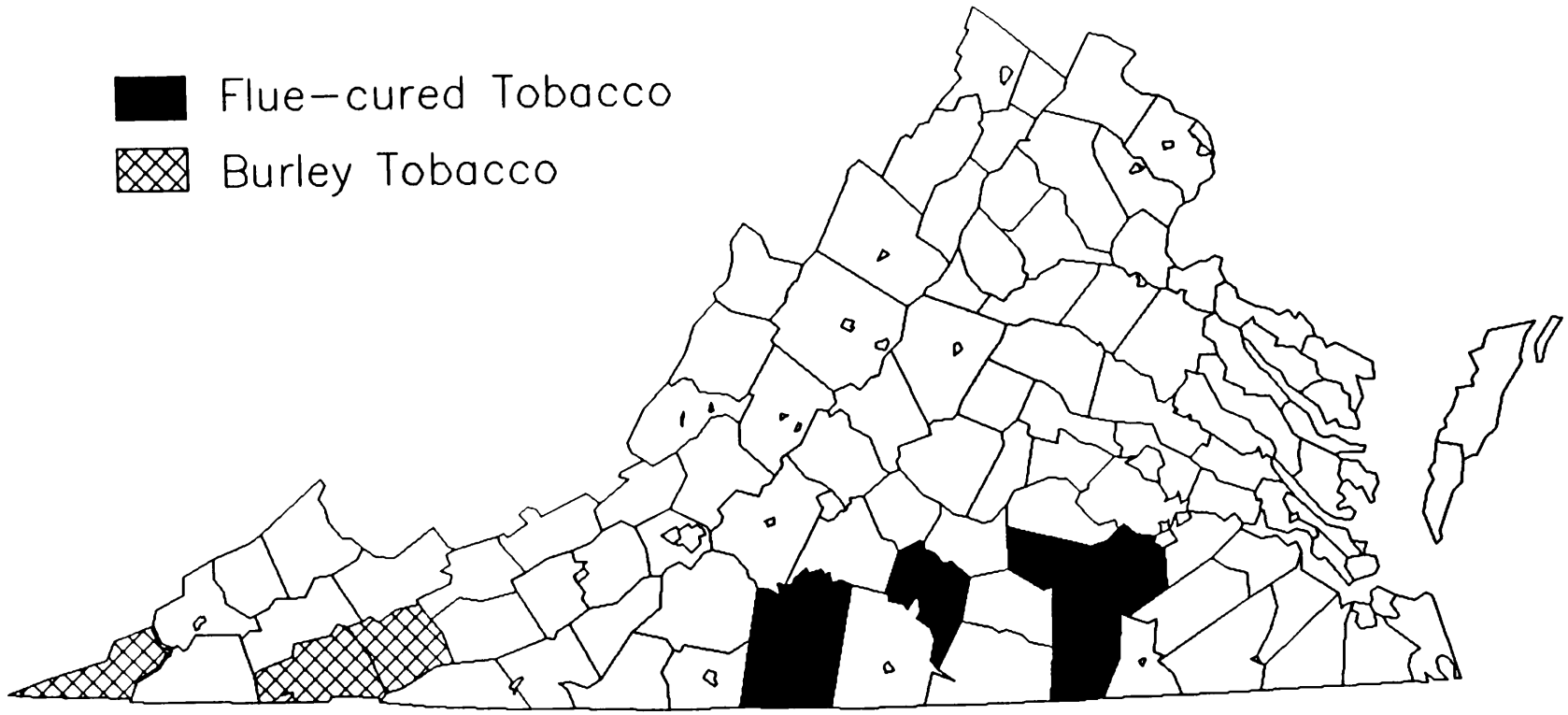

Figure 3.1. Counties in Virginia Where Tobacco Aphids Were Sampled, 1988.

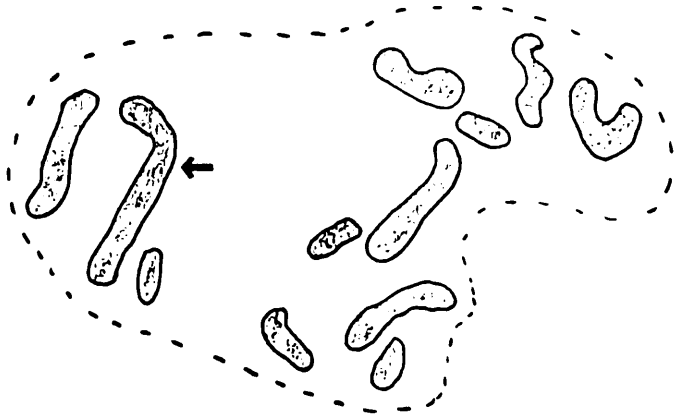

Figure 3.2. 2N = 12 translocated karyotype of the tobacco aphid, Myzus nicotianae Blackman. The arrow denotes the translocated chromosome.

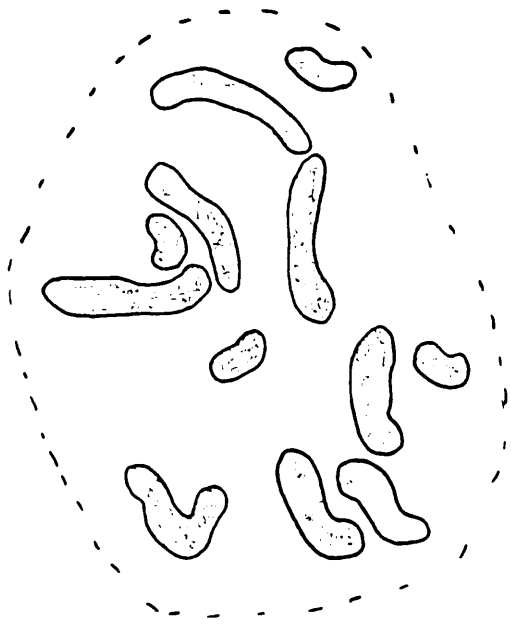

Figure 3.3. 2N = 12 normal karyotype of the tobacco aphid, Myzus nicotianae Blackman.

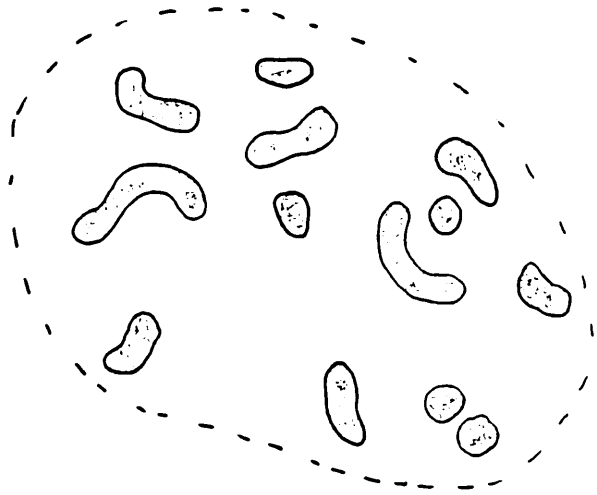

Figure 3.4. 2N = 13 karyotype of the tobacco aphid, Myzus nicotianae Blackman.

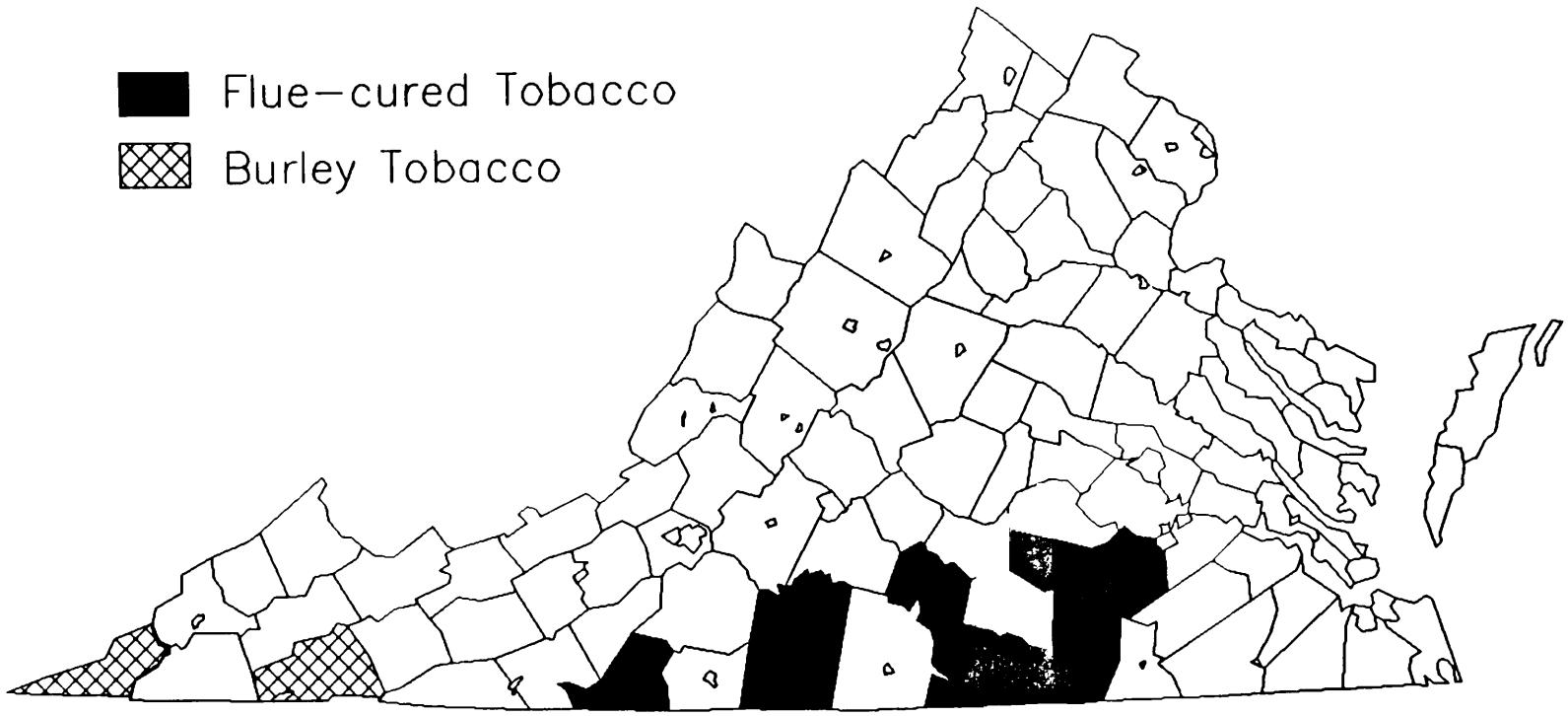

Figure 3.5. Counties in Virginia Where Tobacco Aphids Were Sampled, 1989.

Chapter 4

Tolerance Levels of the Tobacco Aphid, Myzus nicotianae Blackman , from Various Counties in Virginia to Acephate, 1989.

The tobacco aphid, Myzus nicotianae, has been a pest of tobacco in the United States for many years. However, it did not become a serious pest on tobacco until 1946 (Dominick 1949, Chamberlin 1958, Kulash 1949). Moderate infestations occurred in 1974 and serious damage developed in North America during 1975 and 1976 (Cheng and Court 1977). Tobacco aphids injure tobacco by removing plant juice and depositing honeydew and exuviae on the leaves (Dominick 1949). Infested leaves yellow prematurely and necrotic tissue may develop along leaf margins and at the base of the petiole (Dominick 1949). These injuries result in reductions in both yield and quality of the cured tobacco leaf.

Insecticide resistance in aphid populations has been a problem for many years. Resistance problems seem inevitable when insect pressure and injury to the plant begin to increase, because higher dosages of the insecticide are used and more frequent sprays are necessary (Georghiou 1963). Thus aphids are becoming better fit to tolerate the insecticides. Resistance in the closely associated M. persicae was first reported in 1955 (Anthon 1955). Koziol and Semtner (1984) found resistance to acephate in several field strains of aphids from Virginia.

In M. persicae a chromosome translocation involving autosome 1 and 3 is often associated with aphids showing resistance to organophosphorous insecticides (Blackman 1980). In many instances the red morphs of M. nicotianae have the translocated karyotype (Blackman 1987). Lampert (pers.

comm.) has found that there is a higher tolerance in the red morphs of the tobacco aphid to monocrotophos (Azodrin) and acephate (Orthene).

Complaints of failure in controlling the red morph of M. nicotianae with acephate have been reported by tobacco growers in Virginia in the past few years. This study was initiated to investigate tolerance levels of M. nicotianae to acephate and determine if the red and green morphs with differing karyotypes differ in their tolerance to acephate.

Materials and Methods

Aphids were collected once or twice monthly from tobacco grown on 11 commercial farms in nine counties in Virginia (Fig 4.1). Aphids were sampled from two farms in the burley tobacco regions and nine farms in the flue-cured tobacco region. Whenever possible both red and green morphs of the tobacco aphid were collected. The aphids were maintained alive on excised tobacco leaves from greenhouse plants. The petioles of the leaves were inserted into water agar in the bottom of a 473 ml styrofoam cup. Cups were covered with translucent plastic lids and placed in growth chambers maintained at 24^{o}C and a 16h photophase. After the karyotype was determined, the colonies were reared for an average of five generations before being used in the dosage mortality study. A laboratory colony established from aphids supplied by Dr. E.P. Lampert at North Carolina State University was used as the reference standard.

Acephate (Orthene Tobacco Insect Spray 75SP) was tested because it is the most commonly used insecticide for aphid control. A 1986 survey in Virginia indicated that almost 90% of the flue-cured tobacco growers used

acephate to control aphids or other pests (C.S. Johnson personal communication). Futhermore, tobacco growers have reported increased difficulty in controlling the red morph of M. nicotianae. Seven concentrations of commercially formulated Orthene Tobacco Insect Spray were tested -- 50, 100, 200, 400, 800, 1600, and 3200 ppm. Deionized water served as the control treatment. Test concentrations were prepared by diluting the formulated insecticide in deionized water.

A leaf disk procedure was used for the study. Leaf disks 5 cm in diameter were cut from whole tobacco leaves and dipped in the various concentrations for 20 sec. The leaf disks were blotted on a paper towel and then placed on a plastic tray lined with a paper towel. Apterous adult aphids were transferred to the treated disks with a camel's hair brush. A 35X10 mm petri dish lid was placed over the leaf with a 33 g hex nut to secure the petri dish and insure that no aphids could escape.

Four hundred aphids per colony -- five replicates of 10 insects each -- were tested at each of the seven concentrations and water control. The treated aphids were held in a growth chamber at 24^{o}C and a 16h photoperiod until mortality was recorded at 24h post-treatment. An aphid was considered dead if it did not walk when prodded with a camel's hair brush.

Probit Analysis was performed using Proc Probit in SAS (1985). Abbott's formula was used to adjust for mortality in the control (Abbott 1925). When LC_{50} and LC_{90} values for a given county were compared, the significant differences between values was determined by the criterion of overlap of the 95% fiducial limits. A tolerance ratio was calculated by

dividing the LC_{50} value of the field strain by the LC_{50} of the laboratory strain.

Results and Discussion

Comparison of LC_{50} and LC_{90} Values (Acephate 75SP) of Red and Green Morphs of the Tobacco Aphid

The LC_{50} values for red and green morphs of the tobacco aphid ranged from a low of 87 ppm to 413 ppm. (Appendix 4A). Six colonies had an LC_{50} significantly higher than that of the laboratory standard. (Table 4.1) These colonies included green morphs from burley tobacco at the Southwest Virginia Agricultural Experiment Station near Glade Springs in Washington County, and from flue-cured tobacco on the Moore farm in Charlotte County; and red morphs on flue-cured tobacco from the Tuck farm in Brunswick County (two collection dates) and the Pittard farm in Mecklenburg County (two collection dates). Red morphs of the tobacco aphid collected from Barnes' farm in Dinwiddie County had LC_{50} values greater than the standard on all three collection dates (5 June, 23 June, 26 July). However, no date was significantly different due to the wide range of the 95% fiducial limits caused by the large amount of variation among treatments.

Four colonies, two green and two red, had significantly lower LC_{50} values than that of the standard (Table 4.2). Highly susceptible red morphs were taken from the Moore farm in Charlotte County on 26 June and 12 July, and green morphs from the Via farm on 27 August, and the Hawthorne farm on 5 September.

Aphids from all farms with significantly higher LC_{50} values, except the Pittard farm, were taken from experimental plots where no insecticide applications were made. Acephate 75SP was used in the transplant water at Moore's farm and this could explain the increased tolerance of the green morphs sampled on 26 June. No insecticide applications were made at the Pittard farm before the collection of aphids on 26 June and 30 June. However, these aphids showed as much tolerance as those collected at later dates after two insecticide applications had been made. This suggests that aphids migrating into the field had an inherent tolerance to acephate and the selection of tolerant aphids was not occurring because of increased insecticide application.

Looking at the LC_{90}, there were nine colonies with values significantly lower than the laboratory colony (Table 4.3). These colonies came from the following locations: Hawthorne in Brunswick County, Southern Piedmont Agricultural Experiment Station (sampling dates 5 June and 1 August) in Nottoway County, Moore (26 June, 12 July) and Crews in Charlotte County, Via in Patrick County, and Pittard in Mecklenburg County. There were no colonies with LC_{90} values significantly higher than that of the standard; although, many farms had aphids with tolerance 1-1.6X greater than the laboratory strain. Aphids from all of the farms (except Pittard) with LC_{90} values significantly lower than the standard came from untreated insecticide plots. This may explain their susceptibility to acephate 75SP. However, the standard has never been exposed to acephate 75SP. The green morphs from Pittard (1 September) had received 0-2 applications of acephate 75SP. The red morphs from the same sampling date (1

September) had the second highest LC_{90}. This supports Blackman's (1987) idea that the red morphs with the translocation are more tolerant than the green morphs to organophosphorous insecticides.

Comparison of LC_{50} and LC_{90} Values (Acephate 75SP) of the Green Morphs of the Tobacco Aphid

Among the green morphs tested seven colonies had LC_{50} values lower than the standard and seven had values higher than the standard (Table 4.4). Via's farm had both the highest and lowest LC_{50} values with the later collection date (27 August) being more susceptible to acephate 75SP and differing significantly from the standard and four other colonies. Aphids from Hawthorne's farm showed a similar trend with the later collection date being more susceptible to acephate 75SP. Aphid populations sampled from Hawthorne and Via's farms came from untreated experimental plots. It seems possible, since no insecticide applications were made, that the aphids from the earlier collection date could have migrated into the field already possessing tolerance to acephate.

Green morphs from two farms in Charlotte County -- Crews' and Moore's showed a somewhat homogeneous response to acephate. Only one colony from Moore's farm (26 June) had an LC_{50} significantly greater than the standard and the green morphs from Crews' (20 July). The homogeneity in response of these populations would be expected because the aphids sampled from Charlotte County were taken from untreated plots, thus no selection for increased tolerance was occurring. This similarity in response to acephate was also seen in green morphs from untreated plots at Southern Piedmont Agricultural Experiment Station.

The standard did not fall in the middle of the LC_{90} values as it did with the LC_{50}; but had the fourth highest value (Table 4.5). Aphid colonies from five farms had LC_{90} values significantly less than the standard and no farms were significantly higher. Here again the aphids (with the exception of those from Pittard's farm) came from untreated experimental plots.

Comparison of LC_{50} and LC_{90} values (Acephate 75SP) of the Red Morphs of the Tobacco Aphid.

The response of red morphs to Acphate 75SP are shown in table 4.6. Two colonies from the Moore farm (26 June, 12 July) had LC_{50} values significantly less than the standard. Four colonies - from the Tuck farm in Brunswick (collection dates 30 June and 25 July) and from the Pittard farm in Mecklenburg County (collection dates 26 June and 7 August) - had LC_{50} values significantly greater than the standard. The other collection dates from Pittard's farm, with the exception of 14 June, had LC_{50} values 1.4 to 1.8X greater than the standard. However, there were no significant differences due to the wide range of the 95% fiducial limits caused by the large amount of variation among treatments. The plots from which aphids were sampled at Pittard's farm had received two applications of acephate. This could contribute to the increased tolerance to acephate.

Red morphs from Tuck's farm came from untreated plots. The increased tolerance of aphids from the untreated plots could be explained by migration of aphids from treated plots within the same field or migration of acephate tolerant aphids from other areas.

Red aphids sampled from Nottoway County showed similar tolerance levels to acephate. There were no significant differences among aphids from

three collection dates on Via's farm. However, there was a decrease in tolerance to acephate from sampling date 18 June to 27 August. The same decrease in tolerance is seen in the response of aphids from Hawthorne's and Tuck's farms in Brunswick County. Although, Pittard's farm showed an increase in tolerance from the first sampling on 14 June to the fifth sampling on 1 September with the first being significantly lower than the other dates. This increase in tolerance may be due to the application of acephate to these plots throughout the tobacco growing season. Futhermore the decrease in tolerance at Tuck's and Hawthorne's farms could be due to the fact that no insecticide applications were made, and there was no selection for tolerance.

The LC_{90} for the red morphs ranged from 241 to 876 ppm (Table 4.7). Four colonies -- two collection dates each in Charlotte and Nottoway Counties -- had significantly lower LC_{90} values than the standard. The red aphids from Nottoway County were collected from the same insecticide plot in the field; however, those collected on 1 August were taken from a plot where methomyl had been applied on 7 July. The response of red morphs from Nottoway were similar with the exception of one sampling date (1 August) which was significantly lower than the other dates. There was a gradual increase in tolerance to acephate from the first collection date (26 June) to the fourth (24 August) for aphids from Moore's farm. A colony collected on 26 June had a significantly lower LC_{50} than that collected on 27 July. An explanation for this increase in acephate tolerance could be the migration of aphids from treated plots in the same experimental test. A decrease in acephate tolerance from earlier to later collection dates was

shown by the response of red morphs from the Hawthorne's, Via's, Tuck's and Barnes' farms.

Of the aphid colonies with LC_{50} values significantly higher than the laboratory standard, there were more red than green colonies. Fifty percent of the green morph colonies tested had LC_{50} values higher than the standard. The red morphs tested had 64% with values higher than the standard. However, there were just as many red colonies as green with LC_{50} values significantly lower than the standard.

Aphids from untreated plots tended to show a decrease in resistance from the early collection dates to the later ones. Migration of aphids from Southern states early in the season with an inherent tolerance to acephate may be the reason for this decrease. Once the aphids colonized the tobacco plant there was no insecticide pressure and thus no need for increased tolerance to acephate. Furthermore aphids from treated plots showed an increase in tolerance to acephate. The application of acephate selected for aphids with increased tolerance to the insecticide.

The red morphs of M. nicotianae from all farm locations had the non-reciprocal translocation associated with insecticide resistance in M. persicae. The green morphs with the exception of morphs from Via's farm (27 August) and Hawthorne's farm (28 July) had the $2n=12$ normal karyotype. Green morphs from collection dates at Crews', Glade Springs, Nottoway and Moore's showed significantly greater LC_{50} values for Acephate 75SP than some red colonies from the same location. Red Colonies from Hawthorne's, Pittard's and Via's farms had significantly greater LC_{50} values than the green colonies. There does not appear to be a strong relation between the red

color morph with the translocated karyotype and insecticide resistance, since green morphs from several farms had LC50 values greater than the red morphs.

This data shows that tolerance to acephate is present at a low level in Virginia, and that the potential for increased tolerance to acephate exists. Additional research is needed to test the influence of time of year and insecticide pressure on acephate tolerance in tobacco aphid populations.

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Table 4.1. Tobacco aphid colonies with LC_{50} values (acephate 75SP) significantly higher than the susceptible colony, Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Clayton	Johnston/2	1983	green	202 (105-253)	-	0.004
Tuck	Brunswick	25 Jul	red	289 (253-338)	1.4	0.006
Pittard	Mecklenburg	7 Aug	red	302 (270-342)	1.5	0.007
Tuck	Brunswick	30 Jun	red	322 (273-378)	1.6	0.004
SVAES/3	Washington	22 Aug	green	335 (298-384)	1.7	0.007
Moore	Charlotte	26 Jun	green	339 (291-394)	1.7	0.004
Pittard	Mecklenburg	26 Jun	red	341 (294-397)	1.7	0.004

/1 Tolerance Ratio = LC_{50} field strain / LC_{50} susceptible strain.

/2 Johnston = the location in North Carolina where the susceptible strain was obtained.

/3 SVAES = Southwest VA Agricultural Experiment Station in Glade Springs, VA.

Table 4.2. Tobacco aphid colonies with LC_{50} values (acephate 75SP) significantly lower than the susceptible colony, Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Moore	Charlotte	12 Jul	red	87 (37-123)	0.4	0.006
Via	Patrick	27 Aug	green	108 (66-142)	0.5	0.006
Moore	Charlotte	26 Jun	red	110 (69-143)	0.5	0.007
Hawthorne	Brunswick	5 Sep	green	129 (111-149)	0.6	0.013
Clayton	Johnston/2	1983	green	202 (150-253)	-	0.004

/1 Tolerance Ratio = LC_{50} field strain / LC_{50} susceptible strain.

/2 Johnston = the location in North Carolina where the susceptible strain was obtained.

Table 4.3. Tobacco aphid colonies with LC_{90} values (acephate 75SP) significantly lower than the susceptible colony, Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{90} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Hawthorne	Brunswick	5 Sep	green	229 (199-280)	0.4	0.013
SPAES IT4/2	Nottoway	1 Aug	red	241 (213-289)	0.4	0.016
Moore	Charlotte	26 Jun	red	302 (251-395)	0.5	0.007
Crews	Charlotte	20 Jul	green	309 (271-370)	0.5	0.010
Moore	Charlotte	12 Jul	red	321 (266-421)	0.6	0.006
Via	Patrick	27 Aug	green	329 (277-419)	0.6	0.006
SPAES IT4	Nottoway	5 Jul	red	371 (322-447)	0.7	0.007
SPAES IT5/2	Nottoway	5 Jul	green	378 (322-472)	0.7	0.006
Pittard	Mecklenburg	1 Sep	green	381 (329-467)	0.7	0.006
Clayton	Johnston/3	1983	green	563 (474-710)	-	0.004

/1 Tolerance Ratio = LC_{90} field strain / LC_{90} susceptible strain.

/2 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/3 Johnston = the location in North Carolina where the susceptible strain was obtained.

Table 4.4. Response of field strains of the green morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance[1] Ratio	Slope
Via	Patrick	27 Aug	108 (66-142)	0.5	0.006
Hawthorne	Brunswick	5 Sep	129 (111-149)	0.6	0.013
SPAES IT1[2]	Nottoway	5 Jul	171 (31-328)	0.8	0.006
Crews	Charlotte	20 Jul	177 (154-204)	0.9	0.010
SPAES IT5[2]	Nottoway	5 Jul	179 (146-215)	0.9	0.006
Pittard	Mecklenburg	1 Sep	179 (148-212)	0.9	0.006
Crews	Charlotte	26 Jun	195 (106-307)	1.0	0.006
Clayton	Johnston[3]	1983	202 (150-253)	-	0.004
Crews	Charlotte	7 Aug	203 (73-402)	1.0	0.006
Hawthorne	Brunswick	28 Jul	251 (118-685)	1.2	0.006
Moore	Charlotte	12 Jul	265 (162-419)	1.3	0.005
SVAES[4]	Washington	22 Aug	335 (298-384)	1.7	0.007

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Table 4.4. Continued.

Table 4.4. Response of field strains of the green morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Moore	Charlotte	26 Jun	339 (291-394)	1.7	0.004
Crews	Charlotte	26 Jul	349 (209-570)	1.7	0.004
Via	Patrick	15 Jul	362 (170-777)	1.8	0.004

/1 Tolerance Ratio = LC_{50} field strain / LC_{50} susceptible strain.

/2 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/3 Johnston = the location in North Carolina where the susceptible strain was obtained.

/4 SVAES = Southwest VA Agricutlural Experiment Station, Glade Springs, VA.

Table 4.5. Response of field strains of the green morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance[1] Ratio	Slope
Hawthorne	Brunswick	5 Sep	229 (199-280)	0.4	0.013
Crews	Charlotte	20 Jul	309 (271-370)	0.5	0.010
Via	Patrick	27 Aug	329 (277-419)	0.6	0.006
SPAES IT1[2]	Nottoway	5 Jul	370 (255-1157)	0.7	0.006
SPAES IT5[2]	Nottoway	5 Jul	378 (322-472)	0.7	0.006
Pittard	Mecklenburg	1 Sep	381 (329-467)	0.7	0.006
Crews	Charlotte	26 Jun	412 (302-831)	0.7	0.006
Crews	Charlotte	7 Aug	430 (297-1366)	0.8	0.006
Hawthorne	Brunswick	28 Jul	468 (318-2240)	0.8	0.006
SVAES[3]	Washington	22 Aug	530 (464-637)	0.9	0.007
Moore	Charlotte	12 Jul	537 (394-1026)	1.0	0.005

-- Continued --

Table 4.5. Continued.

Table 4.5. Response of field strains of the green morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Clayton	Johnston/4	1983	563 (474-710)	-	0.004
Moore	Charlotte	26 Jun	660 (576-786)	1.2	0.004
Via	Patrick	15 Jul	687 (466-2039)	1.2	0.004
Crews	Charlotte	26 Jul	689 (500-1340)	1.2	0.004

/1 Tolerance Ratio = LC_{90} field strain / LC_{90} susceptible strain.

/2 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/3 SVAES = Southwest VA Agricutlural Experiment Station, Glade Springs, VA.

/4 Johnston = the location in North Carolina where the susceptible strain was obtained.

Table 4.6. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance[1] Ratio	Slope
Moore	Charlotte	12 Jul	87 (37-123)	0.4	0.006
Moore	Charlotte	26 Jun	110 (69-143)	0.5	0.007
Crews	Charlotte	26 Jun	158 (116-198)	0.8	0.005
SPAES IT4[2]	Nottoway	1 Aug	162 (144-184)	0.8	0.016
Via	Patrick	27 Aug	169 (18-306)	0.8	0.005
Hawthorne	Brunswick	5 Sep	171 (45-303)	0.8	0.006
SVAES[3]	Washington	26 Jul	180 (108-271)	0.9	0.008
SPAES IT1[2]	Nottoway	1 Aug	183 (146-220)	0.9	0.005
SPAES IT4	Nottoway	5 Jul	189 (160-220)	0.9	0.007
Pittard	Mecklenburg	14 Jun	194 (163-228)	1.0	0.006
SPAES IT5[2]	Nottoway	5 Jul	199 (75-374)	1.0	0.006

-- Continued --

Table 4.6. Continued.

Table 4.6. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Moore	Charlotte	7 Aug	199 (167-235)	1.0	0.006
Clayton	Johnston/4	1983	202 (150-253)	-	0.004
Easley	Pittsylvania	15 Jul	202 (113-321)	1.0	0.006
Via	Patrick	31 Jul	213 (181-251)	1.1	0.006
SPAES IT1	Nottoway	5 Jul	225 (140-361)	1.1	0.007
Easley	Pittsylvania	8 Aug	227 (197-262)	1.1	0.007
Greene	Lee	8 Aug	235 (206-269)	1.2	0.007
Crews	Charlotte	20 Jul	245 (91-938)	1.2	0.007
Moore	Charlotte	27 Jul	248 (209-297)	1.2	0.005
SPAES/5	Nottoway	15 Jul	249 (221-282)	1.2	0.008

-- Continued --

Table 4.6. Continued.

Table 4.6. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Moore	Charlotte	24 Aug	275 (36-730)	1.4	0.004
SPAES	Nottoway	13 Oct	277 (82-495)	1.4	0.003
Pittard	Mecklenburg	27 Jul	289 (252-338)	1.4	0.006
Tuck	Brunswick	25 Jul	289 (253-335)	1.4	0.006
Hawthorne	Brunswick	28 Jul	298 (248-354)	1.5	0.004
Pittard	Meckenburg	7 Aug	302 (270-342)	1.5	0.007
Barnes	Dinwiddie	23 Jun	310 (242-392)	1.5	0.003
Via	Patrick	18 Jun	319 (35-3768)	1.6	0.005
Tuck	Brunswick	30 Jun	322 (273-378)	1.6	0.004
Pittard	Mecklenburg	26 Jun	341 (294-397)	1.7	0.004

-- Continued --

Table 4.6. Continued.

Table 4.6. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{50}), Virginia, 1989.

Farm	County	Collection Date	LC_{50} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Barnes	Dinwiddie	26 Jul	363 (247-525)	1.8	0.003
Pittard	Mecklenburg	1 Sep	373 (246-559)	1.8	0.003
Barnes	Dinwiddie	5 Jun	413 (103-1956)	2.0	0.003

/1 Tolerance Ratio = LC_{50} field strain / LC_{50} susceptible strain.

/2 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/3 SVAES = Southwest VA Agricutlural Experiment Station, Glade Springs, VA.

/4 Johnston = the location in North Carolina where the susceptible strain was obtained.

/5 SPAES = Southern Piedmont Agricultural Experiment Station, Blackstone, VA.

Table 4.7. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance[1] Ratio	Slope
SPAES IT4[2]	Nottoway	1 Aug	241 (213-289)	0.4	0.016
Moore	Charlotte	26 Jun	302 (251-395)	0.5	0.007
Moore	Chalotte	12 Jul	321 (266-421)	0.6	0.006
SVAES[3]	Washington	26 Jul	346 (259-633)	0.6	0.008
SPAES IT4	Nottoway	5 Jul	371 (322-447)	0.7	0.007
Hawthorne	Brunswick	5 Sep	376 (264-987)	0.7	0.006
Pittard	Mecklenburg	14 Jun	394 (340-481)	0.7	0.007
SPAES IT1[2]	Nottoway	5 Jul	406 (299-821)	0.7	0.007
SPAES[4]	Nottoway	15 Jul	410 (365-478)	0.7	0.008
Greene	Lee	8 Aug	412 (363-486)	0.7	0.007
Moore	Charlotte	7 Aug	412 (355-506)	0.7	0.006

-- Continued --

Table 4.7. Continued.

Table 4.7. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance/1 Ratio	Slope
Easley	Pittsylvania	15 Jul	414 (303-845)	0.7	0.006
Easley	Pittsylvania	8 Aug	414 (362-494)	0.7	0.007
SPAES IT5/2	Nottoway	5 Jul	416 (291-1204)	0.7	0.006
SPAES IT1	Nottoway	1 Aug	421 (357-530)	0.7	0.005
Crews	Charlotte	26 Jun	424 (354-550)	0.8	0.005
Via	Patrick	31 Jul	427 (367-523)	0.8	0.006
Crews	Charlotte	20 Jul	432 (289-3330)	0.8	0.007
Via	Patrick	27 Aug	439 (304-1285)	0.8	0.005
Pittard	Mecklenburg	7 Aug	476 (423-558)	0.8	0.007
Tuck	Brunswick	25 Jul	503 (436-612)	0.9q	0.006

-- Continued --

Table 4.7. Continued.

Table 4.7. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance[1] Ratio	Slope
Moore	Charlotte	27 Jul	505 (426-643)	0.9	0.005
Pittard	Mecklenburg	27 Jul	514 (443-631)	0.9	0.006
Clayton	Johnston[5]	1983	563 (474-710)	-	0.004
Moore	Charlotte	24 Aug	594 (384-2759)	1.1	0.004
Via	Patrick	18 Jun	596 (374-17130)	1.1	0.005
Hawthorne	Brunswick	28 Jul	654 (564-791)	1.2	0.004
Pittard	Mecklenburg	26 Jun	660 (576-785)	1.2	0.004
Tuck	Brunswick	30 Jun	666 (577-799)	1.2	0.004
SPAES	Nottoway	13 Oct	714 (495-1653)	1.3	0.003
Barnes	Dinwiddie	26 Jul	737 (562-1187)	1.3	0.003

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Table 4.7. Continued.

Table 4.7. Response of field strains of the red morph of the tobacco aphid to acephate 75SP (LC_{90}), Virginia, 1989.

Farm	County	Collection Date	LC_{90} (95% Fid. Limits)	Tolerance[/1] Ratio	Slope
Barnes	Dinwiddie	23 Jun	749 (622-961)	1.3	0.003
Pittard	Mecklenburg	1 Sep	749 (562-1275)	1.3	0.003
Barnes	Dinwiddie	5 Jun	876 (552-8324)	1.6	0.003

/1 Tolerance Ratio = LC_{90} field strain / LC_{90} susceptible strain.

/2 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/3 SVAES = Southwest VA Agricutlural Experiment Station, Glade Springs, VA.

/4 SPAES = Southern Piedmont Agricultural Experiment Station, Blackstone, VA.

/5 Johnston = the location in North Carolina where the susceptible strain was obtained.

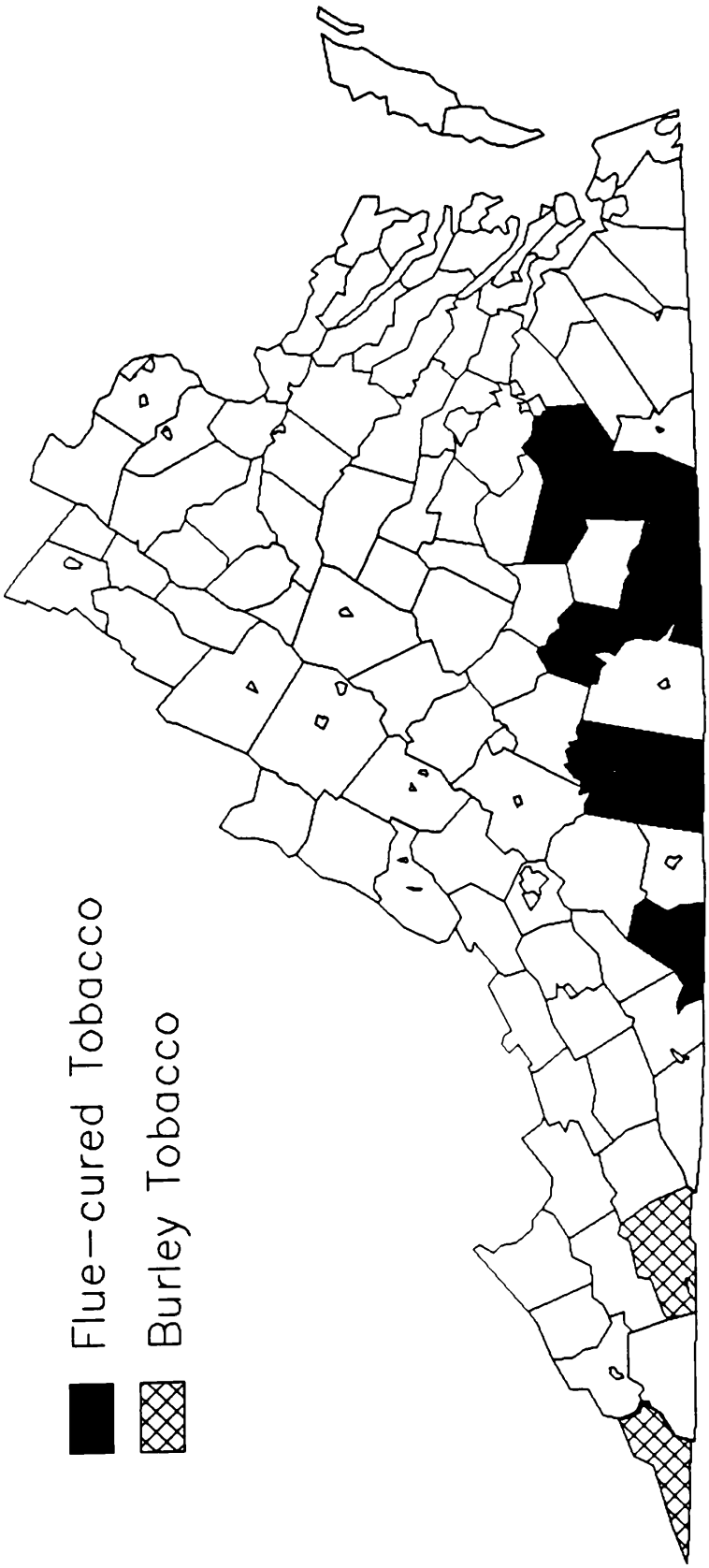

Figure 4.1. Counties in Virginia Where Tobacco Aphids Were Sampled for the Dosage Mortality Study, 1989.

Appendix 4A.

Response of field strains of the tobacco aphid to acephate 75SP (LC_{50} and LC_{90}), Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% F.L./1)	LC_{90} (95% F.L.)	Tolerance/2 Ratio	Slope
Moore	Charlotte	12 Jul	red	87 (37-123)	321 (266-421)	0.4	0.006
Via	Patrick	27 Aug	green	108 (66-142)	329 (277-419)	0.5	0.006
Moore	Charlotte	26 Jun	red	110 (69-143)	302 (251-395)	0.5	0.007
Hawthorne	Brunswick	5 Sep	green	129 (111-149)	229 (199-280)	0.6	0.013
Crews	Charlotte	26 Jun	red	158 (116-198)	424 (354-550)	0.8	0.005
SPAES IT4/3	Nottoway	1 Aug	red	162 (144-184)	241 (213-289)	0.8	0.016
Via	Patrick	27 Aug	red	169 (18-306)	439 (304-1285)	0.8	0.005
Hawthorne	Brunswick	5 Sep	red	171 (45-303)	376 (264-987)	0.8	0.006
SPAES IT1/3	Nottoway	5 Jul	green	171 (31-328)	370 (255-1157)	0.8	0.006
Crews	Charlotte	20 Jul	green	177 (154-204)	309 (271-370)	0.9	0.010
SPAES IT5/3	Nottoway	5 Jul	green	179 (146-215)	378 (322-472)	0.9	0.006

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Appendix 4A. Continued

Response of field strains of the tobacco aphid to acephate 75SP (LC_{50} and LC_{90}), Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% F.L./[1])	LC_{90} (95% F.L.)	Tolerance/[2] Ratio	Slope
Pittard	Mecklenburg	1 Sep	green	179 (148-212)	381 (329-467)	0.9	0.006
SVAES/[4]	Washington	26 Jul	red	180 (108-271)	346 (259-633)	0.9	0.008
SPAES IT1	Nottoway	1 Aug	red	183 (146-220)	421 (357-530)	0.9	0.005
SPAES IT4	Nottoway	5 Jul	red	189 (160-220	371 (322-447)	0.9	0.007
Pittard	Mecklenburg	14 Jun	red	194 (163-228)	394 (340-481)	1.0	0.006
Crews	Charlotte	26 Jun	green	195 (106-307)	412 (302-831)	1.0	0.006
SPAES IT5	Nottoway	5 Jul	red	199 (75-374)	416 (291-1204)	1.0	0.006
Moore	Charlotte	7 Aug	red	199 (167-235)	412 (355-506)	1.0	0.006
Easley	Pittsylvania	15 Jul	red	202 (113-321)	414 (303-845)	1.0	0.006
Clayton	Johnston/[5]	1984	green	202 (150-253)	563 (474-710)	-	0.004
Crews	Charlotte	7 Aug	green	203 (73-402)	430 (297-1366)	1.0	0.006

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Appendix 4A. Continued

Response of field strains of the tobacco aphid to acephate 75SP (LC_{50} and LC_{90}), Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% F.L./1)	LC_{90} (95% F.L.)	Tolerance/2 Ratio	Slope
Via	Patrick	31 Jul	red	213 (181-251)	427 (367-523)	1.1	0.006
SPAES IT1	Nottoway	5 Jul	red	225 (140-361)	406 (299-821)	1.1	0.007
Easley	Pittsylvania	8 Aug	red	227 (197-262)	414 (362-494)	1.1	0.007
Greene	Lee	8 Aug	red	235 (206-269)	412 (363-486)	1.2	0.007
Crews	Charlotte	20 Jul	red	245 (91-938)	432 (289-3330)	1.2	0.007
Moore	Charlotte	27 Jul	red	248 (209-297)	505 (426-643)	1.2	0.005
SPAES/6	Nottoway	15 Jul	red	249 (221-282)	410 (365-478)	1.2	0.008
Hawthorne	Brunswick	28 Jul	green	251 (118-685)	468 (318-2240)	1.2	0.006
Moore	Charlotte	12 Jul	green	265 (162-419)	537 (394-1026)	1.3	0.005
Moore	Charlotte	24 Aug	red	275 (36-730)	594 (384-2759)	1.4	0.004
SPAES	Nottoway	13 Oct	red	277 (82-495)	714 (495-1653)	1.4	0.003

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Appendix 4A. Continued

Response of field strains of the tobacco aphid to acephate 75SP (LC_{50} and LC_{90}), Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% F.L./1)	LC_{90} (95% F.L.)	Tolerance/2 Ratio	Slope
Pittard	Mecklenburg	27 Jul	red	289 (252-338)	514 (443-631)	1.4	0.006
Tuck	Brunswick	25 Jul	red	289 (253-335)	503 (436-612)	1.4	0.006
Hawthorne	Brunswick	28 Jul	red	298 (248-354)	654 (564-791)	1.5	0.004
Pittard	Mecklenburg	7 Aug	red	302 (270-342)	476 (423-558)	1.5	0.007
Barnes	Dinwiddie	23 Jun	red	310 (242-392)	749 (622-961)	1.5	0.003
Via	Patrick	18 Jun	red	319 (35-3768)	596 (374-17130)	1.6	0.005
Tuck	Brunswick	30 Jun	red	322 (273-378)	666 (577-799)	1.6	0.004
SVAES	Washington	22 Aug	green	335 (298-384)	530 (464-637)	1.7	0.007
Moore	Charlotte	26 Jun	green	339 (291-394)	660 (576-786)	1.7	0.004
Pittard	Mecklenburg	26 Jun	red	341 (294-397)	660 (576-785)	1.7	0.004
Crews	Charlotte	26 Jul	green	349 (209-570)	689 (500-1340)	1.7	0.004

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Appendix 4A. Continued

Response of field strains of the tobacco aphid to acephate 75SP (LC_{50} and LC_{90}), Virginia, 1989.

Farm	County	Collection Date	Color Morph	LC_{50} (95% F.L./1)	LC_{90} (95% F.L.)	Tolerance/2 Ratio	Slope
Via	Patrick	15 Jul	green	362 (170-777)	737 (466-2039)	1.8	0.004
Barnes	Dinwiddie	26 Jul	red	363 (247-525)	737 (562-1187)	1.8	0.003
Pittard	Mecklenburg	1 Sep	red	373 (246-559)	749 (562-1275)	1.8	0.003
Barnes	Dinwiddie	5 Jun	red	413 (103-1956)	876 (552-8324)	2.0	0.003

/1 F.L. = Fiducial Limits.

/2 Tolerance Ratio = LC_{50} field strain / LC_{50} susceptible strain.

/3 SPAES IT = Southern Piedmont Agricultural Experiment Station, Blackstone, VA, Insecticide Test Plot.

/4 SVAES = Southwest VA Agricultural Experiment Station in Glade Springs, VA.

/5 Johnston = the location in North Carolina where the susceptible strain was obtained.

/6 SPAES = Southern Piedmont Agriculturl Experiment Station, Blackstone, VA.

Chapter 5

Influences of Insecticide Exposure under Field Conditions on the Chromosomes of Red and Green Morphs of the Tobacco Aphid, Myzus nicotianae

The tobacco aphid, Myzus nicotianae Blackman, is an economically important pest on tobacco in Virginia (Semtner 1983). Tobacco aphids have been pests of tobacco in North America since the mid 1940's (Dominick 1949, Chamberlin 1958). Heavy infestations of the tobacco aphid can result in reductions in yield and quality of the cured tobacco leaf. Research in Virginia has shown that aphid populations may reduce tobacco yield by 20% and returns to the grower by 25%.

Tobacco aphids injure tobacco by removing plant sap and depositing honeydew (Chamberlin 1958). Infested leaves yellow prematurely and necrosis may occur along leaf margins (Dominick 1949). Black sooty mold (Fumago vagans) can develop on the leaves coated with honeydew and cause additional leaf discoloration (Dominick 1949). Aphids also transmit several viral diseases (Chamberlin 1958, Lucas 1975)

There are two color morphs associated with M. nicotianae. The green morph was the predominant form found on tobacco until 1983, when a red morph was infrequently observed. In 1986 the red morph of M. nicotianae became the most prevalent form infesting tobacco (McPhereson 1989, Reed and Semtner 1989).

There are three karyotypes characteristic of M. nicotianae: 1) 2n=12 normal karyotype; 2) 2n=12 translocated form; 3) 2n=13 dissociated

karyotype (Blackman 1987). In many instances the red morph of M. nicotianae has the translocated karyotype which is associated with an increased resistance to organophosphorous insecticides in M. persicae (Blackman et al. 1978). Lampert (pers. comm.) has found that there is a higher resistance in the red morph to monocrotophos (Azodrin) and acephate (Orthene).

Due to the occurrence of the red morph and a translocated karyotype associated with insecticide resistance in M. persicae, this study was conducted to determine the effect of various insecticides on the karyotype of red and green morphs of M. nicotianae. The efficacy of five insecticides in 1988 and four insecticides in 1989 was also studied.

Materials and Methods

1988. Experimental plots were established at the Southern Piedmont Agricultural Experiment Station. Flue-cured tobacco 'Coker 319' used for this study was transplanted into the field on 9 May. Experimental plots consisted of two 12.2 m rows of tobacco planted on 1.2 m centers. Adjacent plots were separated by single untreated border rows. Treatments included:

1. Acephate (Orthene Tobacco Insect Spray 75SP) (840g (AI)/ha).
2. Endosulfan (Thiodan 2L) (560g (AI)/ha)
3. Methomyl (Lannate 1.8L) (504g (AI)/ha)
4. Monocrotophos (Azodrin 5WM) (1120g (AI)/ha)
5. Permethrin (Pounce 3.2EC) (224g (AI)/ha)
6. Untreated Control

The treatments were replicated four times in a randomized complete block design. Insecticide treatments were applied with a CO_2-pressurized backpack sprayer operated at 414 kPa and delivering 187 l/ha through three TX-8 (Spraying Systems) hollow cone nozzles/row on 24 June and 224.4 l/ha through three TX-10 hollow cone nozzles/row on 12 July and 3 August. At the time of application, temperatures were 24, 26, and 32 ^{o}C on 24 June, 12 July, and 3 August respectively. There was 0.89cm of rainfall 2 days after the insecticide application on 24 June, 1.27cm on 14 July and 0.76cm on 6 August.

Tobacco aphids were counted on the uppermost four leaves with a minimum length of 10 cm (Reed, 1987) on 10 plants/plot before treatment and at 3 day to 2 week intervals after insecticide application. Aphids were collected from each of the experimental plots on 23 June, 6 July, and 3 August. When it was possible both red and green morphs were collected. The live aphids were maintained on excised leaves of tobacco in growth chambers monitored at 24^{o}C and a 16h photophase. The leaves were inserted into water agar in the bottom of a 473 ml styrofoam cup and covered with a translucent plastic lid. The karyotype of each aphid was determined using the chromosome squash procedure described in Chapter 3 pg. 26.

1989. Experimental plots were established at the Southern Piedmont Agricultural Experiment Station. Flue-cured tobacco 'NC 567' used for this study was transplanted on 23 May. Experimental plots consisted of two 12.2 m rows of tobacco planted on 1.2 m centers. Adjacent plots were separated by single untreated border rows. Treatments included:

1. Acephate (Orthene Tobacco Insect Spray 75SP) (840g (AI)/ha)
2. Endosulfan (Thiodan 0.75EC) (1120g (AI)/ha)
3. Methomyl (Lannate 1.8L) (504g (AI)/ha)
4. RH-7988 50WP (Proposed common name - Triazuron) (280g (AI)/ha)
5. Untreated Control

The treatments were replicated four times in a randomized complete block design. Insecticide treatments were applied with a CO_2-pressurized backpack sprayer operated at 414 kPa and delivering 234 l/ha through 3 TX-10 hollow cone tips on 7 July. At the time of application, the temperature was 26^{o}C. Only one insecticide application was made. The aphid pressure in the test did not warrant further applications. Myzus nicotianae were counted on the uppermost 4 leaves with a minimum length of 10 cm (Reed 1987) on 20 plants/plot before treatment (22 and 29 June) and at 4 day to 3 week intervals after treatment.

Tobacco aphids were collected from each of the experimental plots on 4 and 5 July and 1 August. Red and green morphs were collected on 4 and 5 July; however, only the red morph was found on 1 August. The live aphids were maintained on excised tobacco leaves from greenhouse plants. The leaves were inserted into the agar in the bottom of a 473ml styrofoam cup. The cups were covered with translucent plastic lids and placed in growth chambers maintained at 24^{o}C and a 16h photophase. Aphid karyotype was determined using the chromosome squash preparation previously described in Chapter 3 pg. 26.

Results and Discussion

Acephate was the most effective insecticide in 1988. Permethrin was not effective. Permethrin is not labelled for control of aphids; but was included in this test to investigate its effect upon the karyotype of the tobacco aphid. Monocrotophos, methomyl, and endosulfan were generally not significantly different in their control of M. nicotianae (Table 5.1). Very high temperatures (daytime highs of 36-39^{o}C) probably killed many aphids and may have improved control after the 12 July treatment.

Only three insecticides tested in 1988 were also used in 1989. Monocrotophos was omitted because it is no longer labelled for aphid control. RH-7988, an experimental aphicide was added. Control with RH-7988 was significantly different from all other insecticides tested at four and seven days post-treatment (Table 5.2). Acephate and endosulfan were generally not significantly different in their control of the tobacco aphid. However, the persistence of control with endosulfan was not as long as acephate and RH-7988. Control with methomyl was significantly different from all other insecticides. The decline in aphid populations in the untreated plots after 14 July was probably caused by a naturally occurring fungus attacking the aphids or due to a natural decline in aphid populations.

Table 5.3 shows the karyotypes of aphids collected in 1988 before and after insecticide application. Aphids sampled on 23 June were collected one day before the first insecticide applications were made. Among the morphs sampled on this pre-treatment date, 90% of the red morphs had the translocation, while only 30% of the green morphs had the translocated

karyotype. The 2n = 12 normal karyotype was most prevalent in the green morphs (70%) and somewhat less in the red morphs (7.5%). The 2n = 13 chromosome complement was only found in the red morphs (2.5%).

Tobacco aphids collected on 6 July about 2 weeks after the first insecticide application had a slight increase in the number of translocated individuals in the red morphs and an increase in the 2n = 12 normal individuals among the green morphs. No morphs contained the 2n = 13 karyotype. This collection date occurred six days before the second insecticide application (12 July).

The final sampling date was 3 August approximately 3 weeks after the second insecticide application. The final spraying of the insecticide plots occurred on 3 August. There were no green aphids left in the plots at this time. The number of translocated individuals made up 94% of the population sampled. There were no morphs with the 2n = 13 karyotype.

Karyotypes of tobacco aphids collected from the insecticide plots in 1989 are shown in Table 5.4. The 2n = 13 chromosome complement was not present in any of the aphids sampled in 1989. As in the 1988 study, the translocated karyotype occurred most often in the red morphs. Of the 200 red morphs sampled 99.5% had the translocation. The 2n = 12 normal karyotype occurred 98.8% of the time in the green morphs. By 1 August the green morphs of M. nicotianae were no longer found in the insecticide plots. All red morphs sampled on 1 August had the translocated karyotype.

The results from the 1988 and 1989 study indicate that karyotype and color morph are highly related. Insecticide exposure on the karyotype of M. nicotianae had little influence except for selecting the translocated

individuals in the population. The increase of red morphs with the 2n=12 translocated karyotype in 1988 indicates such selection. All insecticides tested, with the exception of Permethrin, gave satisfactory to excellent control of red and green morphs of the tobacco aphid. There did not appear to be any insecticide tolerance in red or green morphs. The decline in the green morphs near the end of the tobacco growing seasons in 1988 and 1989 is probably due to the high temperatures at this time. Research in Virginia has shown that the red morph of the tobacco aphid is more tolerant of high temperatures than the green morph (Reed 1987).

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Table 5.1. Control of the tobacco aphid with various insecticides applied as foliar sprays, Blackstone, Virginia 1988.

Myzus nicotianae / 4-leaf sample*

	Treatment (g (AI) / ha)					
Date	Acephate 840	Endosulfan 560	Methomyl 504	Monocrotophos 1120	Permethrin 224	Untreated Control
21 Jun	16.2a**	20.3a	25.7a	41.3a	31.6a	27.2a
27 Jun	2.7c	17.6b	4.9bc	14.1b	60.7a	148.4a
30 Jun	1.5c	21.0b	18.9b	40.4ab	106.8a	202.2a
12 Jul	5.9c	100.3b	166.3ab	166.5ab	532.6a	488.0ab
18 Jul	0.1c	0.1c	0.5c	3.0b	106.9a	147.6a
22 Jul	0.4c	0.2c	32.1b	3.2bc	137.7a	123.2a
3 Aug	2.4c	12.7bc	67.6b	73.3b	209.2a	333.7a
5 Aug	0.6c	5.9b	13.2b	4.8bc	209.9a	302.8a
10 Aug	0.3d	6.7bc	22.9b	3.3c	94.3a	138.8a

* Means within a row followed by the same letter are not significantly different (P = 0.05, DMRT)

** Data were transformed to $\log_{10}(x + 1)$. Untransformed means are presented.

Table 5.2. Control of the tobacco aphid with various insecticides applied as foliar sprays, Blackstone, Virginia 1989.

Myzus nicotianae / 4-leaf sample*

	Treatment (g (AI) / ha)				
Date	Acephate 840	Endosulfan 560	Methomyl 504	RH 7988 280	Untreated Control
22 Jun	8.4ab**	6.6b	11.6ab	13.8ab	14.6a
29 Jun	28.0a	21.6a	27.5a	30.1a	36.5a
11 Jul	5.2b	4.3b	13.0b	0.0c	666.8a
14 Jul	3.5c	3.9c	18.9b	0.0d	864.8a
21 Jul	7.6c	6.4c	27.5b	3.0c	457.1a
1 Aug	6.5c	28.0b	44.3b	6.0c	103.4a

* Means within a row followed by the same letter are not significantly different (P = 0.05, DMRT)

** Data were transformed to $\log_{10}(x + 1)$. Untransformed means are presented.

Table 5.3. Influence of five insecticides on the karyotype of red and green morphs of the tobacco aphid, Blackstone, Virginia, 1988.

Treatment	Collection Date	Color Morph	N/1	Karyotype/2		
				12N	12T	13
Permethrin	23 Jun	green	10	7	3	0
Acephate	23 Jun	red	30	3	27	0
Methomyl	23 Jun	red	30	2	27	1
Monocrotophos	23 Jun	red	30	1	28	1
Permethrin	23 Jun	red	20	2	17	1
Untreated Control	23 Jun	red	10	1	9	0
Methomyl	6 Jul	green	10	7	3	0
Untreated Control	6 Jul	green	20	19	1	0
Endosulfan	6 Jul	red	20	0	20	0
Methomyl	6 Jul	red	10	1	9	0
Monocrotophos	6 Jul	red	20	2	18	0
Permethrin	6 Jul	red	30	0	30	0
Untreated Control	6 Jul	red	20	1	19	0

-- Continued --

Table 5.3. Continued.

Table 5.3. Influence of five insecticides on the karyotype of red and green morphs of the tobacco aphid, Blackstone, Virginia, 1988.

Treatment	Collection Date	Color Morph	N/1	Karyotype/2 12N	12T	13
Acephate	3 Aug	red	10	0	10	0
Endosulfan	3 Aug	red	40	4	36	0
Methomyl	3 Aug	red	40	1	39	0
Monocrotophos	3 Aug	red	30	3	27	0
Permethrin	3 Aug	red	20	2	18	0
Untreated Control	3 Aug	red	40	1	39	0

/1 N = number of aphids karyotyped

/2 12N: 2n = 12 karyotype; 12T: 2n = 12 translocated karyotype; 13: 2n = 13 karyotype

Table 5.4. Influence of four insecticides on the karyotype of red and green morphs of the tobacco aphid, Blackstone, Virginia, 1989.

Treatment	Collection Date	Color Morph	N/1	Karyotype/2		
				12N	12T	13
Acephate	5 Jul	green	20	20	0	0
Endosulfan	5 Jul	green	30	29	1	0
Methomyl	5 Jul	green	10	10	0	0
Untreated Control	5 Jul	green	20	20	0	0
Acephate	4 Jul	red	40	0	40	0
Endosulfan	4 Jul	red	40	0	40	0
Methomyl	4 Jul	red	40	0	40	0
RH-7988	4 Jul	red	40	1	39	0
Untreated Control	4 Jul	red	40	0	40	0
Acephate	1 Aug	red	30	0	30	0
Endosulfan	1 Aug	red	30	0	30	0

-- Continued --

Table 5.4. Continued.

Table 5.4. Influence of four insecticides on the karyotype of red and green morphs of the tobacco aphid, Blackstone, Virginia, 1989.

Treatment	Collection Date	Color Morph	N/1	Karyotype/2 12N	12T	13
Methomyl	1 Aug	red	40	0	40	0
RH-7988	1 Aug	red	40	0	40	0
Untreated Control	1 Aug	red	40	0	40	0

/1 N = number of aphids karyotyped

/2 12N: 2n = 12 karyotype; 12T: 2n = 12 translocated karyotype; 13: 2n = 13 karyotype

Chapter 6

Life Cycle of the Tobacco Aphid, Myzus nicotianae Blackman in Virginia -- Male Production Study

The green peach aphid, Myzus persicae (Sulzer), has a worldwide distribution and its life cycle varies between different regions of the world (Blackman 1974). Life cycle variation of an aphid can be explained by climatic differences and the effect of these differences on gene and genotype frequencies within the species (Blackman 1974). The green peach aphid can overwinter on winter hosts as parthenogenetically reproducing females (anholocycly) or overwinter as eggs on peach trees (holocycly). In androcyclic populations, reproduction occurs throughout the year by parthenogenesis; however, some males are produced to contribute to the sexual phase (Blackman 1971).

The ability of an aphid to reproduce asexually enables the aphid to take advantage of short-term food supplies and thus acquire considerable importance as an agricultural pest (Blackman 1979). In Britain the migrants of M. persicae from peach trees are less important in intially infesting commercial crops than are those that have overwintered parthenogenetically on secondary hosts (Heathcote 1962). Aphids are serious pests on tobacco in the United States (Dominick 1949, Chamberlin 1958). The tobacco aphid is an economically important pest on tobacco in Virginia (Semtner 1983). Heavy infestations of the tobacco aphid can result in reduction in yield and quality of the cured tobacco leaf (Cheng and Court 1977). Tobacco aphids

injure tobacco by removing plant sap and depositing honeydew and exuviae on the leaves (Chamberlin 1958). Black sooty mold (Fumago vagans) can develop on leaves coated with honeydew. Infested leaves yellow prematurely and necrotic tissue may develop along leaf margins and at the base of the petiole (Dominick 1949).

Sexual morphs of M. persicae are produced in response to certain photoperiods and temperatures (Blackman 1971b). The production of males occurs parthenogenetically. This type of reproduction is termed arrhentoky (Blackman 1987b). Male production results from the loss of half of the sex chromatin of the parent female in a single maturation division producing an XO individual (Blackman 1980).

Mittler et al. (1979) reported that males of M. persicae are produced as a result of a reduction in juvenile hormone produced by the corpora allata. A precocene analogue: 6-methoxy-7-ethoxy-2,2-dimethylchromene has been used successfully to produce males in M. persicae since precocene reduces juvenile hormone levels (Hales and Mittler 1983).

Color morphs of M. persicae can differ in their response to photoperiod and temperature. A study by Takada (1982a) indicated that suppression of males and alate viviparae at high temperatures and long photoperiods was more prominent in the red morphs than in the green morphs.

Blackman (1987) reported that males are not present in tobacco aphid populations. However, the aphids that he tested were from European populations. Thus this study was initiated to determine whether the tobacco

aphid in Virginia produces males under short day length and cool temperatures that induce male production in M. persicae.

Materials and Methods

Tobacco aphids were collected from field tobacco during August, September and October. Aphids were collected at these times because fall is when males are produced in holocyclic species. Twenty colonies in 1988 and ten colonies in 1989 were established by collecting ten aphids from each collection site and placing them on individual excised tobacco leaves. Aphids were reared in cages in growth chambers maintained at 15^{o}C and an 8 hour photoperiod.

The rearing cage consisted of a plastic box (17.8 X 12.7 X 4.4 cm) with two ventilation holes (10.2 X 8.9 cm) cut in the top and bottom and covered with organdy cloth. A sponge with a slit cut in the top center was placed in the bottom portion and the petiole of the excised leaf was placed into the slit of the sponge. The cages were aligned in a plastic tray filled with water. The slit in the bottom of the plastic boxes allowed for uptake of water by the sponge which provided a continual supply of moisture to the leaf.

Winged aphids (alate) were collected from each colony as they developed. Alate aphids were then checked for the presence of embryos, which indicated that they were female.

In 1989 an additional experiment was conducted using a slightly different technique developed by Blackman (1974). Green peach aphids and red and green morphs of the tobacco aphid were used in this experiment. Red and green morphs of the tobacco aphid were taken from aphid colonies

collected on 1 August from untreated plots in the insecticide test at the Southern Piedmont Agricultural Experiment Station. The green peach aphids were taken from an aphid colony collected from a turnip plant in an experimental vegetable plot in November 1989.

The rearing cage was the same as described above. One third or fourth instar nymph was placed on a leaf in each cage and exposed to 15^{o}C and 8 hour photoperiod. After the nymph had become an adult and produced 20 progeny, she was removed from the cage. When these offspring became adults, their young were removed every day for the first 10 days of reproduction. Then the adults were allowed to produce 20 progeny. When 20 young were produced the adults were removed. The alate that developed from these offspring were examined for males. Five replications of each aphid colony (green peach aphid, red and green tobacco aphids) were tested.

Results and Discussion

Embryos were present in each of the winged aphids examined (369 in 1988 and 952 in 1989). Therefore all alates examined were females. Males were not produced in any of the aphid colonies even though the colonies were kept under conditions that favored the development of males in species with holocyclic life cycles.

The study in 1989 investigating male production in green peach aphids and red and green morphs of the tobacco aphid also revealed that only females were present. Twenty (F_3) adults were examined from each aphid colony. Embryos were present in each aphid. Only five of the aphids

produced wings; all others remained apterous. The data indicates that tobacco aphids in Nottoway County, Virginia have an anholocyclic life cycle.

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Chapter 7

Summary and Conclusions

The red and green morphs of the tobacco aphid had different chromosomal make-ups. The red morph usually possessed the translocated karyotype while, the normal karyotype was most prevalent in the green morphs. The $2n=13$ chromosome complement was found in very low numbers in both the red and green morphs. Geographic location in Virginia and tobacco type did not have an impact on the karyotype of the tobacco aphid.

There were no seasonal changes in the karyotype of red and green morphs of M. nicotianae in 1989. Research was initiated in 1989 to study the seasonal change in the aphid karyotype throughout the growing season. It was expected that a low proportion of red morphs with the translocation would occur early in the season and then the proportion would increase with increased insecticide pressure. However, only 3 of 100 red aphid examined early in June had the normal karyotype. All other red morphs sampled in 1989 had the translocated karyotype (99.2%). The normal chromosome complement was most commonly found in the green morphs (96%). Only 6 of 150 green morphs sampled had the translocation.

The presence of the translocated karyotype in the red morphs did not pose a serious problem in controlling the tobacco aphid. If the translocation was associated with increased tolerance to organophosphorous insecticides, it could cause a serious problem for tobacco growers in the future.

Some tolerance to acephate was found in both the red and green morphs of the tobacco aphid. However, there was not a strong relation between the red color morph and tolerance to acephate. Green morphs from several farms had LC_{50} values greater than the red morphs and the North Carolina standard used in this test. These findings were not as expected, since the tobacco aphid has sometimes been difficult to control during the past four years and since the translocated karyotype found in red morphs of the tobacco aphid is associated with increased tolerance to organophosphorous insecticides in the closely related green peach aphid, Myzus persicae (Sulzer).

The present data show that tolerance to acephate is present at a low level in Virginia, and that the potential for increased tolerance to acephate exists. It is necessary for acephate to be used judiciously in Virginia to reduce the possibility that the tobacco aphid will acquire tolerance to this insecticide. Three foliar insecticides and one soil insecticide are currently recommended for aphid control in Virginia. Of the recommended insecticides, acephate is most often chosen by the tobacco grower due to its relatively low toxicity, high efficacy, control of other important pests, and low residues on the cured tobacco. Additional research is needed to test the influence of time of year and insecticide pressure on acephate tolerance in tobacco aphid populations.

Males were not found in tobacco aphid populations from Nottoway County, Virginia. Embryos were present in alate aphids examined in 1988 (369) and 1989 (952). It appears that the tobacco aphid in Virginia has an anholocyclic life cycle. Additional life cycle studies using secondary hosts of

the tobacco aphid and aphids from other counties in Virginia are necessary to determine if this would influence the production of males.

VITA

Martha Letcher Barnes was born to William T. and Betty R. Barnes on July 21, 1964 in Farmville, Virginia. She attended private school at Kenston Forest School in Blackstone, Virginia and graduated from high school in 1982. She enrolled in Randolph-Macon Woman's College in 1982 and graduated cum laude with a B.A. in biology in May 1986. While at Randolph-Macon Woman's College, she was elected to membership in the Phi Beta Kappa Honor Society.

In September of 1987, she enrolled in the graduate school of Virginia Polytechnic Institute and State University to pursue a M.S. degree in entomology. Under the direction of Dr. Paul J. Semtner at the Southern Piedmont Agricutlural Experiment Station in Blackstone, VA, she conducted research on the karyotypic diversity of red and green morphs of the tobacco aphid in Virginia. While at VPI & SU, she was elected to membership in Phi Kappa Phi and Sigma Xi and was awarded the James McD. Grayson Scholarship Award.

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