Intraclonal Morphological Plasticity within the *Myzus persicae* (Sulzer) Complex Related to Host Plant and Temperature

by

Joan Marie

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Advisory Committee: Paul J. Semtner, Chair – Professor of Entomology, Virginia Tech
Donald Mullins – Professor of Entomology, Virginia Tech
Douglas Pfeiffer – Professor of Entomology, Virginia Tech

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Abstract

Blackman (1987) used life cycle and morphology to separate *Myzus nicotianae* Blackman, a tobacco-feeding species of aphid, from *Myzus persicae* (Sulzer). In the present study, the first objective was to investigate the influence of temperature and host plant on the morphology of *M. nicotianae* and *M. persicae*. The second objective was to assess Blackman's 1987 key to *Myzus* for separating tobacco and non-tobacco originating morphs under different environmental conditions. Four host plants were used: tobacco, turnip, pepper, and okra, and three temperatures, 15°C, 20°C, and 25°C. The intraclonal plasticity of two tobacco collected morphs and one turnip collected morph was investigated in relation to these combinations of host and temperature in a 4 x 3 x 3 factorial experimental design. Fifth generation mature apterous aphids were mounted on slides and 10 different morphological structures utilized in morphometric analysis were measured.

Data support a morphologically distinct, host-adapted tobacco race but not a separate tobacco-feeding species of *M. persicae*. The key developed by Blackman (1987) did not discriminate between the tobacco and non-tobacco originating clones but the canonical variates generated from the analysis successfully separated the tobacco and non-tobacco groups. Other studies have used many different clones to investigate the possible distinctions between *M. persicae* and *M. nicotianae*; the objective here was to see how much morphological perturbation may be induced within a clone by rearing at different temperatures and on different host plants.

Temperature and host plant had substantial influences on the morphology of these aphids. The physiological interactions of temperature-host plant-aphid morphology are very complex yet controlling only for temperature and host plant was sufficient to group specimens according to these independent variables with remarkable accuracy using the linear discriminant functions generated with these data. Percent of aphids in which rearing temperature was correctly identified using linear discriminant functions generated for temperature classes was 87%, 63%, and 64% for 15°C, 20°C, and 25°C, respectively. Random designations would be 33%. Correct identification of host plant was 65%, 45%, 47%, and 48% successful for tobacco, turnip, pepper, and okra, respectively. Random designations for host plant would be 25%.

Canonical variates produced clusters by host, temperature, morph, and combinations of these independent variables with varying degrees of discreteness. CV1 by CV2 for host plants gave a very distinct cluster for tobacco and also separate groupings for aphids reared on turnip and pepper. Aphids from the host plant okra were scattered quite widely across the CV1 by CV2 graph. CV1 by CV2 for temperature conditions showed a tight cluster for aphids from 15°C and still distinct though less closely grouped clusters for both 20°C and 25°C rearing temperatures. CV1 by CV2 for the three morphs gave substantial overlap for the two tobacco originating morphs and a more separate cluster for the morph originally collected from turnip.

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1.1.1 Agricultural Importance of *Myzus persicae* (Sulzer)

The *Myzus persicae* (Sulzer) complex is a serious pest of several major agricultural crops including peaches, potatoes, sugar beets, and tobacco, and various ornamental crops grown in landscapes and in glasshouses (Mason 1940, Chamberlin 1958, van Emden et al. 1969, Blackman and Eastop 2000). High populations of *M. persicae* cause injury by removing large volumes of sap from plants and depleting them of nutrients. They also cause indirect injury via the production of sugary honeydew that makes the leaves susceptible to sun scald and provides food for sooty mold, *Fumago vagans* Pers., which then reduces leaf quality (Dominick 1949). *M. persicae* is also one of the most important vectors of several persistent and non-persistent viruses that cause serious losses in tobacco and other crops (Sylvester 1954).

1.1.2 Separation of Related Species

Two closely related species were separated from *M. persicae*, *Myzus antirrhinii* (Machiattii) (the snapdragon aphid) and *Myzus nicotianae* (Blackman) (the tobacco aphid). Blackman and Patterson (1986) separated *M. antirrhinii* from *M. persicae* based on differences in life cycle, karyotype (number of chromosomes) and morphology. Blackman (1987) used life cycle and morphology to separate *M. nicotianae* from *M. persicae*.

Speculations regarding separate species status within the *M. persicae* complex specifically adapted to tobacco were associated with field observations in the 1980's in tobacco fields in the United States (Blackman 1987, Lampert and Dennis 1987, McPherson 1989, Reed and Semtner 1989). *M. persicae* has several color morphs. When it first became a problem in tobacco fields in the United States in the 1940's, the light green morph was predominant and

dark green and red forms rarely occurred (Dominick 1949, Chamberlin 1958, Blackman 1987). In the early 1980's, two things happened concurrently or nearly so, significant insecticide resistance was found in the tobacco fields and the red form became the dominant morph (McPherson 1989, Reed and Semtner 1989, Harlow and Lampert 1991). This red morph was also noticeably larger than the light green morph (Blackman 1987). So a serious agricultural problem, insecticide resistance, arose at the same time that a striking visual change took place (Blackman 1987, Lampert and Dennis 1987, Harlow and Lampert 1990, McPherson and Bass 1990). These developments set the stage for scientists to explore both the particulars of the applied problem (insecticide resistance) and the basic biology/life history of the organism.

1.1.3 Identification Problems for *Myzus nicotianae* Blackman

Several difficulties followed the designation of the tobacco aphid, *M. nicotianae*, as a separate species. From the beginning, there were populations that did not fit into one group or the other. Questions about life cycle differences and, therefore, degree of reproductive isolation have been presented several times. The morphological key developed by Blackman (1987) proved difficult for researchers to use. When clonal populations derived from single individuals of *M. persicae* and *M. nicotianae* were keyed out using the key to *Myzus* developed by Blackman (1987), the identifications were often inconsistent (Clements et al. 2000a,b). Researchers identified aphid groups to the level of *M. persicae* from earlier keys and then designated them as *M. persicae* or *M. nicotianae* depending on host plant association, *M. nicotianae* if collected from tobacco and *M. persicae* if collected from other hosts (Clements et al. 2000a,b).

This was an awkward and confusing situation in that species identification should be more consistent and the key to distinguish *M. persicae* from *M. nicotianae* should be more useful. Not only was this problematic for scientists working on this species, it kept the status of

M. nicotianae tenuous and several groups took this up as an explicit research question itself. Is *M. nicotianae* really a separate species? If so, could a more reliable key be constructed? For this study the hypotheses are that (1) there is a morphologically distinct, host-adapted tobacco race but not a separate tobacco-feeding species of *M. persicae,* and (2) that temperature and host plant have significant effects on the morphology of aphids in the *M. persicae* complex.

Investigations into the basic biology of *M. persicae* have helped researchers understand its potential for population growth on various crops, mechanisms of disease transmission, the processes associated with the development of insecticide resistance, and mechanisms of host-plant resistance to *M. persicae*. Of course, the fundamental assumption when conducting these important experiments is that researchers know that they are studying the correct organism, i.e., the species *M. persicae* and not another, closely related but significantly different species. Excellent keys exist to identify *M. persicae* to species in both alate (winged) and apterous (non-winged) forms (Smith et al. 1992, Blackman and Eastop 2000).

1.2 Research Objectives

This investigation addressed two questions: Whether or not environmental factors such as temperature and host plant affect the utility of the taxonomic keys and whether or not the taxonomic status of *M. nicotianae* is supported with morphometric multivariate analysis. The purpose of the analysis was twofold. The first part was to see if Blackman's approach was replicable with these groups of *M. persicae* and *M. nicotianae* in Virginia. Secondly, the analysis was used to evaluate the impact of temperature and host on the size of the characters used in discrimination.

2. Review of Literature

2.1 *Myzus persicae* (Sulzer) as Agricultural Pest and Taxonomic Problem

Aphids are insects in the family Aphididae and the order Homoptera. They are very small, rarely over 5 mm in length, with piercing-sucking mouthparts that enable them to feed on the fluid (sap) transported in the phloem of plants. There are thousands of species of aphids within numerous genera. Because they feed on plants, many are important pests of garden, orchard and field crops (Ilharco and van Harten 1987, Blackman and Eastop 2000).

Aphids are highly variable in both form and life cycle. Within the same species, there are winged and non-winged forms and there are often both asexual and sexual life cycles within which there are distinct phenotypes (Ilharco and van Harten 1987, Blackman and Eastop 2000). This great variety of basic characteristics has made the field of aphid systematics highly complicated.

The green peach aphid, *M. persicae*, has been a particularly challenging taxon for over 200 yr. It has characteristics that make it both an important agricultural pest and difficult to discriminate. It is one of the most significant pests on agricultural crops worldwide (Blackman and Eastop 2000). Its highly polyphagous nature is one reason it is such a problem in so many areas of agricultural production and also why it has been difficult to specify taxonomically.

Sulzer first described *M. persicae* as *Aphis persicae* in 1776 (Mason 1940). Passerini transferred *Aphis persicae* to the genus *Myzus* in 1860 (Mason 1940). The genus *Myzus* has several distinct morphological characteristics, including: six-segmented antennae; convergent antennal tubercles; generally long, cylindrical siphunculi; and generally short, conical cauda without much constriction (Mason 1940, Blackman and Eastop 2000).

In addition to its morphological characters, *M. persicae* is noted for its association with the genus *Prunus* in the family Rosaceae. The peach, *Prunus persicae,* is the origin of one of *M. persicae's* common names, the green peach aphid, and it serves as the primary host in regions where the aphid completes the sexual phase of its life cycle. Many species of *Myzus*, including *M. persicae*, have holocyclic life cycles where the sexual phase is completed on a primary host plant that is different from the secondary host plant(s) that is used for the parthenogenic (asexual) phase. Aphids that no longer undergo a sexual reproductive phase are considered anholocyclic (Ilharco and van Harten 1987).

Secondary hosts of *M. persicae* include species from more than 60 plant families (Patch 1938). Some *Myzus* are only described in association with their secondary host plants (Mason 1940). One of the reasons that *M. persicae* has so many synonyms is because it has an especially large number and wide range of secondary hosts, including grasses, lilies, legumes, peppers, mustards, geraniums, spurges, dogbanes, nettles, cacti, and nightshades (Patch 1938). *M. persicae* has been linked to tobacco from at least the early part of the 20th century (Blackman 1987). It has been managed as a pest of tobacco in the United States since the 1940's (Chamberlin 1958).

Permanent anholocyclic life cycles appear to be associated with geographic/climatic factors that fail to trigger the production of one or both sexuparae (the male and oviparous female forms). There are also intermediate life cycles, such as an androcyclic life cycle in which sexual males are produced, but no sexual females (Ilharco and van Harten 1987). This complicates the question of reproductive/genetic isolation of a separate species since it is possible for males from one population to mate with females of another population.

Myzus persicae occurs as several distinct color morphs and phenotypes. It is always described as some or several variations of green and descriptions also sometimes include pink or various shades of red (Mason 1940, Blackman and Eastop 2000). It has both alate and apterous forms. The alates may or may not be associated with the sexual reproductive phase. In his 1940 revision, Mason described seven phenotypes for *M. persicae*.

2.2 A New Species: *Myzus nicotianae* Blackman

Blackman (1987) designated a tobacco-adapted form of *M. persicae* as a new species. *M. nicotianae* was separated from *M. persicae* by multivariate morphometric analysis using the method of canonical variates. This is an approach based on overall similarity of characters, providing multidimensional representation, such as a scatter diagram, of the degree of dissimilarity between groups (Cranston et al. 1991).

The tobacco-adapted form of *M. persicae* is the most serious pest of tobacco in Virginia and is a major pest of tobacco worldwide. During severe infestations, the aphid can reduce yields and returns over 20% and 30% respectively (Reed and Semtner 1992). This aphid is the target of about two-thirds of the insecticides applied to tobacco in Virginia. Annual losses attributed to the aphid and its control average $500,000 to $1 million each yr in Virginia (Semtner, unpublished) and $15 million in North Carolina (Southern 1990).

2.2.1 Morphological Distinctions

In the approach used by Blackman (1987) to separate *M. nicotianae* from *M. persicae*, 14 measurements of morphological characters (Blackman and Patterson 1986) were taken from various laboratory cultures and field collections originating from tobacco and 61 other host

plants from around the world. Linear discriminant functions (LDFs) were calculated from data sets using a reduced set of characters (Table 1).

Table 1. Blackman's 1987 Linear Discriminant Functions Used to Separate Species within the *Myzus persicae* complex

No.	Function[1]
1	(204 x urs) - (53 x ht II)
2	(185 x urs) - (37 x base VI)
3	(208 x urs) + (7 x cauda) - (6 x hf) - (117 x max. w. siph.)
4	(708 x urs) + (138 x pt) - (53 x ant. III) - (500 x base VI)

[1]**urs**=length of ultimate rostral segment
ht II= length of second segment of hind tarsus
base VI=length of base of sixth antennal segment;
cauda=length of cauda;
hf=length of hind femur
max. w. siph.=maximum width of distal half of siphunculi
pt=length of processus terminalis
ant. III=length of third antennal segment
min. w. siph.=minimum width of siphunculus at or near midpoint.

Five LDFs were calculated, two, LDFs 1 and 2 separated *M. persicae* from *M. nicotianae* with 95% confidence. LDF 3 was used to separate *M. nicotianae* from *M. antirrhinii* and LDF 5 separated *M. antirrhinii* from *M. persicae*. Blackman (1987) included LDFs 1 to 4 in his key to the *M. persicae* complex.

The ranges of values for the LDFs, from the closely related *Myzus* with which Blackman was working, allow for substantial overlap when used for identification (Blackman 1987). Blackman and others note that environmental factors such as host plant and temperature can significantly affect morphological characters of aphids (Blackman and Spence 1994, El Din 1976, Margaritopoulos et al. 2000, Moran 1986, Semtner et al. 1998, Woodford 1977). One of the most heavily weighted morphological measurements used in all five of the LDFs is the length

of the ultimate rostral segment (urs). This body part has been shown to be highly variable with environmental conditions in other aphids (Moran 1986).

2.2.2 Life Cycle Differences

When Blackman originally distinguished *M. nicotianae* from *M. persicae*, part of his argument was that, unlike *M. persicae*, *M. nicotianae* is permanently anholocyclic. Permanent parthenogenesis maintains separate species in two ways. It separates the two groups based on life cycle differences and removes the possibility of interbreeding between the groups. In Japan and the Middle East there are holocyclic and/or androcyclic tobacco feeding forms of *M. persicae* (Blackman 1987). These did not fit well within Blackman's analyses and he did not considered them to be *M. nicotianae*.

Other discrepancies in Blackman's study (1987) include two samples originally collected from tobacco and then maintained on a *Brassica* that did not group with *M. nicotianae* in some of Blackman's analyses. This suggests that morphology may be so sensitive to environmental conditions, e.g. host plant, that this needs to be more thoroughly investigated. In addition, three samples from non-tobacco hosts grouped with *M. nicotianae* in some of his analyses. Three samples from tobacco overlapped with *M. antirrhinii*. Because of difficulties in separating *M. persicae* from *M. nicotianae* based on the 1987 morphological key, Blackman and Spence (1992) published electrophoretic techniques to separate *M. persicae* and *M. nicotianae*. They found that populations of tobacco-feeding *Myzus* in Greece are holocyclic, also involving peach trees like *M. persicae*. Blackman and Spence (1992) found that these tobacco-feeding *Myzus* grouped with anholocyclic *M. nicotianae* by the canonical variate analysis. These results may be evidence of morphometrics being influenced by host plant rather than representing species differences.

2.2.3 Enzymatic Separation

Blackman and Spence (1992) also found that most *Myzus* identified morphometrically as *M. nicotianae* were polymorphic for the enzyme glutamate oxaloacetate transaminase 1 (GOT-1), while *M. persicae* was monomorphic. *M. antirrhinii* was polymorphic like *M. nicotianae,* but with unique esterases. This may be further evidence of the species separation or genotypes with host plant adaptations.

2.3 Review of Research to Distinguish Between *M. nicotianae* and *M. persicae*

Since Blackman's 1987 publication describing the new species *M. nicotianae*, there has been a series of publications explicitly addressing the question of difference and similarity between *M. nicotianae* and *M. persicae*. A historical summary of research on the discrimination of *M. nicotianae* is provided in Table 2.

Genotypic variability is another factor to consider when looking for evidence of separation of species. Weber (1985) addressed this issue for *M. persicae*. He approached genotypic variability from an agricultural perspective, instead of the taxonomic standpoint of Blackman (1987). To conduct useful research addressing such questions as plant resistance and optimum cultural methods, it is important to understand biological aspects of a pest including genetic variability and adaptability (Weber 1985). Weber was specifically concerned with the adaptation of *M. persicae* to sugar beets and potatoes. Weber (1985) found evidence for both substantial phenotypic plasticity and genetic variability for *M. persicae* clones.

Other scientists have conducted research suggesting that *M. nicotianae* and *M. persicae* are conspecific. In Greece, Margaritopoulos et al. (1998) sought evidence of species distinction for *M. nicotianae* using RAPD PCR (random amplified polymorphic DNA polymerase chain reaction) techniques. Clones from different hosts were first identified by morphometric analysis

Table 2. Summary of Publications Regarding the Species Status of *Myzus nicotianae*

Date	Author	Title	Finding
1987	Blackman, R. L.	Morphological discrimination of a tobacco-feeding form of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Nectarosiphon) species.	A new species
1992	Blackman, R. L. and Spence, J. M.	Electrophoretic distinction between the peach-potato aphid, *Myzus persicae* and the tobacco aphid, *Myzus nicotianae* (Homoptera: Aphididae).	Different Enzymes
1998	Margaritopoulos, J.T., et al.	Attempted discrimination of Myzus persicae and Myzus nicotianae (Homoptera: Aphididae) by random amplified polymorphic DNA polymerase chain reaction technique.	Same Species
1999	Clements, K. M. et al.	*Myzus nicotianae* Blackman, a new junior synonym of *Myzus persicae* (Sulzer) (Homoptera: Aphididae)	Same Species
2000a	Clements, K. M., et al.	Genetic, biochemical, and behavioral uniformity among populations of *Myzus nicotianae* and *Myzus persicae*.	Same species
2000b	Clements, K. M., et al.	Genetic variation in the *Myzus persicae* complex (Homoptera: Aphididae): evidence for a single species.	Same species
2000	Margaritopoulos, J. T., et al.	Host-correlated morphological variation of *Myzus persicae* (Hemiptera: Aphididae) populations in Greece.	Same species
2002	Kephalogianni, T. E., et al.	Variation in the life cycle and morphology of the tobacco host-race of *Myzus persicae* (Hemiptera: Aphididae) in relation to its geographical distribution.	Same species
2002	Margaritopoulos, J. T., et al.	Life cycle variation of *Myzus persicae* (Hemiptera: Aphididae) in Greece.	Same species
2003	Margaritopoulos, J. T., et al.	Co-existence of different host-adapted forms of the *Myzus persicae* group (Hemiptera: Aphididae) in southern Italy.	Host-adapted forms

using Blackman's key, and then subjected to RAPD PCR to see if banding patterns agreed in separation of the two groups. Their technique failed to separate *M. persicae* and *M. nicotianae* although it distinguished all six outgroups (known to be distinct species of *Myzus*) included in their analysis.

In the United States, Clements et al. (1999, 2000a, and 2000b) used RAPD PCR, mitochondrial cytochromic oxidase II (COII), and EF-1a to detect differences between tobacco associated *Myzus* and non-tobacco *Myzus* in the *M. persicae* complex. They did not find evidence indicating that the two groups were separate species.

Blackman and Patterson (1986) used the multivariate morphometric analytic technique to distinguish *M. antirrhinii* from *M. persicae*. *M. antirrhinii* has unique karyotypes, 2n=13 or 14 versus 2n=12 for *M. persicae* and *M. nicotianae*. It is further described as a dark green form that is permanently anholocyclic. They were able to separate European populations with 2n=13 or 14 versus 2n=12 karyotypes as predicted, but not the non-European populations that they considered separately. Ffrench-Constant et al. (1988) developed an electrophoretic technique to identify *M. antirrhinii*.

Blackman (1987) revisited the issue of the non-European samples that he had expected to group with *M. antirrhinii*. After experimenting with variable rearing conditions, host plants and temperatures, he found that *M. antirrhinii* has greater within species morphological plasticity than originally thought. It is reasonable to expect similar variability with other closely related *Myzus*; particularly for such a highly adaptable aphid such as *M. persicae* is known to be.

Several other researchers focused on this area to assess the effects of host plant and other environmental conditions on the morphology of these aphids. Margaritopoulos et al. (2000) presented evidence for a host race of *M. persicae* on tobacco, but not a separate species. Their

investigations centered on discriminating genetic effects from environmental effects. If morphological characteristics are influenced more by environment than by genotype, evidential support for a separate species diminishes.

Unfortunately the situation isn't so simple. Because of the rapid and prolific reproductive capacity of this aphid and because it reproduces by parthenogenesis, 'types' which are better able to exploit a given host plant can be quickly established. Each of these environmentally selected types can become a clonal colony numbering in the millions and dominating in an agricultural field in a matter of wk. This is why the life cycle question is so fundamental. If no *M. nicotianae* are found to reproduce sexually, then regardless of the above stated problem, and even given difficulties with morphometric keys, it could justify a separate species.

Kephalogianni et al. (2002) investigated the life cycle and morphological variations associated with geographical distribution. They showed evidence of isolation between populations of *M. persicae* in western and southeastern Europe. They found that the establishment of stable characteristics is evidence that *M. persicae* can form distinct types that might be mistaken for separate species.

Margaritopoulos et al. (2003) found that populations of *M. persicae* complex from southern Italy, an area very similar climatically to Greece, had a stable anholocyclic life cycle and morphological differences between tobacco-feeding clones and clones from other host plants. Even though the climate and daylength conditions are substantially equivalent to the regions studied in Greece they did find a stable anholocyclic type associated with tobacco in southern Italy. Notwithstanding these results, the following is taken from the introduction:

> "…the tobacco-feeding form was given the status of a separate species by Blackman (1987), but evidence of identical DNA sequence at some loci (Field et al. 1994; Clements et al. 2000) indicates that some interbreeding has occurred between the two forms, and that subspecific status (as *M. persicae*

ssp. *nicotianae* Blackman) would be more appropriate (Blackman & Eastop, in press)."

M. nicotianae Blackman is now considered by most as regrouped under *M. persicae.* It had brief status as a separate species but with the naming author, Blackman, a joint author on the above paper, convinced that the evidence does not warrant such a distinction it is not likely to maintain this designation. Indeed, in most papers published following Margaritopoulos et al. (2003), the name *M. nicotianae* is no longer used as a designator.

2.4 Recent Work with *Myzus persicae*

The story does not end for *M. persicae*. It is one of the most important agricultural pests worldwide and research into its genetics, resistance mechanisms, morphological plasticity, and more continues to proceed apace. Developments in genetic fingerprinting techniques for *M. persicae* were published recently (Fenton et al. 2003) and more research supporting host-plant related morphological plasticity of *M. persicae* clones was reported from Brazil (Peppe and Lomonaco 2003). Both of these publications support the decision of Blackman to revise his earlier description of *M. nicotianae*.

2.5 Present Research into Influence of Host Plant and Temperature

There are two key questions throughout this controversy, the effect of environment on morphological characters and reproductive isolation. If it could be shown that host plants have such a significant impact on the phenotype that morphological character measurements do not remain stable within a clonal population, it would seriously undermine the species status of *M. nicotianae*. In the United States the life cycle question remains a critical issue because of the increased potential for insecticide resistance with the genetic recombination of sexual

reproduction (Harlow and Lampert 1990, Guillemaud et al. 2003). The androcyclic form may be of particular concern. Doherty and Hales (2002) found that males from an androcyclic clone with insecticide resistance do not share the decline in reproductive potential exhibited by males from a holocyclic clone that have developed resistance.

Semtner et al. (1998) found that *M. persicae/M. nicotianae* reared on tobacco weighed more than twice as much as those reared on several other host plants. This research focuses on the problem of intraclonal morphological plasticity. The objective was to investigate the influence of temperature and host plant on the morphology of *M. persicae/M. nicotianae* to gain a better understanding of which body parts, and to what extent, these two environmental conditions affect taxonomically important measurements.

3. Materials and Methods

3.1 Aphid Clones and Host Plants

In this study three independent factors were investigated, aphid morph, temperature, and host plant. Three forms of the *M. persicae* complex were used in these tests, red and dark green morphs of *M. nicotianae* collected from tobacco at the Virginia Tech Southern Piedmont AREC, Blackstone, VA; and a light green morph of *M. persicae* collected from turnip in the greenhouse at the Southern Piedmont AREC, Blackstone, VA. The red and green morphs were designated *M. nicotianae* based on being originally collected from tobacco and the light green morph of *M. persicae* was so designated as it originated on a non-tobacco host, turnip (*Brassica napus*). This has been the classification approach used by others when the key fails to consistently separate *M. nicotianae* from *M. persicae* (Clements et al. 2000a, b). The green morph of *M. persicae* was a lighter, paler green than the green morph designated *M. nicotianae.*

Test clones of each type were started from single individuals and reared under controlled temperature and light conditions for five generations on four host plants: tobacco, *Nicotiana tabacum* L. ('K 326') (Solanaceae); turnip, *Brassica napus* L. ('Purpletop White Globe') (Brassicaceae); bell pepper*, Capsicum annuum* L. ('Keystone Giant') (Solanaceae); and okra, *Abelmoschus esculenus* L. ('Clemson Spineless') (Malvaceae). The experimental host plants were chosen for a range of favorability to the tobacco-feeding form of the *M. persicae* complex based on fecundity, longevity, reproduction, generation time, and body weight (Semtner et al. 1998). Tobacco and turnip are considered the most favorable hosts, pepper is intermediate and okra is marginal.

3.2 Host Plant Propagation

Seedlings were started in moist vermiculite in aluminum loaf pans placed in aluminum cake pans containing fertilizer solution. New plantings of tobacco and turnip were transplanted every 2 wk and okra and pepper were transplanted every 4 wk to maintain a continuous supply of leaves. Plants were grown in 15 cm plastic pots filled with vermiculite (Palmetta Vermiculite Co. Inc.). The pots were placed in cake pans containing a solution of 50 ppm Peters 20-20-20 (N-P-K) (Scotts-Sierra Horticultural Products, Co., Marysville, OH). The fertilizer solution was replenished every 2 to 3 d depending on the temperatures inside the greenhouse. Plantings were rotated between two greenhouses to manage infestations of whiteflies and aphids.

3.3 Rearing Temperatures

Each aphid clone was maintained on the excised leaves of the four host plants at three temperatures, 15°C, 20°C, and 25 °C, and a photophase of 14:10 (L:D) h (Reed and Semtner 1991). All three temperatures are favorable for green peach aphid development (DeLoach 1974, Reed and Semtner 1991). At 15°C, generation time is longest and reproductive capacity and body size generally falls between those at either 20°C or 25°C (Reed and Semtner 1991). At 25°C, generation time is shortest but reproductive capacity and body size tend to be less than that at 20°C (DeLoach 1974). Three runs of this experiment were conducted and the temperature groups were rotated among the environmental chambers (Nor-Lake Scientific, Hudson, WI; Percival, Boone, IA and Revco, Asheville, NC) to lessen the chance of an effect resulting from idiosyncratic conditions of a particular chamber.

3.4 Aphid Colony Maintenance

The petiole of each leaf was placed in 20 ml of water agar (10 g agar/liter of water) in the bottom of a 473 ml Styrofoam® cup with a translucent lid. All possible combinations of host plant and aphid morph were randomly set up in two cups per combination within each temperature group in an environmental chamber. Each temperature group was kept in a separate environmental chamber with independent temperature and light controls. The experiment was repeated three times, so that there was one run in each chamber at each temperature.

The water agar base maintained the leaves of the host plants as viable food sources for aphids for 7 to 14 d. Leaves were changed about once ever 7 d for the 20°C and 25°C chambers and about once every 2 wk for the 15°C chamber**.** Approximately 20 nymphs or young adults were transferred to new leaves using a moistened camel's hair brush. Great care was taken to prevent contamination of colonies from other colonies and greenhouse plants. Each cup was securely closed between aphid transfers and transfers were made at a lab bench covered with white paper. Leaves were carefully inspected and washed if necessary to clear them of other aphids. As a further precaution, notes were made at each transfer of the number and age of the group moved to the new leaf.

3.5 Collection, Preparation, and Measurement of Aphids

Fifth generation apterous adults were collected at maturity as they were beginning to reproduce. These were placed in aphid preservative (3 parts methanol: 1 part glacial acetic acid) and subsequently cleared and mounted on slides. The ten morphological characters described by Blackman and Patterson (1986) and used in Blackman's 1987 *Myzus* key were measured; length of the processus terminalis (pt), the length of the base of the sixth antennal segment including the

primary sensorium (base VI), length of the third antennal segment (ant III), the length of the ultimate rostral segment (urs), length of the hind femur (hf), the length of the second segment of the hind tarsus (ht II), length of the siphunculus (siph.), minimum width of the siphunculus at or proximal to midpoint (min. w. siph.), maximum width of the distal half of the siphunculus (max. w. siph.), length of the cauda. The processus terminalis is the apical segment of the antennae. The urs is the apical segment of the aphid mouth part, the rostrum. The siphunculi are the two tube like structures projecting back from the rear of aphid, also sometimes referred to as cornicles. The cauda is the tail-like structure at the distal end of the aphid. The measurements were taken according to Ilharco and van Harten (1987). These morphological characters were measured using a Boekeler/Zeiss Filar eyepiece digital micrometer mounted on a Bausch and Lomb compound microscope. The digital micrometer was calibrated with a stage micrometer at the beginning of each measuring session and after each group of 20 slides.

3.6 Experimental Design and Research Challenges

My aim was to have three runs over 3 yr (1998, 1999 and 2000) of a 4 X 3 X 3 factorial experiment, testing the impacts of four host plants (tobacco, turnip, pepper and okra), and three temperatures (15°C, 20°C, and 25°C) on three different morphs (red morph from tobacco, green morph from tobacco, and green peach aphid from turnip). There were some technical difficulties that prevented me from having three complete runs. In 1998, a severe whitefly infestation in the greenhouse near the end of the study reduced host plant quality so much for the 15°C group that the test aphids died and the test could not be completed. The maintenance colony of the green morph from tobacco was lost between the 1998 and 1999 tests and wasn't reestablished until it

was found again in the late summer in the tobacco fields. In 1999, the 25°C environmental chamber malfunctioned and this temperature group was lost.

The most complete run was in 2000 with all morphs and all temperatures completing the test. There are a few missing host/temp/morph combinations from each yr due to low survival. If there were fewer than five slides from a combination that allowed for measurement of all ten characters, I did not include that combination in the analysis. The cauda was the most difficult character to measure. Much care must be taken during slide preparation to position the specimen such that all characters are in an alignment that will allow for accurate measurements.

The target was to collect at least 20 mature aphids from each of the 36 rearing (test combinations) conditions, slide mount 20 of these, and select ten slides with all ten morphological characters needed for Blackman's key in measurable positions. These character measurements were then used to run multivariate morphometric analysis (Blackman 1987). The factors that precluded this were greatly reduced survivability for some morphs at some temperatures and on some host plants and positional problems for some body parts after the aphids were slide mounted.

3.7 Statistical Analysis

Multivariate analysis of variance (MANOVA) allows for multiple independent and dependent variables to be analyzed at the same time and minimizes the probability of type I errors, which increases when a series of ANOVAs on each dependent variable is run. While minimizing the chance for type I errors, MANOVA can find combined effects within the dependent variables even if no single dependent variable indicates an effect.

Multivariate morphometric analysis was conducted to assess the host plant and temperature influences on the morphological characters measured and on the classification of aphids reared at the different temperatures and on the different hosts (SAS 1999). Analysis of variance was used to measure the effects of temperature and host plant on the character measurements and classification results for each aphid morph.

Canonical variates are created from a canonical correlation using linear discriminant functions put together from weighted combinations of the dependent and independent variables. These can then be used in cluster analysis and if warranted the linear discriminant functions themselves can be used in a classification key. Use of the linear discriminant functions becomes warranted if the cluster analysis shows that there is significant separation of the groups (one or more of the dependent variables) by one or more of the independent variables. This is how Blackman (1987) analyzed the data he used to establish *M. nicotianae* as a separate species from *M. persicae* and produced the linear discriminant functions in the resulting key.

ANOVA was performed on the morphological characters to determine which ones, if any, were influenced by the varying environmental conditions. ANOVA was also used on the key results to see how consistently these groups (morph X temp X host) were distinguished on the basis of the key developed by Blackman (1987). I consulted with the Virginia Tech Department of Statistics' consulting service regarding these analyses.

4. Results and Discussion

Table 3 is a summary of the experiment and shows how the aphids keyed out within each morph, temperature, and host plant combination each yr using the *Myzus* key of Blackman (1987).

Findings in this study support the hypothesis that there is a morphologically distinct, host-adapted tobacco race but not a separate tobacco-feeding species of *Myzus persicae*. The key developed by Blackman (1987) did not satisfactorily discriminate between the tobacco and non-tobacco originating clones, but canonical variates generated from the test data successfully separated the tobacco and non-tobacco groups. This work explored the extent of morphological variation within clones exposed to different environmental conditions. Unlike other studies that have used many different clones to investigate possible distinctions between *M. persicae* and *M. nicotianae*, the concern here was to see how much morphological perturbation may be induced within a clone by rearing at different temperatures and on different host plants.

The hypothesis that temperature and host plant have significant effects on the morphology of these aphids in the *M. persicae* complex was also supported by the results. While physiological interactions of temperature-host plant-aphid morphology are very complex, even controlling for two simple environmental factors (temperature and host plant) was sufficient to group specimens according to these independent variables at a much higher frequency than would be expected if the assignments were random (Figs. 1 and 2).

4.1 Application of Blackman's 1987 Key

This key did not successfully distinguish between the morphs designated 'tobacco aphid' versus the non-tobacco morph. When the morphs did not key to *M. nicotianae*, they were more likely to key to *M. antirrhinii* than to *M. persicae* (Figs. 3, 4, and 5). The linear discriminant

Table 3. Species designation based on Blackman (1987) of three morphs of *Myzus persicae* reared on four host plants at three temperatures by year.

			Key Results 1998[1]				Key Results 1999[1]				Key Results 2000[1]			
Host	Temp (°C)	Morph[2]	No.	*nic.*	*per.*	*anti.*	No.	*nic.*	*per.*	*anti.*	No.	*nic.*	*per.*	*anti.*
Tobacco	15	RTA					7	5	2	0	10	9	1	0
Tobacco	15	GPA					10	10	0	0	10	4	0	6
Tobacco	15	GTA									10	8	0	2
Tobacco	20	RTA	7	4	0	3	9	9	0	0	10	10	0	0
Tobacco	20	GPA	10	4	4	2	10	10	0	0	10	9	1	0
Tobacco	20	GTA	6[3]	0	2	3					10	8	1	1
Tobacco	25	RTA	6	6	0	0					10	4	0	6
Tobacco	25	GPA	5	5	0	0					10	9	0	1
Tobacco	25	GTA	m								10	9	0	1
Turnip	15	RTA					10	10	0	0	8	3	2	3
Turnip	15	GPA					10	9	1	0	10	10	0	0
Turnip	15	GTA									10	9	1	0
Turnip	20	RTA	1	3		7	9	9	0	0	10	10	0	0
Turnip	20	GPA	10	9	1		10	10	0	0	10	10	0	0
Turnip	20	GTA	10	2	1	7					10	10	0	0
Turnip	25	RTA	9	9	0	0					10	10	0	0
Turnip	25	GPA	m								10	9	0	1
Turnip	25	GTA	10	8	0	2					10	10	0	0
Pepper	15	RTA					9	4	4	1	10	4	1	5
Pepper	15	GPA					10	9	1	0	10	8	2	0
Pepper	15	GTA									10	1	5	4
Pepper	20	RTA	9	3	4	2	10	6	0	4	10	10	0	0
Pepper	20	GPA	9	7	2	0	10	8	1	1	10	10	0	0
Pepper	20	GTA	10	1	5	4					10	8	0	2
Pepper	25	RTA	6	5	0	1					10	10	0	0
Pepper	25	GPA	10	5	1	4					9	9	0	0
Pepper	25	GTA	10	9	0	1					10	8	0	2

-- Continued—

Table 3. Continued.

Table 3. Species designation based on Blackman (1987) of three morphs of *Myzus persicae* reared on four host plants at three temperatures by year.

			Key Results 1998[1]				Key Results 1999[1]				Key Results 2000[1]			
Host	Temp (°C)	Morph[2]	No.	*nic.*	*per.*	*anti.*	No.	*nic.*	*per.*	*anti.*	No.	*nic.*	*per.*	*anti.*
Okra	15	RTA					10	4	3	3	10	7	2	1
Okra	15	GPA					10	8	1	1	10	10	0	0
Okra	15	GTA									10	10	0	0
Okra	20	RTA	7	3	1	3	m				m			
Okra	20	GPA	6	2	1	3	10	4	0	6	10	10	0	0
Okra	20	GTA	9	6	2	1					m			
Okra	25	RTA	10	6	1	3					10	9	0	1
Okra	25	GPA	7	7	0	0					m			
Okra	25	GTA	m								10	10	0	0

[1]Key results*: nic=Myzus nicotianae, per=Myzus persicae; anti=Myzus antirrhinii*

[2]Morph: RTA=red morph from tobacco, GPA=yellow-green morph from turnip, GTA=dark green morph from tobacco
m=missing samples.

[3]One aphid keyed out to *Myzus dianthicola*

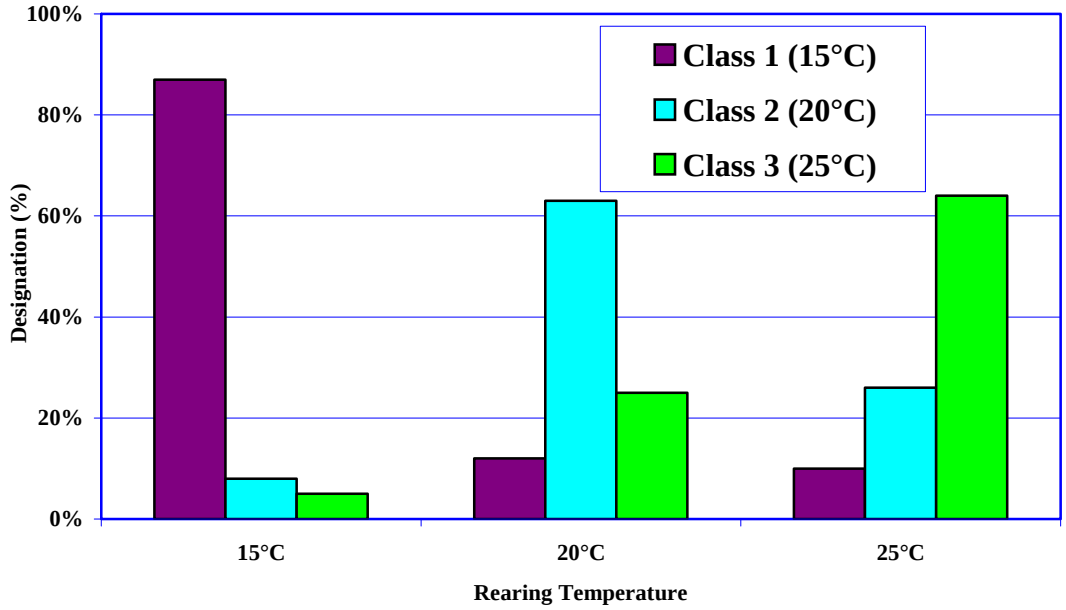

Fig. 1. Percent of Aphids in which Rearing Temperature was Correctly Identified Using Linear Discriminant Functions Generated for Temperature Classes. Random Designations would be 33%.

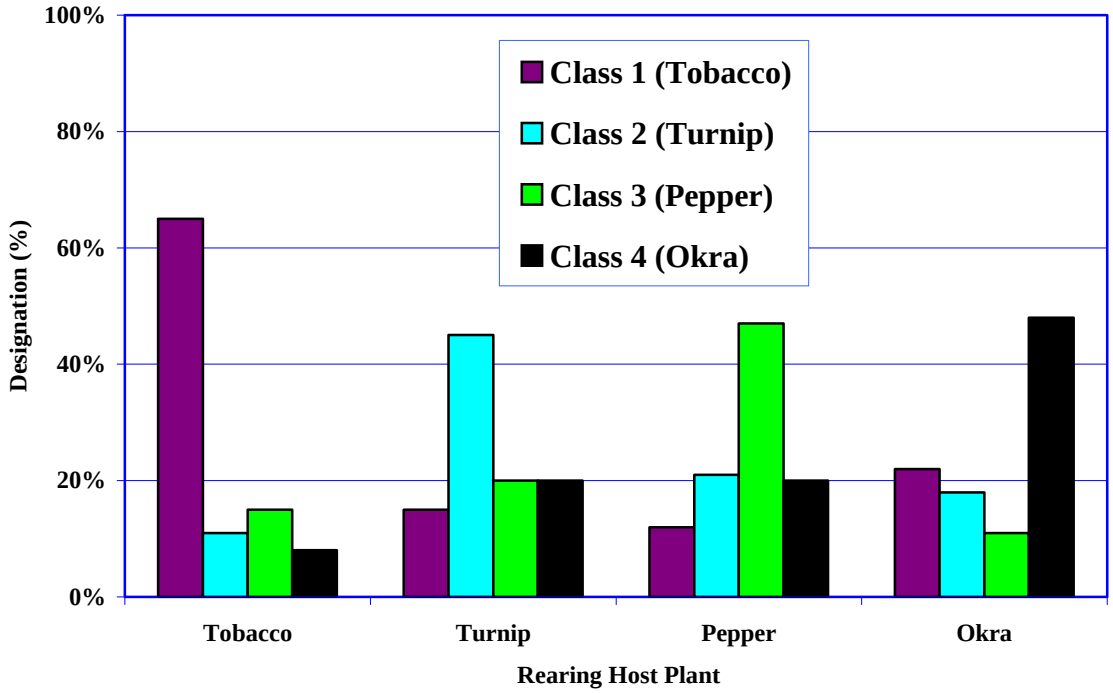

Fig. 2. Percent of Aphids in which Rearing Host Plant was Correctly Identified Using Linear Discriminant Functions Generated for Temperature Classes. Random Designations would be 25%

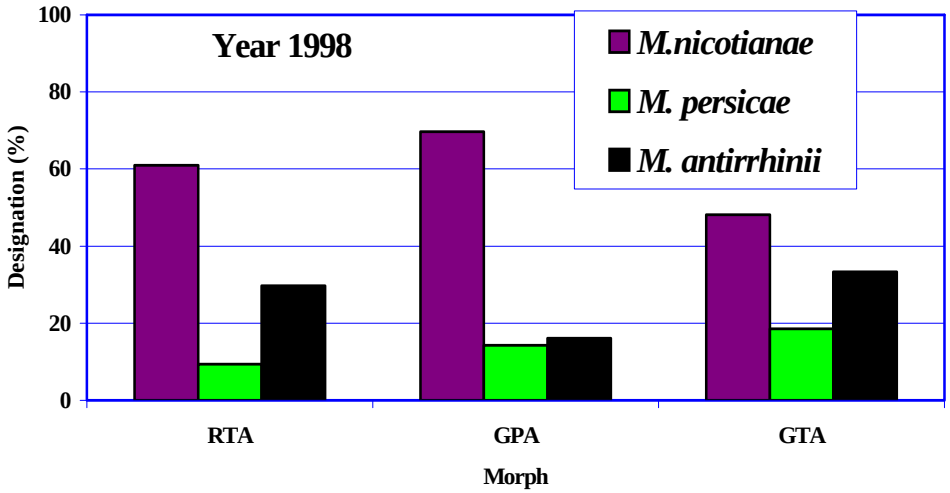

Fig. 3. Percent of *Myzus persicae* Complex within Three Morphs (RTA=Red Morph from Tobacco, GPA=Light Green Morph from Turnip, and GTA=Dark Green Morph from Tobacco) Keyed out to *M. nicotianae*, *M. persicae*, and *M. antirrhinii* with the Key of Blackman (1987) for 1998

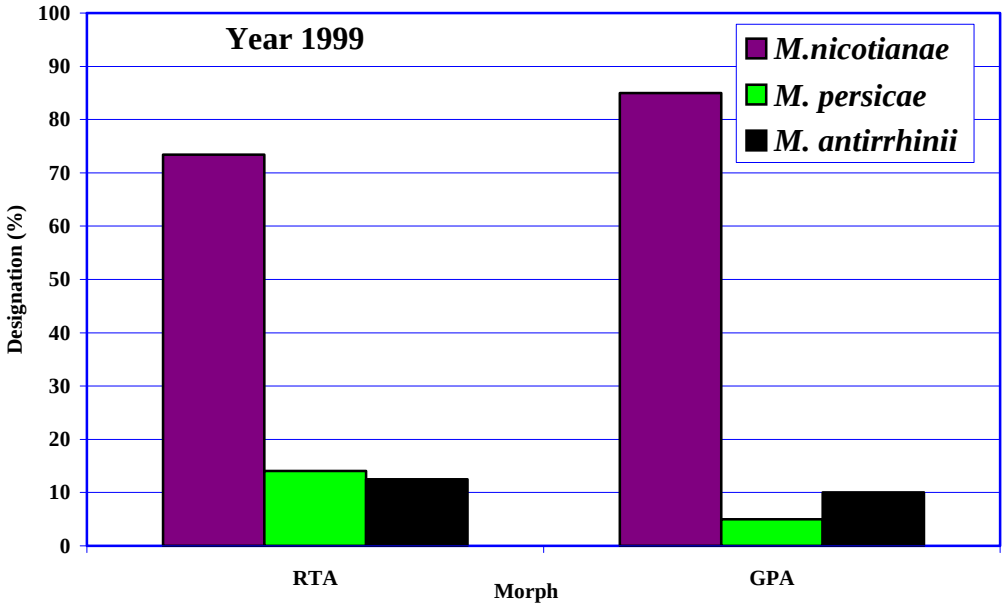

Fig. 4. Percent of *Myzus persicae* Complex within Three Morphs (RTA=Red Morph from Tobacco, GPA=Light Green Morph from Turnip, and GTA=Dark Green Morph from Tobacco) Keyed out to *M. nicotianae*, *M. persicae*, and *M. antirrhinii* with the Key of Blackman (1987) for 1999

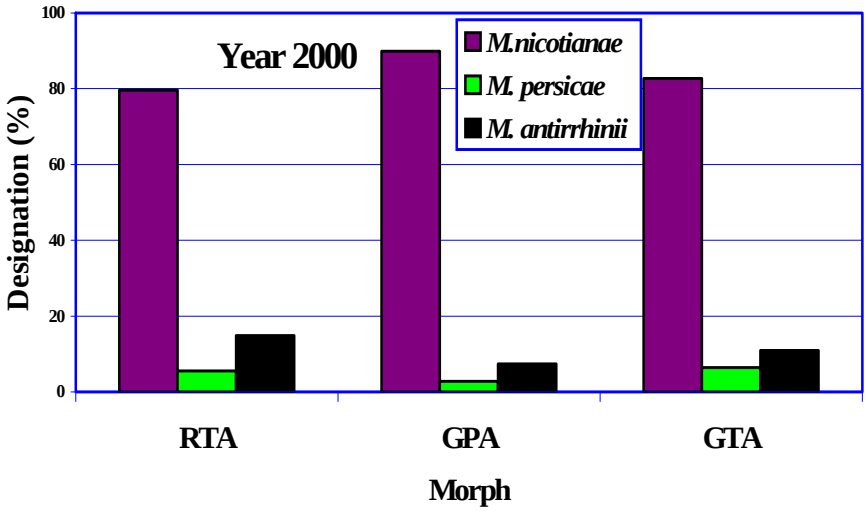

Fig. 5. Percent of *Myzus persicae* Complex within Three Morphs (RTA=Red Morph from Tobacco, GPA=Light Green Morph from Turnip, and GTA=Dark Green Morph from Tobacco) keyed out to *M. nicotianae*, *M. persicae*, and *M. antirrhinii* with the Key of Blackman (1987) for 2000

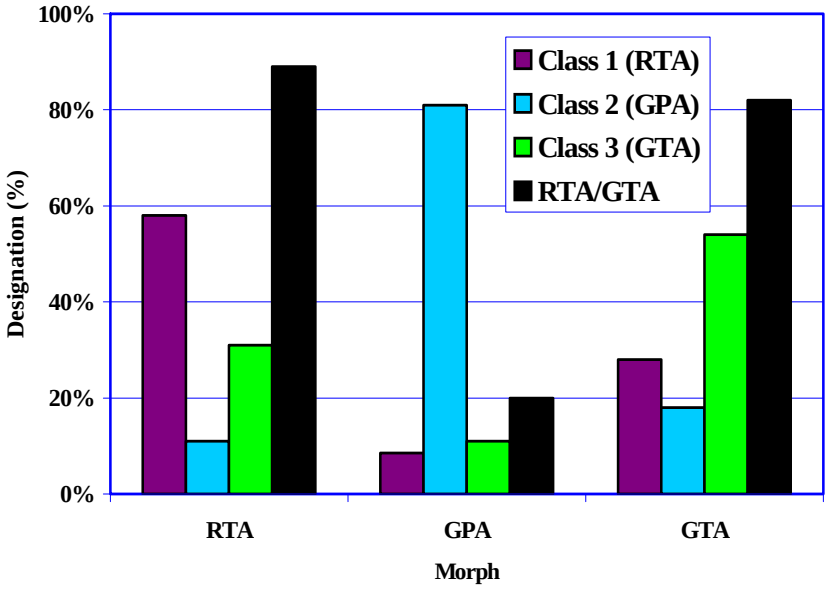

Fig. 6. Percent of Aphids in which Morph was Correctly Identified Using Linear Discriminant Functions Generated for Morph Classes. Random Designations would be 33%. RTA/GTA is a Columned Combined for the Results of the Tobacco Originating Morphs.

functions generated from the multivariate analysis of variance (MANOVA) separated the turnip morph from the tobacco morphs but the two tobacco morphs had substantial overlap (Fig. 6).

The LDFs used in Blackman's key (1987) are greatly influenced by the size of the urs. It is used in every one of the LDFs and is always the character with the largest coefficient within an LDF. A small difference in the size of this character will be greatly magnified by the coefficients used in the LDFs and consequently have a much greater impact on the key results. In these tests the urs was consistently largest on tobacco, even as it was one of the least variable of the characters (Table 4). Another important character in Blackman's key (1987) is the ht II. This character appears very early in the couplets of the key and has a coefficient of 127 and 53, couplets 4 and 6 respectively. The ht II was also relatively stable and largest on tobacco in each test. Couplets 4/5 and 7 separate out *M. antirrhinii* from the others and are greatly affected by the size of the urs and ht II; couplet 7 is the final place where *M. persicae* separates from *M. antirrhinii* (Fig. 7). The generally large size of the urs for the test specimens resulted in a designation of *M. antirrhinii* for many specimens that did not separate at couplet 6 as *M. nicotianae*.

4.2 Influence of Host Plant and Temperature

The impacts of environmental conditions on aphid morphology were statistically significant (p-value ≤ 0.05) when analyzing all specimens together and as separate groups by yr. However, the effects were not consistent in regards to which host and which temperature correlated with greatest and/or least size of the measured morphological characters. The MANOVA from all 3 yr combined showed a significant 'yr' effect, as would be expected given the complexity of

Table 4. Influence of Host Plant on the Size of Characters Used to Separate *Myzus persicae* Morphs, 1998 to 2000.

Character	Host	1998 Means ±SD[1,2]	1999 Means±SD	2000 Means±SD	Character	Host	1998 Means ±SD	1999 Means ±SD	2000 Means ±SD
ultimate	Tobacco	120 ± 7a	125 ±6 a	125±4 a	third antennal	Tobacco	515±50a	497±32 a	471 ±44b
rostral segment	Turnip	119 ± 5a	122 ±5 b	125±6 a	segment	Turnip	511±38 a	481±39 b	499±61a
	Pepper	119 ± 5a	122 ±4 b	122±6 b		Pepper	498±39 b	495±43	478±61b
	Okra	117 ± 5b	117 ±4 c	124±5 a		Okra	459±42c	423±34 c	479±31b
base of sixth	Tobacco	138 ± 15a	136 ±14 b	128±11c	maximum width of	Tobacco	50±4 a	51±4 a	49a
antennal	Turnip	131 ± 10b	130c	132±11b	siphunculus (distal	Turnip	47±4 b	48±4 c	49±5 a
segment	Pepper	136 ± 9a	140 ±10 a	135±13a	half)	Pepper	50±5 a	50±4 b	49±4 a
	Okra	125 ± 10d	128 ±8 d	133±10 b		Okra	47±4 b	45±4 d	49±4 a
hind femur	Tobacco	743 ± 59a	725 ±39 a	699±59a	processus	Tobacco	518±52a	505±49	469±54
	Turnip	702 ± 53b	662 ±38 c	695±70a	terminalis	Turnip	526±36 a	503±49	510±50
	Pepper	694 ± 35b	695 ±39 b	682±69b		Pepper	523±30a	508±38a	501±48
	Okra	632 ± 67c	592 ±44d	700±40		Okra	492±33b	459±48	493±28 c
cauda	Tobacco	238 ± 21 a	225 ±13a	230±18 a	siphunculus	Tobacco	549±45	551±28 a	523±43b
	Turnip	223 ± 15 b	211 ±15 b	226±20 b		Turnip	525±34 b	516±32 b	524±44ab
	Pepper	223 ± 13 b	223 ±15 a	221±15 c		Pepper	531±30 b	543±25 a	514±49
	Okra	202 ± 19 c	192 ±15 c	226±16 ab		Okra	495±51	451±40	527±30
second segment	Tobacco	123 ± 9 a	119 ±8 a	120±6 a	minimum width of	Tobacco	46±4 a	45±4 a	45±3 a
of hind tarsus	Turnip	118 ± 7 b	114 ±8 b	120±8 a	siphunculus	Turnip	42±4 c	40±4 c	43±5 c
	Pepper	116 ± 9 b	120 ±10 a	119±9 a	(midpoint)	Pepper	44±4 b	43±4 b	44±4 ab
	Okra	109 ± 7 c	109 ±7 c	118±7 a		Okra	40±4 d	38±4 d	44±3 b

[1]Means and standard deviations (SD) given in μm.

[2]Means within a year and character not followed by the same letter(s) are significantly different as indicated by Waller-Duncan k-ratio-t-test (k-ratio=100)

Beginning with couplet four from Blackman's 1987 key to apterous new world species of *Myzus*. key ends with couplet seven.

4 Value of function (306 x urs) – (127 x ht II) less than 17. Deep yellow-green in life, anholocyclic on *Dianthus caryophyllus*. 2*n* = 14 (heterozygous) .....*dianthicola* Hille Ris Lambers

\- Value of function (306 x urs) – (127 x ht II) greater than 17 5

5 Pt in range 0.22-0.40 mm, urs in range 0.090-0.112 mm. Value of function (25 x pt) + (205 x urs) – (13 x ant. III) – (9 x siph.) less than 23. Brown or red-brown in life, usually on Caryophyllaceae and Violaceae. Monoecious holocyclic with apterous males, or anholocyclic. 2*n* = 12.....*certus* (Walker)

\- Pt in range 0.33-0.59 mm, urs in range 0.100-0.128 mm. Value of function (25 x pt) + (205 x urs) – (13 x ant. III) – (9 x siph.) greater than 23. Mid-grey-green to dark green, rarely dark red. Polyphagous, but often feeding on Scrophulariaceae, Buddlejaceae, Pittosporaceae and woody Rosaceae. Anholocyclic, males unknown and alate females only produced sporadically. 2*n* = 13 or 14.....*antirrhinii* (Macchiati) (partim)

6 Urs 0.113-0.139 mm long. Value of function (204 x urs) – (53 x ht II) more than 18, that of function (185 x urs) – (37 x base XI) more than 17. In borderline cases, value of function (208 x urs) + (7 x cauda) – (6 x hf) – (117 x max. w. siph.) greater than 16.7, *or* max. w. siph. less than 0.11 of length of siph. Greyish-green or pink in life, mainly on tobacco. Permanently anholocyclic?.....*nicotianae* sp. n.

\- Urs 0.090-0.128mm long. Value of function (204 x urs) – (53 x ht II) less than 18, that of function (185 x urs) – (37 x base VI) less than 17. In borderline cases, value of function (208 x urs) + (7 x cauda) – (6 x hf) – (117 x max. w. siph.) less than 16.7, *or* max. w. siph. more than 0.11 of its length 7

7 Urs in range 0.102-0.128 mm. Siphunculi usually dusky over entire length with max. width of swollen part more than 0.11 of length of siphunculus. Value of function (138 x pt) + (708 x urs) – (53 x ant. III) – (500 x base VI) usually greater than 58. Mid-grey-green to dark green in life, rarely dark red. Polyphagous (but see couplet 5). Anholocyclic. 2*n* = 13 or 14 ..... *antirrhinii* (Macchiati) (partim)

\- Urs in range 0.090-0.122 mm. Siphunculi variably pigmented but usually pale except at apices, with max. width of swollen part less than 0.11 of length of siphunculus. Value of function (138 x pt) + (708 x urs) – (53 x ant. III) – (500 x base VI) usually less than 58. Pale green, yellow-green or straw-coloured. Polyphagous. Androcyclic or holocyclic heteroecious with sexual phase on *Prunus persica* or *P. nigra*. 2*n* = 12.....*persicae* (Sulzer)

Fig. 7. Couplets 4-7 Adapted from Blackman's 1987 Key to the New World species of apterous *Myzus* (Nectarosiphon**)**

aphid response to host plant physiology. The MANOVA also indicated significance from host, temperature, and most independent variable combinations at the $p \leq 0.05$ level.

In order to find potential systematic influences from temperature and host plant, a MANOVA was conducted for each yr and then p-values were compared for host, temperature, and the host x temperature, host x morph, and temperature x morph interactions (Table 5). Again, most of the morphological characters showed significant effects for the independent variables and their interactions. I then looked for consistent trends in response to each of the hosts and temperatures. The ANOVA for host and temperature was examined to see if certain temperatures or host plants were ranked consistently across the years in relation to size of morphological character.

The influence of temperature on each character is more difficult to address given the gaps in the tests for 1998 and 1999. For temperature, yr 1998 and 1999 were combined and compared with the results from 2000 (Table 6). A trend in both of these sets for temperature was that the size of five characters, ant III, cauda, pt, ht II, and hf was smallest at 15°C.

ANOVA for host plant was compared across the three yr for the same purpose. Four characters consistently ranked tobacco first in relation to size, cauda, urs, max. w. siph., and min. w. siph. (Table 4). The siphuncular measurements, max. w. siph. and min. w. siph., also consistently ranked pepper second for size. While the rankings show consistency for these characters and hosts, there was not always a significant difference between each host for this group of characters. The urs in particular was quite stable and only showed minimal variation in relation to host plant.

In general, the variation in rankings and assignment of significant difference in relation to influence of temperature and host plant was so great between yr that these few consistencies

Table 5. p-Values of Impact of Independent Variables on Characters from MANOVA[1] for Each Year.

Character	Variable	1998 p-value	1999 p-value	2000 p-value	Character	Variable	1998 p-value	1999 p-value	2000 p-value
ultimate	Host	0.0003	0.0001	0.0002	second	Host	0.0001	0.0010	0.3004
rostral	Temp	0.0001	0.2169	0.0001	segment	Temp	0.0001	0.1315	0.0001
segment	Host*Temp	0.0429	0.0050	0.0001	of hind tarsus	Host*Temp	0.0691	0.8314	0.0001
	Morph	0.0001	0.0001	0.0001		Morph	0.0002	0.0001	0.0001
	Host*Morph	0.0272	0.0791	0.0032		Host*Morph	0.0517	0.0200	0.0001
	Temp*Morph	0.0958	0.6664	0.0001		Temp*Morph	0.0547	0.0046	0.0001
	Host * Temp* Morph	0.0001	0.2075	0.0343		Host * Temp* Morph	0.0001	0.9727	0.0001
base of sixth	Host	0.0001	0.0001	0.0001	third	Host	0.0001	0.0001	0.0001
antennal	Temp	0.0009	0.0025	0.0001	antennal	Temp	0.0001	0.0001	0.0001
segmentl	Host*Temp	0.0033	0.0013	0.0001	segment	Host*Temp	0.0574	0.1258	0.0001
	Morph	0.0001	0.0001	0.0001		Morph	0.0168	0.0001	0.0001
	Host*Morph	0.0193	0.0002	0.0001		Host*Morph	0.0001	0.0001	0.0001
	Temp*Morph	0.4379	0.7048	0.4037		Temp*Morph	0.0082	0.6596	0.0001
	Host * Temp* Morph	0.0016	0.0899	0.0001		Host * Temp* Morph	0.0001	0.5352	0.0001
hind femur	Host	0.0001	0.0001	0.0143	maximum width of siphunculus (distal half)	Host	0.0008	0.0001	0.2013
	Temp	0.0001	0.0041	0.0001		Temp	0.5611	0.0001	0.0011
	Host*Temp	0.1048	0.3055	0.0001		Host*Temp	0.0021	0.5484	0.0001
	Morph	0.7468	0.4785	0.0235		Morph	0.3883	0.0082	0.0001
	Host*Morph	0.0001	0.0016	0.0001		Host*Morph	0.0028	0.0834	0.0001
	Temp*Morph	0.0001	0.9572	0.0001		Temp*Morph	0.5914	0.0001	0.0001
	Host * Temp* Morph	0.0005	0.0730	0.0001		Host * Temp* Morph	0.1472	0.1138	0.0168

--- Continued ---

Table 5. Continued.

Table 5. p-Values of Impact of Independent Variables on Characters from MANOVA[1] for Each Year

cauda	Host	0.0001	0.0001	0.0008	processus	Host	0.0001	0.0001	0.0001
	Temp	0.0001	0.0001	0.0001	terminalis	Temp	0.0001	0.0001	0.0001
	Host*Temp	0.2836	0.0117	0.0001		Host*Temp	0.0372	0.0142	0.0001
	Morph	0.7132	0.5313	0.2321		Morph	0.0001	0.0001	0.2274
	Host*Morph	0.0001	0.0037	0.0001		Host*Morph	0.0017	0.0030	0.0001
	Temp*Morph	0.0016	0.5685	0.0004		Temp*Morph	0.0153	0.8098	0.0001
	Host * Temp* Morph	0.0085	0.4614	0.0002		Host * Temp* Morph	0.0001	0.0308	0.0001
siphunculus	Host	0.0001	0.0001	0.0687	minimum width of siphunculus (midpoint)	Host	0.0001	0.0001	0.0001
	Temp	0.0148	0.0004	0.0001		Temp	0.0001	0.0001	0.0001
	Host*Temp	0.0057	0.0696	0.0001		Host*Temp	0.0834	0.2724	0.0001
	Morph	0.4781	0.0704	0.0806		Morph	0.9011	0.0001	0.0001
	Host*Morph	0.0001	0.0002	0.0001		Host*Morph	0.0001	0.9402	0.0001
	Temp*Morph	0.0001	0.6609	0.0752		Temp*Morph	0.4036	0.0659	0.0001
	Host * Temp* Morph	0.0037	0.4414	0.0001		Host * Temp* Morph	0.0001	0.2211	0.0001

[1]MANOVA=Multivariate Analysis of Variance, SAS 1999.

Table 6. Influence of temperature on the size of characters used to separate *Myzus persicae* morphs, 1998/1999 and 2000.

Character	Temp °C	1998/9 Means±SD[1,2]	2000 Means±SD	Character	Temp °C	1998/9 Means± SD	2000 Means± SD
ultimate rostral	15	121±7 b	121± 6 c	third antennal	15	451± 39 c	444± 40 c
segment	20	119± 6 c	126± 5 a	segment	20	489± 39 b	525± 40 a
	25	122± 5 a	125± 4 b		25	523± 46 a	483± 45 b
base of sixth	15	135±13 a	132± 11 b	maximum width of	15	51± 4 a	48± 5 a
antennal segment	20	132±11 b	138± 11 a	siphunculus (distal	20	48± 5 b	49± 3 a
	25	134±13 a	127± 10 c	half)	25	48± 3 b	48± 4 b
hind femur	15	661±68 c	669± 61 b	terminal process	15	462± 37 c	463± 33 c
	20	682±57 b	739± 43 a		20	516± 35 b	533± 34 a
	25	712±64 a	678± 56 b		25	530± 43 a	488± 52 b
cauda	15	208±20 c	220± 20 c	siphunculus	15	508± 53 b	513± 48 b
	20	219±18 b	233± 17 a		20	526± 43 a	548± 34 a
	25	225±20 a	226± 15 b		25	527± 42 a	507± 34 b
second segment	15	115±11 b	116± 8 c	minimum width of	15	43± 5 b	43± 4 c
of hind tarsus	20	115±9 b	124± 7 a	siphunculus	20	41± 5 c	45± 3 a
	25	119±8 a	118± 6 b	(midpoint)	25	45± 4 a	44± 4 b

[1]Means and standard deviations (SD) given in µm.

[2]Means within a year and character not followed by the same letter(s) are significantly different as indicated by Waller-Duncan k-ratio t test (k-ratio=100).

could equally well be considered in two divergent manners. It might be interpreted as an indication of a very stable effect that is strong enough to overwhelm the highly complex and substantial influence from other aspects of host plant physiology. However, these results could also be viewed as anomalies, the sort of random consistencies that are bound to turn up on occasion given such a data set. Sorting out which of these interpretations is more appropriate will require additional experimentation, especially with an experimental set-up that can control for some of the physiological variation found in relation to age and nutrition for host plants.

4.3 Discrimination by Temperature and Host Plant

4.3.1 Linear Discrimant Functions

While there was no straightforward accounting for effects on individual morphological characters from temperature and host plant, discriminate analysis could separate aphids according to these environmental conditions. A discrimination analysis (STEPDISC, SAS 1999) was performed with classes designated as the temperature groups and one with classes as the host plant groups, LDF's were generated that could place individuals into these classes with much greater accuracy than random assignments to groups. Random assignment to groups, called 'prediction priors', for the temperature class would be 33.33% for each temperature (100%/3 temperatures). The 15°C temperature group did far better at 87% successful prediction (Fig. 1). Temperatures 20°C and 25°C were about the same at 63% and 64% respectively, nearly twice a random assignment (Fig. 1).

Host plant results were remarkably similar. Random assignments to groups for host plants would be 25% for each host class (100%/4 host plants). Tobacco was much greater, having 65% prediction success (Fig. 2). The other three host plants, turnip, pepper, and okra,

had prediction rates of 45%, 47%, and 48% respectively - again, nearly twice a random assignment for these groups (Fig. 2).

4.3.2 Canonical Variate Analysis

The results of the canonical variate analysis (CANCORR in SAS 1999) also shows that host plant and temperature had sufficient impact on the morphology of the aphids to produce clusters according to these factors. In a plot of CV1 by CV2 using the means of each test group, aphids reared on tobacco are clustered very closely, with only two outliers and a few overlapping groups. Aphids reared on turnip and pepper have looser clusters but are still distinct. Okra does not show a distinct cluster and is quite scattered across the CV1 and CV2 graph (Fig.8). This is likely due to the highly variable ability of aphids within these clones to thrive on okra. Because the okra reared aphids on the graph are so scattered that they obscure some of the other points I have included a graph of CV1 by CV2 for host plant without okra for better viewing of the other groups (Fig. 9).

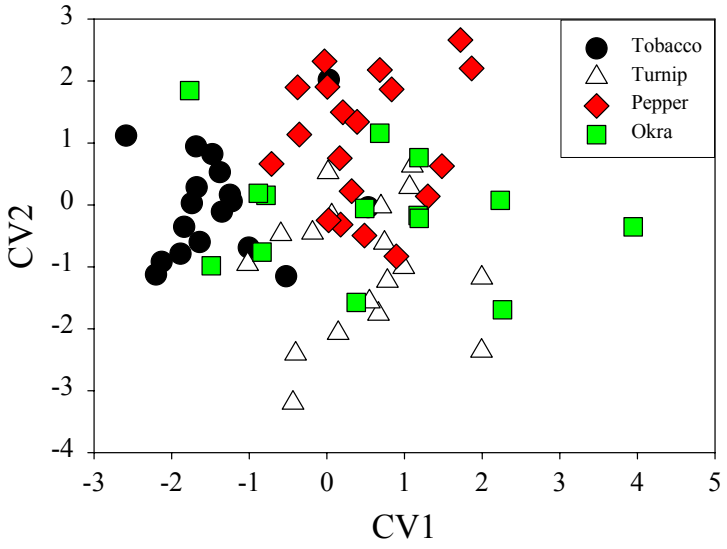

Fig. 8. CV1 by CV2 for Host Plant, Plotting Means for Each Aphid Test Group

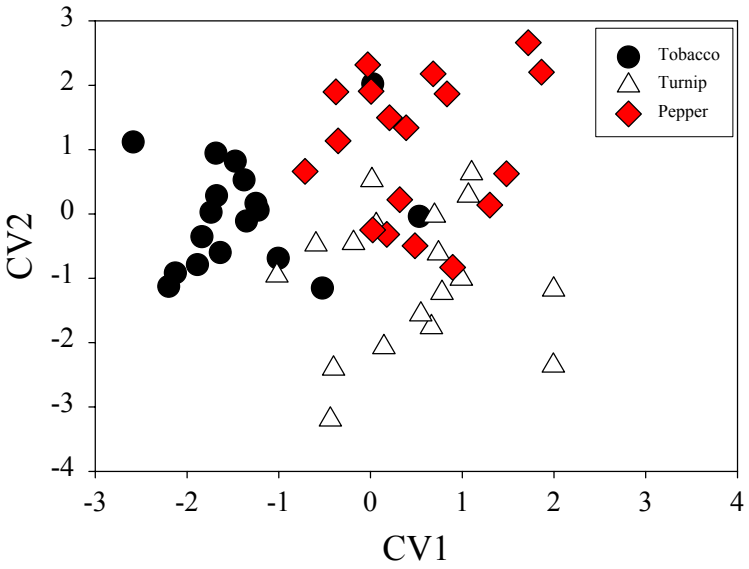

Fig. 9. CV1 by CV2 for Host Plant without Okra, Plotting Means for Each Aphid Test Group

A plot of CV1 by CV2 for rearing temperatures shows three distinct clusters for each of the temperature groups. There is some overlap between the 20°C and 25°C groups but each is still substantially in its own region of the graph. The 15°C groups are quite closely clustered with just a few overlapping points with the 20°C groups (Fig. 10).

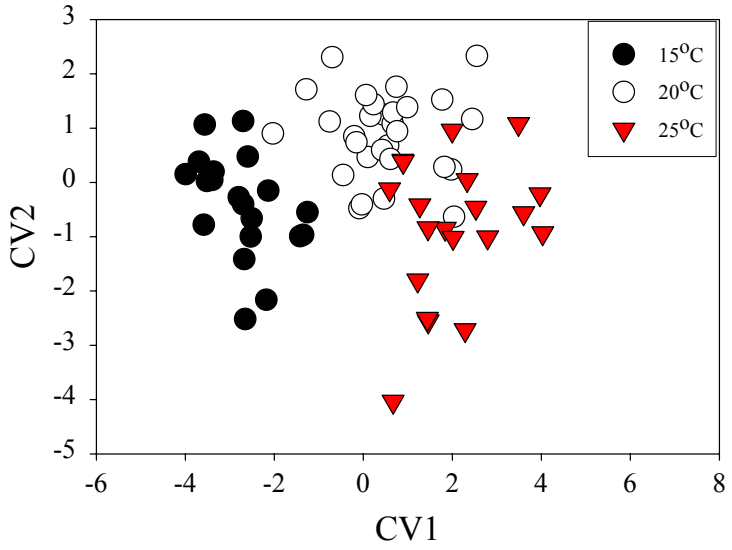

Fig. 10. CV1 by CV2 for Temperature, Plotting Means for Each Aphid Test Group

4.4 Tobacco and Non-Tobacco Morph Separation

4.4.1 Linear Discriminant Functions

While host plant and temperature showed substantial impact on aphid morphology, enough that the analysis could recover host plant and temperature groupings with considerable success, the morph groupings were most predictive (Fig. 6). The RTA morph and GTA morph are the 'red tobacco aphid' and the 'green tobacco aphid' respectively and the GPA morph is the 'green peach aphid'. When combined as the 'tobacco aphid group' RTA and GTA have a successful prediction rate of 89% into the RTA morph and 82% for the GTA morph. The 'green peach aphid' resolves into its group, GPA morph, with equivalent success at 81%.

4.4.2 Canonical Variate Analysis

The tobacco and non-tobacco morphs groups show up in distinct regions of the CV1 by CV2 graph. The GTA morph has the most widely ranging values and has a few outliers and a few groups that overlap with the GPA morph. Three of the GPA morph groups overlap with the non-tobacco groups (Fig. 11). Plots of CV1 by CV2 for morph x host plant combinations and morph x temperature combinations give clusters that group these test combinations with some distinctness, evidence that each morph had some what different responses to these environmental conditions (Fig. 12 and 13).

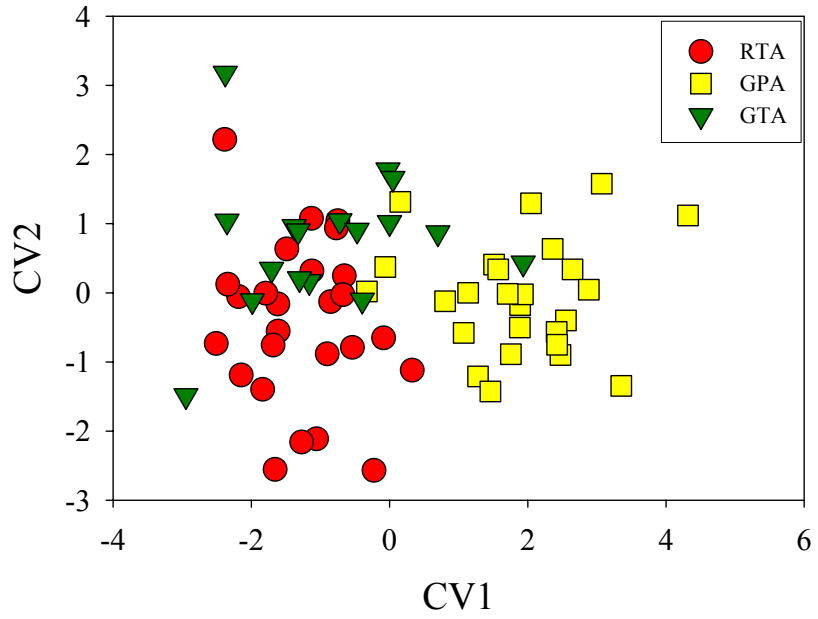

Fig. 11. CV1 by CV2 for Morph, Plotting Means for Each Aphid Test Group

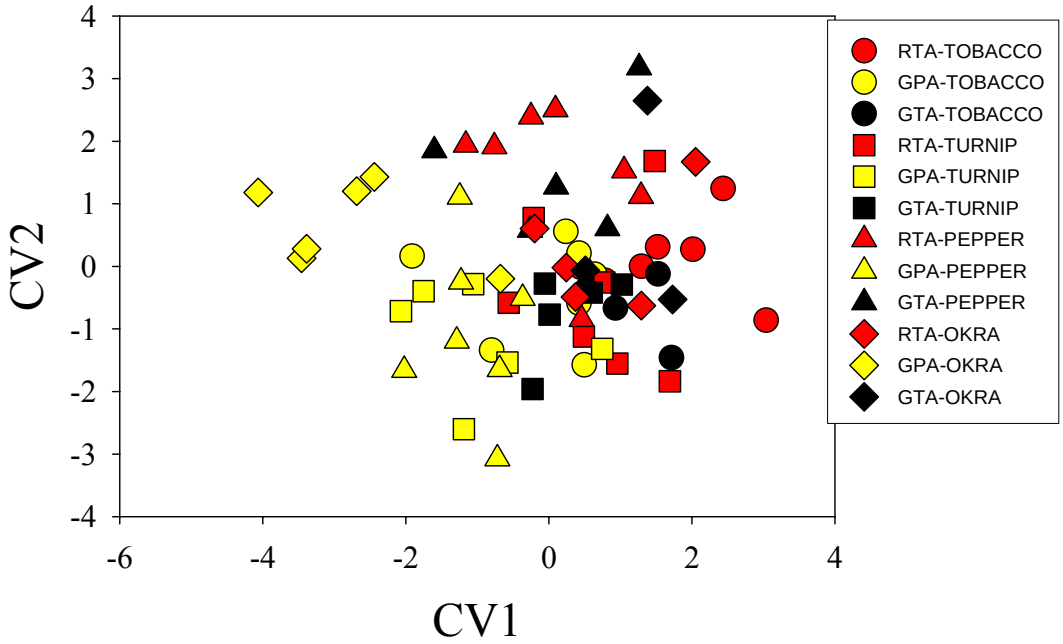

Fig. 12. CV1 by CV2 for Morph x Host, Plotting Means of Each Aphid Test Group

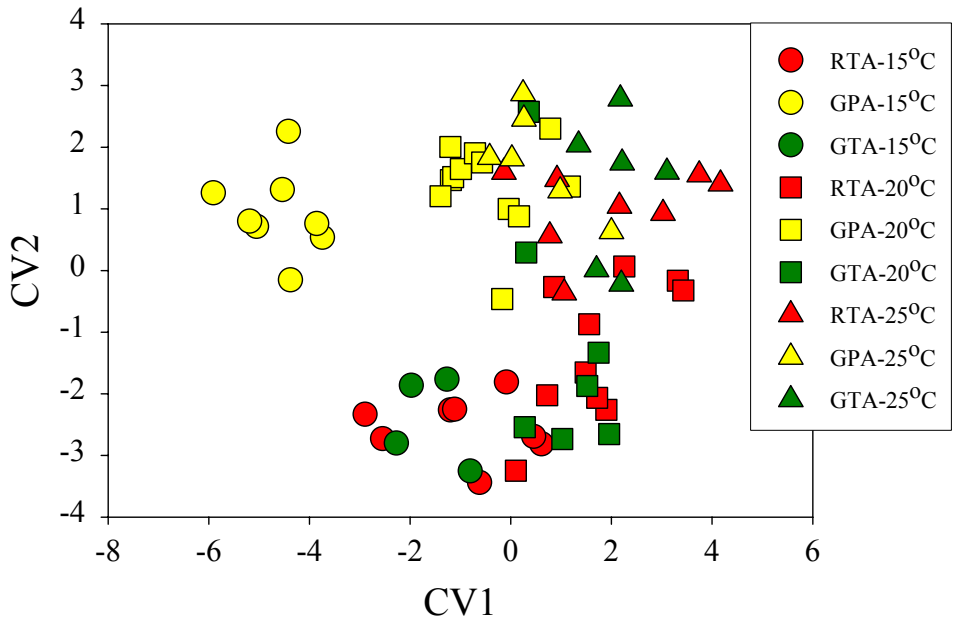

Fig. 13. CV1 by CV2 for Morph x Temperature, Plotting Means of Each Aphid Test Group

4.5 Conclusions

These results are compatible with the prevailing understanding that there is a tobacco host-adapted race or subspecies, but not a separate species. The morphology of the tobacco-adapted form is stable enough compared with non-tobacco forms so that these different clonal groups can continue to be successfully separated after several generations and exposure to different temperatures and host plants, but not with the key developed by Blackman (1987). This suggests that the clones that can establish on tobacco do have some unique morphological characteristics that allow for success on tobacco, but the characteristic differences between tobacco-adapted forms and non-tobacco-adapted forms are not universal. Indeed, these results may indicate that clonal morphology is more stable than expected. Analogous, perhaps, to the physiological and morphological differences between groups of the same species of mammals adapted to living at higher altitudes versus those at lower altitudes.

4.6 Suggestions for Future Research

Further research with more morphs and more clonal groups of both tobacco-adapted forms and non-tobacco-adapted forms would be useful in answering the question of clonal stability versus host-adapted form stability. It would also be extremely helpful, albeit very difficult, to unravel some of the host plant physiology-aphid morphology interactions. A similar experimental set-up that controlled for leaf age/stage would be a good next step. Other plant physiological parameters that are known to impact aphids include leaf turgor pressure, leaf photoperiod exposure, and many different nutritional components of the leaf. Some of these are more easily controlled for than others but unraveling these interactions would shed much light on the very complex biology of this fascinating and agriculturally important insect.

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VITA

Joan Marie was born to Kenneth Morgan Thompson and Camille Celeste Marie Thompson on December 27, 1966 in Portland, Oregon. She grew up on a small farm outside of McMinnville, Oregon where she graduated from high school in 1985. In 1996 she received a B.A. in biology from the University of Montana, Missoula, Montana. She worked for the U.S. Forest Service for several field seasons in Montana and Idaho conducting plant and animal surveys.

In 1995 she joined the Peace Corps and worked with communities living in and around a wildlife reserve in Uganda, East Africa. In 1998 she was hired as a technician to work with Dr. Paul J. Semtner at the Southern Piedmont Agricultural Experiment Station in Blackstone, Virginia. Becoming interested in his research on the aphid *Myzus persicae*, she enrolled in the graduate program in the Department of Entomology at Virginia Tech to pursue a M.S. degree, continuing the taxonomic research begun with Dr. Paul J. Semtner. She served as president of the W.B. Allwood Society, the entomology graduate student organization, from April 1998 to April 1999. While in the department of entomology at Virginia Tech she was awarded the James McD. Grayson Scholarship and the Michael T. Kosztarab Scholarship.

Beginning in 2001 she has been in a Ph.D. program in Science and Technology Studies in the Department of Science, Technology, and Society. Her dissertation project is a social, political, and historical analysis of the environmental movement in the United States.