To the Graduate Council:

I am submitting herewith a thesis written by Peter Joseph Obenauer entitled "The Biological Mechanisms of Resistance in Selected Lines of *Nicotiana tabacum* L. to *Myzus nicotianae* Blackman (Homoptera: Aphididae). I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Charles D. Pless, Major Professor

We have read this thesis and
recommend its acceptance:

Accepted for the Council:

Associate Vice Chancellor
and Dean of the Graduate School

THE BIOLOGICAL MECHANISMS OF RESISTANCE IN SELECTED LINES OF *NICOTIANA TABACUM* L. TO *MYZUS NICOTIANAE* BLACKMAN (HOMOPTERA: APHIDIDAE)

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Peter Joseph Obenauer

August 1998

DEDICATION

This thesis is dedicated to my parents

Mr. Peter James Obenauer

and

Mrs. Patricia Ryan Obenauer

who have always given me their

never ending love and support.

ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Charles D. Pless, for his friendship, guidance, and support throughout this project. I would also like to thank the members of my graduate committee, Dr. Reid R. Gerhardt, Dr. Robert D. Miller, and Dr. Roberto M. Pereira, for their attention and consideration of my research. In addition, I would like to thank the personnel at the Tobacco Experiment Station in Greeneville, TN, who helped make my research a success. I am especially indebted to Dr. Arnold Saxton for his aid with all statistical analyses performed on data, and time he spent in developing a computer program used in this thesis. In addition, many thanks to the following people for assistance with collection of data throughout this project: Stephanie French, Dr. Pereira, Derek Puckett, and Greg Wiggins. I would like to thank Lindey Hamlett, who first introduced me to the world of burley tobacco, and for his friendship and undying support during these past five years. I wish to express appreciation to the University of Tennessee Agricultural Experiment Station and the Department of Entomology and Plant Pathology for awarding me a graduate research assistantship and the opportunity to earn the Master of Science Degree. Finally, I would like to thank my parents, Peter J. Obenauer and Patricia R. Obenauer, for their support and love throughout my life.

ABSTRACT

Since 1993, several aphid-resistant breeding lines of tobacco, *Nicotiana tabacum* L. have been developed at the Tobacco Experiment Station, Greeneville, TN. Crosses were developed between TI 1068, a resistant to the tobacco aphid, *Myzus nicotianae* Blackman, and 'TN 86' or 'KY 17,' two tobacco cultivars susceptible to the tobacco aphid. A research project was conducted to: 1) reevaluate levels of resistance of selected tobacco lines to the tobacco aphid, 2) determine the population growth parameters of the tobacco aphid that are affected by resistant tobacco, and 3) simulate aphid population growth on resistant and non-resistant tobacco using a computer-generated model. In 1996 & 1997, several entries were evaluated for aphid population growth in open field plots, greenhouse, and growth room. Field plots were allowed to become infested naturally by aphids. In greenhouse and growth experiments, adult aphids were placed individually onto each plant and allowed to colonize. Results indicated high resistance to the tobacco aphid on TI 1068 for a variety of experiments, compared to other resistant tobacco lines.

Initially 3 aphid-resistant tobacco entries (TI 1068, 301, 3001) were evaluated to determine their mechanisms of resistance. Four aphid growth parameters (life cycle, fecundity, reproductive longevity, survival) were investigated for their effects on aphid populations. Aphid development was significantly slower on leaf discs of TI 1068, 301, and 3001 than on TN 86 leaf discs. Aphid development, fecundity, reproductive longevity, and survival did not differ significantly among all tobacco entries tested when reared on excised leaves from greenhouse-grown plants. Aphids reared on leaves

excised from field-grown TI 1068 plants had a longer life cycle, lower reproductive rate, shorter reproductive longevity, and shorter survival time than aphids reared on the standard cultivar, TN 86.

A computer simulation, based on data from laboratory experiments, was developed to predict seasonal population development of tobacco aphids on TN 86 and TI 1068 aphid populations under field conditions, but in the absence of limiting factors such as weather, predators, disease, and parasitoids. It was estimated using this model that progeny from one aphid on TN 86 could exceed to 10,000,000 individuals within 40 days; whereas, less than 700 aphids would develop on TI 1068. Future work on the effects of weather, predators, diseases, and parasitoids on an aphid populations could prove useful in more accurately predicting aphid population growth in a typical tobacco field.

TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION	1
II. LITERATURE REVIEW	11
III. SCREENING SELECTED LINES OF *NICOTIANA TABACUM* FOR RESISTANCE TO *MYZUS NICOTIANAE*	20
IV. LIFE CYCLE, FECUNDITY, REPRODUCTIVE LONGEVITY, AND SURVIVAL OF *MYZUS NICOTIANAE* ON SELECTED LINES OF RESISTANT AND NON-RESISTANT *NICOTIANA TABACUM*	36
V. CONCLUSIONS	73
REFERENCES CITED	78
APPENDICES	84
APPENDIX A	85
APPENDIX B	87
VITA	89

LIST OF FIGURES

FIGURE **PAGE**

1. Tobacco aphid population densities on tobacco lines maintained in a growth room, 21 days after initial infestation 27

2. Development of tobacco aphid populations on selected tobacco entries in a greenhouse, for 26 days after initial infestation 29

3. Populations of tobacco aphids on selected tobacco entries in a greenhouse, 21 days after initial infestation 30

4. Seasonal field populations of alate tobacco aphids on selected entries at the Tobacco Experiment Station, Greeneville, TN 1997 32

5. Seasonal field populations of apterous tobacco aphids on selected entries at the Tobacco Experiment Station, Greeneville, TN 1997 33

6. Field population of apterous tobacco aphids on selected tobacco entries, at the Tobacco Experiment Station, Greeneville, TN, August 19, 1997. 34

7. Insect rearing container containing an excised leaf and water pic 40

8. Clip-on cage attached to the leaf of a tobacco plant 40

9. Tobacco leaf discs embedded in agar 40

10. Program used to predict field aphid populations based on biological mechanisms of resistance of tobacco to the tobacco aphid 45

11. Life cycle of tobacco aphid on excised leaf of TN 86, October 1996 48

12. Fecundity of the tobacco aphid on excised leaf of TN 86, November 1996 49

13. Life cycle of the tobacco aphid on excised leaves of resistant (301) and non-resistant (TN 86) tobacco grown in a greenhouse, May 1997 50

14. Fecundity of the tobacco aphid on excised leaves of resistant (301) and non-resistant (TN 86) tobacco grown in a greenhouse, May 1997 52

15. Life cycle of the tobacco aphid on resistant (TI 1068, 301,3001) and non-resistant (TN 86) tobacco grown in the greenhouse, June 1997 53

16. Life cycle of the tobacco aphid on resistant and non-resistant tobacco leaf discs from plants grown in a greenhouse, June 1997. 55

17. Life cycle of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from the field, September 1997 57

18. Time for aphid development, from birth to adult, on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997 58

19. Fecundity of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997 60

20. Number of progeny per female aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997 61

21. Reproductive longevity of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from field, September 1997 62

22. Survival of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from field, September 1997 63

23. Life cycle of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco grown in a greenhouse, March 1998 64

24. Fecundity of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco grown in a greenhouse, March 1998 65

25. Computer-generated deterministic simulation of adult tobacco aphid populations, beginning with one 1st instar 69

26. Computer-generated deterministic simulation of immature tobacco aphid population, beginning with one 1st instar 70

LIST OF TABLES

TABLE **PAGE**

1. Levels of leaf surface chemicals of tobacco entries used in the insect resistant breeding line study, Tobacco Experiment Station, Greeneville, TN, 1994 22

2. Parameter values derived from data on excised leaves from field and used in computer simulation of field populations of tobacco aphids, Tobacco Experiment Station, Greeneville, TN, 1997 68

CHAPTER I

INTRODUCTION

History of Tobacco

Tobacco is a very important agricultural crop in many parts of the United States. In recent years however, tobacco has been the focus of controversy concerning health issues related to the use of cigarettes, cigars, and other tobacco products. Although tobacco is commercially grown for the sole purpose to manufacture such products, it is widely used for scientific experiments ranging from plant physiology and biochemistry to insect resistance (Tso 1972).

Tobacco belongs to the family Solanaceae and was placed in the genus *Nicotiana* in 1753 by Linnaeus (Tso 1972). It is widely believed that tobacco originated in portions of South America and was first introduced to the civilized world when the Arawaks offered it to Columbus on October 11, 1492 (Akehurst 1981). In 1612 John Wolfe introduced tobacco from the Orinoco region of Venezuela to Jamestown, VA. Early colonists of Virginia cultivated tobacco and exported tobacco products, resulting in the birth of the American tobacco industry. Tobacco is commercially grown in 97 countries, and worldwide production continues to increase (Tso 1972). China is the leading producer of tobacco, followed by the United States, India, Brazil, and the former Soviet Union (Akehurst 1981). Although tobacco is considered to be a tropical plant, it is

cultivated in many temperate climates, but usually not beyond 60° North or 45° South (Akehurst 1981).

Origin and Types of Tobacco

There are approximately 63 species of *Nicotiana* found around the world, and forty-four are indigenous to North or South America (Tso 1972). The most commonly grown species of tobacco is *Nicotiana tabacum*. According to Gerstel (1961), *Nicotiana tabacum* is an amphidiploid, developed from the hybridization of *N. sylvestris* and *N. tomentosifomis*. Many species of tobacco contain different levels of nicotine. Therefore, it is believed that *N. tabacum* did not originate in the wild, but was developed by early ancestors to have high levels of nicotine, aroma, and taste that was not found in wild species (Gerstel 1961).

Nicotiana tabacum is comprised of several different varieties, and each variety is characterized by its unique size, shape, texture, color, taste, and curing method (Tso 1972). Three major types of tobacco are grown in the United States: flue-cured, fire-cured, and air-cured. Air-cured tobacco can be broken down even further into five major classes: light air-cured, dark air-cured, cigar filler, cigar binder, and cigar wrapper. Flue-cured tobacco is generally considered a light tobacco and is mainly used in cigarettes. Dark tobacco is comprised of fire-cured and air-cured types. Although dark tobacco is also used in cigarettes, it is primarily used in pipe tobacco, cigars, chewing tobacco, and snuff (Akehurst 1981).

Flue-cured tobacco is the leading type of tobacco grown in the United States (Tso 1972). Burley tobacco, a light air-cured tobacco, is the most widely grown tobacco in Kentucky and Tennessee. According to Tennessee Agriculture (1997) over 109,888,000 pounds of tobacco, worth over 217,837,000 dollars in net revenue, were grown in the state in 1996.

Burley tobacco is perhaps the most widely and best known light air-cured tobacco. Burley tobacco is derived from a mutant, known as white burley, which was named for its creamy white seedlings (Akehurst 1981). Burley tobacco has many advantages: it is very light and fluffy, absorbs added flavorings, and has a high nicotine content. All of these factors make burley tobacco very desirable to the industry and therefore very popular among farmers (Akehurst 1981).

Insect Pests on Tobacco

Many insects feed on tobacco from emergence of the plant through harvesting. Some insects are capable of causing significant amounts of damage resulting in economic losses. According to Reich (1986) major insect pests of tobacco include: cutworms, wireworms, flea beetles, budworms, hornworms, and aphids. Cutworms, particularly the black cutworm, *Agrotis ipsilon* (Hafnagel), cut off newly set transplants at ground level. They are especially damaging in tobacco beds (Reich 1986). Wireworms are worm-like larvae of click beetles. The tobacco wireworm, *Conoderus vespirtinus* F., and the southern potato wireworm, *C. falli* Lane, are important pests in the tobacco regions of the United States (Akehurst 1981). Wireworms injure tobacco plants by cutting off

underground stems and roots and boring into larger stems and roots (Reich 1986). Tobacco flea beetles, *Epitrix hirtipennis* (Melsheimer) and potato flea beetles, *E. cucumeris* (Harris) feed on the leaves of the tobacco plant leaving a small shot-hole appearance on the surface of the leaf. Heavy infestations on small plants may cause stunting, resulting in uneven strands (Reich 1986). The damage to leaves is the most important, especially on cigar-wrapper type tobacco, where only a few holes are sufficient to prevent its use as a wrapper (Arkehurst 1981).

The tobacco budworm, *Heliothis virescens* (Fabricius), and the corn earworm, *Helicoverpa zea* (Boddie), are very destructive to the tobacco plant. The larvae will defoliate leaves and damage the terminal bud, causing the bud to become ragged and distorted (Reich 1986). The tobacco hornworm, *Manduca sexta* L., and the tomato hornworm, *M. quinquemaculata* (Haworth), can also feed on the leaves of tobacco plants. Heavy infestations can quickly strip plants of their leaves, leaving only the stalk and the midribs of the leaf (Akehurst 1981).

The tobacco aphid, *Myzus nicotianae* Blackman, is considered to be one of the most important tobacco pests. Large number of aphids can significantly reduce the amount of tobacco produced and affect the quality of the leaves. Aphids are also known to transmit various viruses to tobacco. In recent years, aphids have become resistant to many conventional insecticides and are harder to control.

General Biology of Aphids

Aphids (Homoptera: Aphididae) are plant-sucking insects, that belong to the superfamily Aphidoidea. There are approximately 4,000 aphid species that have been described worldwide (Blackman 1984). Although over 250 species feed on agricultural and horticultural crops throughout the world, only 50 species are considered to be a serious threat to crops (van Lenteren 1990) . Aphids are usually divided into two groups according to their life cycle: non-host-alternating (monoecious) species which feed on the same host plant year round, and host-alternating (heteroecious) species which migrate between more than one host plant (Miyazaki 1987).

Most aphids are soft-bodied and ovoid, reaching lengths of 1-4 mm. Wings, if present, are membranous. Aphids are characterized by having a pair of tube-like structures on the posterior part of the abdomen known as a siphunculi or cornicles (Miyazaki 1987). Aphids feed on phloem sap from vascular plants. In order to accomplish this task, aphids have a specialized stylet mouthpart, which is inserted between plant cells until they reach a sieve tube within the veins of the host plant. Because phloem sap is rich in carbohydrates but is low in amino acids, which are used to build proteins and essential for growth, an aphid must ingest a large amount of food to acquire sufficient amino acids, which are essential for growth (Dixon 1973). The most common carbohydrate in plant sap is sucrose, and is found in concentrations between 5 and 25% inside the stylet (Srivastava 1987).

Aphids undergo four instars in developing from birth to adult. The development of an aphid is dependent on two extrinsic factors, food quality and temperature, and two

intrinsic factors, birth weight and whether the morph is apterous or alate (Dixon 1987). Good nutrition increases the size and reproductive potential of aphids (Srivastava 1987). Food quality is influenced by the condition of the host plant. Factors such as age, nutrients, and condition of the host plant, can affect the development of the aphid (Dixon 1987).

The excretory system of aphids is unique among other insects. Most insects have malpighian tubules which regulate the balance of water and ions and remove nitrogenous waste in the form of uric acid. Aphids, however, do not have malpighian tubules and excrete nitrogenous waste in the form of ammonia (Dixon 1973). Waste is excreted through the anus as a droplet of liquid known as honeydew. The sugar content of honeydew is relatively high and attracts insects such as flies and ants. Ants harvest honeydew and protect aphids from predators. Honeydew is composed of 20 amino acids and has high concentrations of carbohydrates and nitrogen (Klingauf 1987).

Aphids are greatly affected by the environment. Studies on all species of aphids indicate that seasonal changes play an important role in the development of different morphs (Dixon 1973). Although sex is determined by genetics, over-riding physiological controls are the maternal reproductive system and the external environment (Lees, 1959). Environmental factors such as temperature, humidity, stress, overcrowding, and day length can stimulate aphids to produce alate forms. Crowding has been established as the prime factor in the production of alates (Shaw 1970). Also, the low nutritional condition of the host plant, which follows a heavy population of aphids, causes alate aphids to

develop (Kawada 1987). The development of alate and apterous morphs is controlled by the endocrine gland, the corpus allatum (Dixon 1973).

Aphids have a very complex life-cycle. Polymorphism and parthenogenesis make aphids unusual among insects. Parthenogenesis in aphids evolved over 200 million years ago during the Permian period (Dixon 1973). Parthenogenetic reproduction allows rapid increase in numbers and is extremely important in shaping the structure of aphid populations (Dixon 1985). The combination of parthenogenesis and the ability of asexual females to produce live offspring (viviparity) enables aphids to develop enormous populations in a short period of time. Polymorphism and the ability to produce multiple generations in one growing season enables aphids to exploit their host plants, and to build resistance rapidly to pesticidal applications (Dixon 1973). Twenty aphid species are documented to be resistant to organophosphates, and several species, such as *Myzus persicae*, are resistant to organophosphates and carbamates (Devonshire 1989).

Damage Caused by Aphids

Aphids are regarded as the most important agricultural insect pests in temperate regions of the world (Minks & Harrewijn 1987). The damage caused by aphids has been estimated to cause a 20% loss overall to the agriculture industry (van Lenteren 1990). Aphids are capable of causing a large number of physiological changes in their host plants and in many cases reducing plant productivity (Wellings et al. 1989). The damage aphids cause varies with the host plant and species of aphid. Most aphids affect plants by draining nutrients, especially nitrogen, which can cause a plant to become stunted and

prevent plant maturity. Aphids are also responsible for transmitting disease causing viruses, inoculating plants with toxins through their saliva, and interfering with photosynthetic efficiency by covering the leaves with cast skins or honeydew, which facilitates fungal growth (Niemeyer 1990).

Developing Host Plant Resistance

The development of host plant resistance is one of the most important methods used to reduce pest problems. Host plant resistance is defined as heritable qualities that a plant possesses to tolerate or influence the degree of damage caused by an insect (Painter 1951). The main objective in breeding for host plant resistance in crop plants is to produce the highest level of sustainable resistance while optimizing crop yield and quality (Dent 1991). Depending on the type of crop, resistant cultivars can be used in several ways: First, they can be used as the principal control method. Second, they can be used in conjunction with other pest management practices. Finally, they can be used as a safeguard against the release of more susceptible varieties (Painter 1951).

One of the best examples of resistant cultivars to control insect damage occurred in France. In the late 1870's, France was devastated by the accidental introduction of the pest Grape phylloxera, *Daktulosphaira vitifoliae L;* Fitch. The destruction of grape vines devastated the wine industry, and by 1884 total losses were estimated at ten billion Francs or two billion dollars (Painter 1951). Grape phylloxera was controlled by grafting European wine grape cultivars onto resistant grape root stock found in the United States. This example is perhaps the most successful example how host plant resistance plays an

important role in the fight against destructive insects. Host plant resistance has also been used in cotton, wheat, tobacco, and various other cash crops. Insect resistant varieties have reduced the damage caused by the Hessian fly and wheat stem sawfly to a minimum and have averted an annual crop loss of 10 million dollars in the United States alone (Kumar 1984).

The development and use of plant resistance has accelerated over the past twenty years. The increased use of host plant resistance is a consequence of the increase of insect resistance to many conventional insecticides. Insecticides usually result in temporary control and may lead to environmental contamination, while the use of insect-resistant cultivars is safe, long lasting, does not leave toxic residues, and is economical (Johnson 1978). Advantages of using resistant cultivars also include reduced dependance on chemicals for pest control and higher yields (Metcalf & Luckmann 1994).

According to Painter (1931), aphids are the most frequently targeted insects for host plant resistance studies. During 1937-1956 aphid-resistant cultivars of alfalfa, barley, corn, and sorghum already existed in the United States (Painter 1958). One of the earliest reported cases of aphid resistance in crop plants was resistance of peas to the pea aphid, *Acyrthosiphon pisum* Zhang (Harris 1979). Plant resistance to aphids is especially important in field crops where the margin of profit for farmers is very narrow (Webster 1990).

During the past decade research on insect resistance in burley tobacco has been in progress at the Tobacco Experiment Station, Greeneville, TN. The development of an aphid-resistant tobacco cultivar through breeding a tobacco introduction (TI), known for

its aphid resistance, with burley varieties could eventually enable farmers to grow tobacco that is resistant to these pests. The biological mechanisms of resistance of tobacco to aphids are not well understood. Investigations on how aphids respond to resistant tobacco lines could contribute to the development of aphid-resistant tobacco.

CHAPTER II

LITERATURE REVIEW

Damage Caused by Aphids on Tobacco

Aphids can cause significant damage to tobacco that is manifested both before and after curing. Heavy infestations of aphids can severely stunt the growth of young tobacco plants (Chamberlin 1958). Aphids feed by inserting their stylets through the surface of the leaves and into the phloem. The younger leaves of the tobacco plant are more susceptible because of the enriched phloem sap (Akehurst 1981). Nitrogen is the most common nutrient of which aphids deprive tobacco plants, resulting in slower development. Chemical analyses also show that tobacco leaves with heavy aphid infestations have reduced amounts of nicotine, alkaloids, sugars, and most phenolic compounds (Mistric & Clark 1978). Feeding by aphids can lead to production of large quantities of honeydew, which facilitates the growth of sooty molds, greatly reducing the value of the leaves. The tobacco aphid, *Myzus nicotianae* (Blackman), is known to be the sole or major vector of various viral diseases including: vein banding or potato virus (PVY), cucumber mosiac (CMV), tobacco vein mottling (TVMV), tobacco etch (TEV), and alfalfa mosaic (Reich 1986).

History of Aphids on Tobacco

The first reported incident of economic damage caused by aphids on tobacco in the United States was during the 1946 growing season (Chamberlin 1958). Aphids in noneconomic numbers had been observed on tobacco prior to that time, but did not pose a serious problem. Heavy infestations were reported but were usually confined to a few fields or a county (Chamberlin 1958). The first damaging infestations in Tennessee occurred on dark fire-cured tobacco in July 1947, and by the end of the season, a general aphid outbreak had occurred from the Gulf states to Canada (Chamberlin 1958). By this time taxonomists agreed that the destructive aphid on tobacco was the green peach aphid, *Myzus persicae* (Sulzer). However, it was later determined by Blackman (1987) that the aphid found on tobacco was another species, *Myzus nicotianae* Blackman.

According to Blackman & Eastop (1984) the genus *Myzus* contains 55 species from the Old World, and three-quarters of them probably originated in Asia. *Myzus persicae* (Sulzer) is described as a small to medium-sized aphid with pigments ranging from yellow green to pink or red. *M. persicae* is found throughout all temperate regions of the world, but is thought to be originally from Asia. The holocycle life-cycle of *M. persicae* (sexual reproduction and overwintering of eggs) is commonly found in temperate regions of every continent (Blackman 1974). The primary host plant for *M. persicae* is *Prunus persica* (peaches). This aphid is also known to feed on secondary hosts from over 40 families, some of which are economically important plants (Blackman & Eastop 1984).

Before 1986, the most common color form of the aphid species found on tobacco was green or yellow green (Chamberlin 1958). However, during the 1985 growing season, at the Central Crops Research Station, Johnston County, North Carolina, a red form of the aphid was observed (Harlow & Lampert 1990). The red form had been previously observed in other countries, but not in the United States (Takada 1982). In 1987, preliminary studies were conducted to compare the two color forms. Initial findings indicated that the red form was more difficult to control, required fewer days to reach adulthood, and produced nymphs sooner than the green forms (Lampert & Dennis 1987). During the 1987 and 1988 growing seasons the red form was present on tobacco in other states and was predominant in the southeastern United States (Lampert & Dennis 1987). In North Carolina, the red form exceeded 80% of reported aphid populations on tobacco (Harlow & Lampert 1990). During that same time period, tobacco farmers were having a difficult time controlling aphid populations with insecticides that had previously been effective. Field tests performed in North Carolina showed that there was a severe decline in mortality of aphids, particularly the red form, when treated with azinphosmethyl, diazinon, malathion, methidathion, methyl parathinion and monocrotophos (Harlow & Lampert 1990). Lampert (1987) investigated the mechanisms involved in the resistance of the red color morph to these chemicals. Apparently, resistance was a function of increased esterase activity, which accelerates the metabolism of insecticides (Harlow & Lampert 1990). Therefore, many organophosphorous insecticides, such as malathion and acephate, were ineffective in controlling the red color morph. Harlow & Lampert (1990) also indicated that the resistance among the red morph

was permanent, i.e., an aphid population would remain resistant even if the insecticide pressure was removed.

Many researchers suspected that populations of *M. persicae* on tobacco in different parts of the world were morphologically different (Blackman 1987). Researchers collected *M. persicae* on tobacco in Germany, Zimbabwe, and Puerto Rico (de jong, 1929; Brain, 1940 Wolcott, 1952; Chamberlin, 1958; Muller, 1958 and Blackman, 1987). Their findings showed that the aphids collected in those regions were very similar with a relatively long terminal process of the antennae, anholocyclic reproduction, high fecundity during midsummer temperatures, and the green color of apterous viviparae aphids. Blackman (1987) investigated the possibility that a red morph of tobacco-infesting aphid resulted from a mutation within the green morph populations and that it represented another species of aphid that was taxonomically different from *M. persicae.* The tobacco-adapted form was closely related to *M. persicae*, but had its own characteristic morphology and was described as a new species of aphid, the tobacco aphid, *Myzus nicotianae* Blackman.

Blackman (1987) determined that both species, *M. persicae* and *M. nicotianae*, have 12 chromosomes and are frequently heterozygous. However, the tobacco aphid differs from the green peach aphid by being permanently parthenogenetic. The green morph of *M. nicotianae* has some selective advantages over the yellow green form of *M. persicae.* When reared on potato, the green morph of *M. nicotianae* had a longer longevity and higher fecundity than the yellow green form of *M. persicae* (Boiteu & Lowery 1989). The tobacco aphid was also more resistant to many insecticides than was

the green peach aphid. Boiteau & Lowery (1989) demonstrated that *M. nicotianae* was 2.2-fold less susceptible to aldicarb and methamidophos and 6.0-fold more tolerant to deltamethrin and fenvalerate when compared to *M. persicae*.

Myzus nicotianae was first introduced into America over 40 years ago (Blackman 1987). Although tobacco is the primary host plant of *M. nicotianae*, it has also been found on other plants including *Capsicum*, *Sesamum*, *Orobanche*, *Brashica*, and *Sisymbrium* (Blackman 1987). Boiteau & Lowery (1989) first reported that *M. nicotianae* was also found feeding on greenhouse grown potato, *Solanum tuberosum* L. The red morph of *M. nicotianae* has been shown to have developmental advantages over the green morph. Reed & Semtner (1990) were the first to investigate the influence of temperature on the development of both aphid morphs on tobacco. Their study indicated that when temperatures were above 25°C, the red morph developed faster, was more fecund, and had a longevity 120% greater than the green morph. The red morph was also able to survive twice as long as the green morph at 32°C (Reed & Semtner 1990). The combination of a faster life cycle, greater longevity, higher fecundity, and the ability to resist insecticides and environmental stresses, such as heat, have enabled the red morph to become a very serious pest.

Mechanisms of Resistance

The mechanisms of resistance of a plant to an insect are important factors to understand when developing plant varieties that are resistant to insect damage. Painter (1951) described three mechanisms that control the phenomena of resistance in the field:

a) nonpreference, a group of plant characteristics and insect responses that have either a negative or positive response by the insect to feed, oviposit, or find shelter; b) antibiosis, effects by the plant to prevent feeding or development, or cause injury or death to the insect; and c) tolerance, abilities of the plant to repair, recover, or withstand infestations by insects. Usually, a plant exhibits one or more of these mechanisms to resist insect damage. The combination of environmental factors, such as light, temperature, and humidity, influence the insect-plant interrelations (Painter 1951). Xia & Johnson (1997) determined that leaf surface moisture and higher levels of humidity significantly affected the efficacy of *N. gossei* Domain sugar esters on tobacco aphids.

The resistance mechanisms in aphid-resistant cultivars are not well understood and usually have a complex chemical basis (Campbell & Dreyer 1990). Plant genotypes that are resistant to aphids have different mechanisms that take effect when the aphid is attacking, walking and probing, penetrating the phloem, and feeding on the phloem (Harrewijn 1986). Many plants have their leaves covered with dense trichomes, which serve as a defensive mechanism against insects. Trichomes can interfere with insect feeding and oviposition. They can also secrete sticky exudates which can contain toxic substances (Norris & Kogan 1980). Plants attract or repel aphids through various volatiles or compounds that are located in different parts of the leaf. According to Niemeyer (1990), the plant wax layer may contain alkanes, which can exert a positive stimulus for aphids, or diketones, which repel aphids. The mesophyll layer may contain phenolics that stimulate aphid colonization, and phloem constituents may also act as a positive or negative stimulus for aphid colonization. Production of volatile compounds

may deter feeding; however, many are produced only when an aphid has wounded a plant (Hilderbrand, et al. 1993).

Plant volatile compounds may also influence aphid life cycle and reproduction. Hilderbrand et al. (1993) demonstrated that volatiles produced from young tomato leaves, *Lycopersicon esculentum* L., reduced the growth rate of an aphid population. Their results showed that resistance was due to volatile six-carbon aldehydes and alcohols contained in the leaves. Both the aldehydes and alcohols affected aphid fecundity and significantly limited an increase in the population (Hilderbrand et al. 1993). Aphid development rate is also influenced on resistant plants. The detection of toxic chemicals usually stimulates the aphid to change feeding sites (van Emden & Wratten 1990). Aphids are usually smaller in size and develop at a lower rate due to their constant probing and walking on the plant (van Emden & Wratten 1990).

Host Plant Resistance in Tobacco

Thurston (1961) first reported that some *Nicotiana* species were highly resistant to the green peach aphid, *M. persicae*. He determined that many aphids were suffering from lack of feeding and believed that there were toxic substances in the sap of the leaves. Thurston et al. (1966) studied tobacco trichomes, which contained high amounts of nicotine and other alkaloids. Their findings demonstrated that these chemicals caused the legs of aphids to become paralyzed; unable to move or feed, the aphids eventually died. These studies indicated that the chemicals found on tobacco leaves were affecting aphid survival. Johnson & Severson (1982) determined that more than one type of surface

chemical was usually responsible for aphid resistance. Their study revealed that tobacco plants with high levels of sucrose esters or duvatrienediols were resistant to aphids.

Several tobacco cultivars have been investigated for possible resistance to insects. In particular, TI 1068 was noted for the large number of dead tobacco flea beetles, *Epitrix* spp., and high resistance to aphids (Thurston &Katanyukul 1971). The leaves of TI 1068 contained large amounts of sticky exudates, which unfortunately, severely inhibited predators, such as coccinellid larvae, from feeding on aphids (Belcher & Thurston 1983). Using electron microscopy Johnson et al. (1985) observed leaf trichomes and found straight and branched trichomes, which contained sucrose esters, diterpenes, and docosanol. It is believed that the combination of chemical exudates and leaf surface are responsible for aphid resistance.

Crossing insect resistant tobacco lines with insect susceptible burley tobacco can result in a decrease in the amount of insecticides needed to control insects on the crop. Resistant tobacco lines have also been used to resist viral and fungal infections. 'TN 86' is the first burley cultivar to be released with high resistance to tobacco vein mottling virus (Miller 1987). The combination of insect resistance and virus resistance in one burley tobacco cultivar could be valuable to the tobacco industry.

The long term goal of this research project is to produce a commercial insect-resistant cultivar. The objectives of my research were to: 1) screen selected breeding lines for resistance to the tobacco aphid, 2) determine the life cycle, fecundity, reproductive longevity, and survival of the tobacco aphid on resistant and non-resistant

tobacco lines, and 3) develop a computer generated simulation using these parameters to predict aphid field populations on resistant and non-resistant tobacco lines.

CHAPTER III

SCREENING SELECTED LINES OF *NICOTIANA TABACUM* FOR RESISTANCE TO *MYZUS NICOTIANAE*

Introduction

The tobacco aphid, *Myzus nicotianae* Blackman, is a serious insect pest on tobacco, *Nicotiana tabacum* L. Heavy infestations of aphids can severely stunt the growth of young tobacco plants (Chamberlin 1958). Chemical analyses show that tobacco leaves with heavy aphid infestations have reduced amounts of nicotine, alkaloids, sugars, and most phenolic compounds (Mistric & Clark 1979). Feeding by aphids can lead to production of large quantities of honeydew, which serves as a substrate for the growth of sooty molds, greatly reducing the value of the leaves. Some selected *Nicotiana* species are known to produce internal alkaloids that confer resistance against many tobacco insect pests, including the tobacco aphid (Thurston et al. 1966). Burk and Stewart (1969) reported that during a four week study, aphid numbers on different *Nicotiana* species varied from as high as 3500 aphids per plant on *N. sylvestris* to as low as 30 aphids on *N. fragrans*. TI 1068, a tobacco introduction, has been shown to be less susceptible to aphid infestations than other types of tobacco, such as 'KY 17' (Belcher & Thurston 1983). TI 1068 is a secreting tobacco that produces high concentrations of leaf exudates, which have been shown to make plants resistant to aphids.

During the past decade, research on insect resistance in burley tobacco has been in progress at the Tobacco Experiment Station (TES), Greeneville, TN. In 1989, crosses between TI 1068 and KY 17 or 'TN 86' were made to incorporate aphid resistance into burley tobacco genotypes. Chemical analyses of the F2 generation revealed that plants were segregating according to the absence or presence of sucrose esters and cis-abienol. The F3 generation produced four genetically stable breeding lines. The four lines were designated 100, 200, 300, and 400 series. Individual plants from each line were selected from the F4 generation and were selfed to produce an F5 generation. Plants from the F5 generation were then backcrossed with GR-135, known for its superior black shank resistance, and were designated 1000, 2000, 3000, and 4000 series (Miller 1998 pers. commun.). Chemical analyses of the leaf surface exudates revealed differences in chemical concentrations and compositions of these eight lines and TI 1068 and TN 86 (Table 1).

In 1994, research was conducted to identify an aphid-resistant breeding line from selfed and backcrossed lines. Plants were tested in an open field, small cages, and greenhouse. Shumate (1994) reported that entries in the 300 and 3000 series were colonized by significantly fewer aphids than any of the other selfed or backcrossed entries. TI 1068 was also colonized by fewer aphids but, the 300 and 3000 entries were the most resistant to aphid colonization (Shumate 1994). Resistance to the tobacco aphid in the 300 and 3000 entries was determined to be a result of sucrose ester content in the absence of cis-abienol. Levels of alpha and beta-duvatrienediols were not important to the resistance to the tobacco aphid (Shumate 1994).

Table 1. Levels of leaf surface chemicals of tobacco entries used in the insect resistant breeding line study, Tobacco Experiment Station, Greeneville, TN, 1994.

	LEAF SURFACE CHEMICALS (ug/cm²) *			
ENTRY	**CIS-AB**	**A-DIOL**	**B-DIOL**	**SE**
94-INS-102	0.0	78.4	42.2	0.0
94-INS-201	56.5	82.7	39.8	0.0
94-INS-301	0.0	91.5	37.1	32.5
94-INS-401	39.2	60.7	27.2	60.0
94-INS-1001	0.0	24.3	10.0	0.0
94-INS-2001	0.0	17.6	7.2	0.0
94-INS-3001	0.0	33.2	13.6	7.0
94-INS-4001	3.8	19.5	8.4	6.9
TI 1068	23.4	32.7	19.7	47.9
TN 86	0.0	31.4	19.6	0.0

* Leaf surface chemicals: cis-ab = cis-abienol, a-diol and b-diol = α- and β- duvatrienediols, and se = surcose esters

Adapted from: Shumate, S. 1995. Role of leaf surface chemistry on aphid resistance in burley tobacco. M. S. Thesis, Univ. Tennessee, Knoxville, pp. 78.

Studies in 1996 & 1997 were conducted to reevaluate degrees of resistance of tobacco to the tobacco aphid on previously selected resistant and non-resistant tobacco lines. Aphid population densities were evaluated to isolate the most resistant tobacco line, which could be used to determine the mechanisms of resistance of the tobacco aphid. Selfed-lines, (94-INS-102, 94-INS-201, 94-INS-301, 94-INS-401) and backcrossed lines, (94-INS-1001, 94-INS-2001, 94-INS-3001, 94-INS-4001) were evaluated for aphid resistance. TI 1068 was used as the standard resistant tobacco introduction and TN 86 was used as the standard non-resistant tobacco cultivar.

Materials and Methods

TOBACCO APHIDS

Red color morph tobacco aphids were used in growth room and greenhouse experiments. Aphids were collected from a colony maintained on tobacco plants in a greenhouse at The University of Tennessee (UTK), Knoxville, TN. The colony was started with aphids from field plants in August 1996 and reared on potted TN 86 tobacco plants at temperatures ranging from 21 to 30° C and a controlled photoperiod of 16:8 (L:D) h. Special care was taken to ensure that field collected aphids were not infected with the fungus *Pandora neoaphidis* that causes epizootics in field populations of the tobacco aphid.

GROWTH ROOM EXPERIMENT

Preliminary evaluations of several tobacco entries were conducted in an insectary at The University of Tennessee Agricultural Experiment Station, Plant Sciences Unit, Knoxville, TN. Four selfed-lines (94-INS-102, 94-INS-201, 94-INS-301, 94-INS-401); four backcrossed lines (94-INS-1001, 94-INS-2001, 94-INS-3001, 94-INS-4001); one tobacco introduction, TI 1068; and one cultivar, TN 86, were evaluated for aphid colonization in a growth room setting. Tobacco plants were started in float-beds in October 1996 in a greenhouse at TES. Plants were transported to UTK and were transplanted into 7.5 cm pots and placed in the growth room.

Plants were allowed to grow to an average height of 15 cm before being infested with aphids. On 6 January 1997, 3 apterous adult aphids were placed onto the top 3 leaves of each plant using a fine camel-hair brush. Each of 4 replicates of 10 randomly arranged lines was maintained in a separate watering tray (1 replicate per tray), measuring 106 X 54 X 6 cm. Aphid-infected plants were maintained at 23 ± 2°C and a photoperiod of 16:8 (L:D) h. Aphids on the upper and lower surfaces of the top 5 leaves of each plant were counted every 2 days, for 3 weeks. The experiment was repeated in March 1997. Aphid count data were analyzed using least significant difference (P=0.05) (SAS Institute Incorporated 1989).

GREENHOUSE EXPERIMENT

Aphid population growth on resistant and non-resistant lines, was studied in a greenhouse environment to be compared with population growth in a growth room and

open field at TES. Four entries: 94-INS-301(301), 94-INS-3001 (3001), TI 1068, and TN 86 were evaluated for aphid colonization. Plants used in this experiment were started in float-beds on 15 May 1997 at TES. On 22 June 1997, plants were transplanted into 7.5 cm pots. The potted plants were maintained in watering trays measuring 106 X 54 X 6 cm. Within each watering tray (block), 4 plants one of each line, were randomized. Each tray was replicated 10 times and placed on a greenhouse bench. Plants were allowed to grow for 15 d to an average height of 29 cm before infesting them with aphids. Greenhouse temperature was not monitored; however, a cooling system kept temperatures from exceeding 34 ° C. On 10 July, 3 apterous aphids were placed onto the top 3 leaves of each plant using a fine camel-hair brush. Aphids were counted on the upper and lower surfaces of the top 5 leaves of each plant every 2 d for 3 weeks. Aphid data were analyzed using least significant difference (P=0.05) (SAS Institute Incorporated 1989).

FIELD EXPERIMENT

A field experiment was conducted to study alate and apterous aphid populations, on resistant and non-resistant tobacco lines in the presence of naturally occurring predators and parasitoids. Plants used in the field experiment were started in float-beds in a greenhouse at TES. The same entries used in the greenhouse experiment were evaluated in the field. Due to a very cold and wet spring in 1997, plants were not transplanted until 25 June, 5 weeks after initial planting in the float bed. Treatments were arranged in a randomized complete block design with 4 replications. Plots were 3 rows spaced 107 cm apart, and plants were spaced 41 cm apart within rows. Each row was

approximately 6.8 m long and contained 12 plants. Plants were not treated with insecticides and were allowed to become naturally colonized by aphids and other insects, including aphid predators and parasitoids. Alate and apterous aphids on the middle row of each plot were counted twice weekly. Only plants that numbered 2-11 within each row (10 plants per row) were counted to avoid end-of-row effects. Counts were made on the upper and lower leaf surfaces from the top 5 leaves of each plant. Aphid populations were recorded on 14, 17, 21, 24, July and 1, 5, 7, 12, 19 August. Aphid data were analyzed using least significant difference (P=0.05) (SAS Institute Incorporated 1989).

Results and Discussion

GROWTH ROOM EXPERIMENT

In the preliminary evaluation of the 10 entries, all aphids had successfully colonized all plants by the 2nd day after initial infestation. By the 7th day after initial infestation, aphid population densities approached 150 insects per plant. Three weeks after initial infestation, the top 5 leaves of all entries became stunted from high densities of aphids. Mean aphid population density ranged from 301 aphids per plant on 94-INS-201 to 490 aphids per plant on TN 86 (Fig. 1). No significant differences ($P>0.05$) were detected among aphid population densities among the experimental lines but, there was a significant difference ($P<0.05$) between 94-INS-201 and the standard TN 86. Experiments in January and March produced similar results. A lack of difference between the standard tobacco cultivar, TN 86, the resistant tobacco introduction, TI 1068,

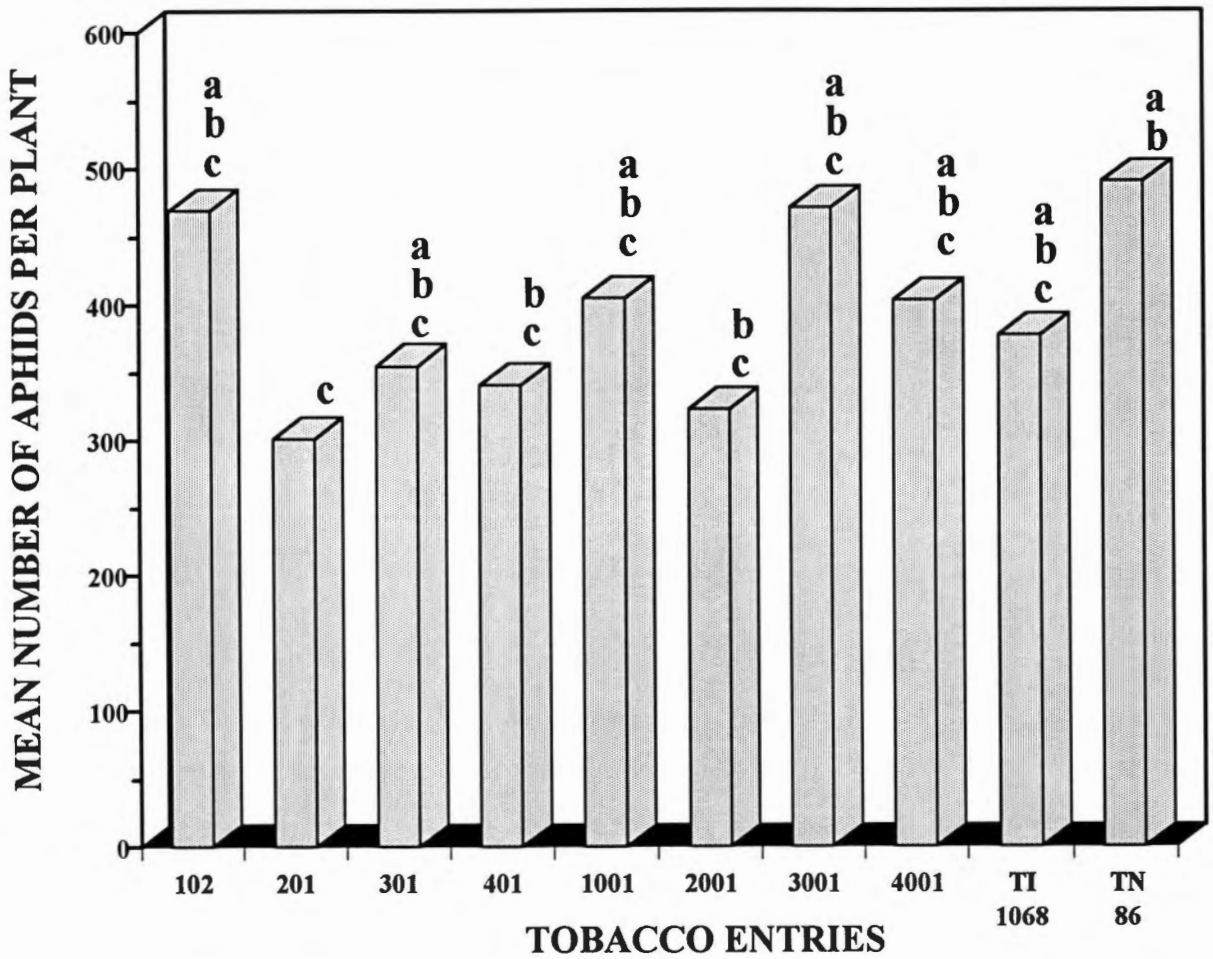

Figure. 1. Tobacco aphid population densities on tobacco lines maintained in a growth room, 21 days after inital infestation. Means with the same letter are not significantly different (P=0.05; Least Significant Difference)[SAS Institute 1989].

and the resistant tobacco lines 301, and 3001, is thought to be in part due to the small size and young age of the tobacco plants. Lack of toxicity in seedlings and young tobacco plants to the tobacco aphid had been documented previously by Abernathy & Thurston (1969).

GREENHOUSE EXPERIMENT

In the greenhouse experiment, aphid population densities increased gradually on all entries until 24 July, 14 d after initial infestation (Fig. 2). Between 1 August and 5 August aphid population densities increased rapidly on all entries except TI 1068 (Fig. 2). The mean population of aphids on TI 1068 never developed above 230 aphids per plant from 24 July to 5 August (Fig. 2). At the conclusion of the experiment, TN 86 had significantly higher aphid populations ($P<0.05$) than TI 1068, 301, and 3001; there was no significant difference between 301 and 3001 (Fig. 3). Although there was a significant difference in aphid densities between TN 86 and selfed the line, 301, and the backcrossed line, 3001, aphid densities were still unusually high on 301 and 3001, compared to past experiments using the same resistant lines (Miller 1997 pers. commun.). A possible explanation for the different magnitudes of aphid densities between TI 1068 and the selfed line, 301, and the backcrossed line, 3001, may have been the amount of leaf surface exudates produced. Although older plants were used, 301 and 3001 plants may have produced lower concentrations of leaf surface exudates compared to TI 1068. Chemical analysis of leaf surface exudates of individual plants was never conducted, so the amount of exudates produced by each plant remained unknown.

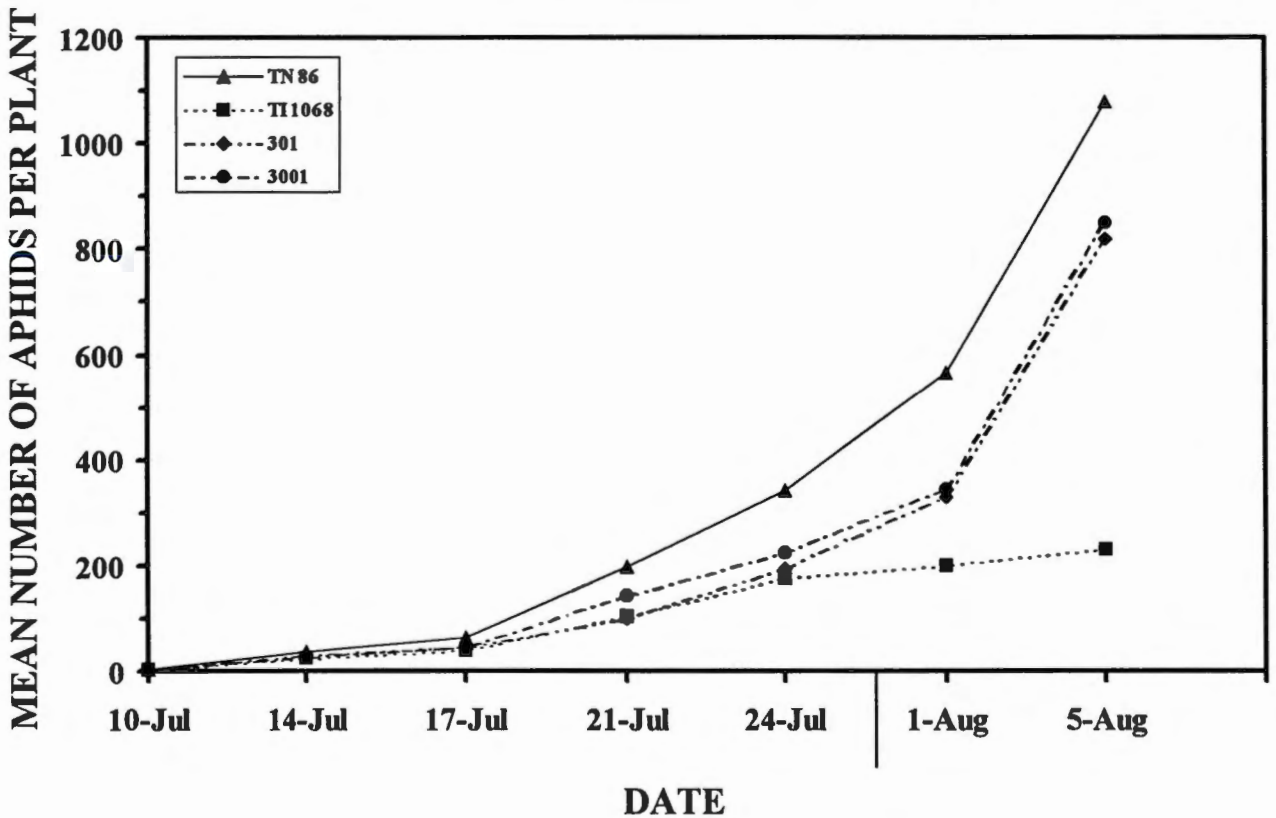

Figure. 2. Development of tobacco aphid populations on selected tobacco entries in a greenhouse, for 26 days after initial infestation.

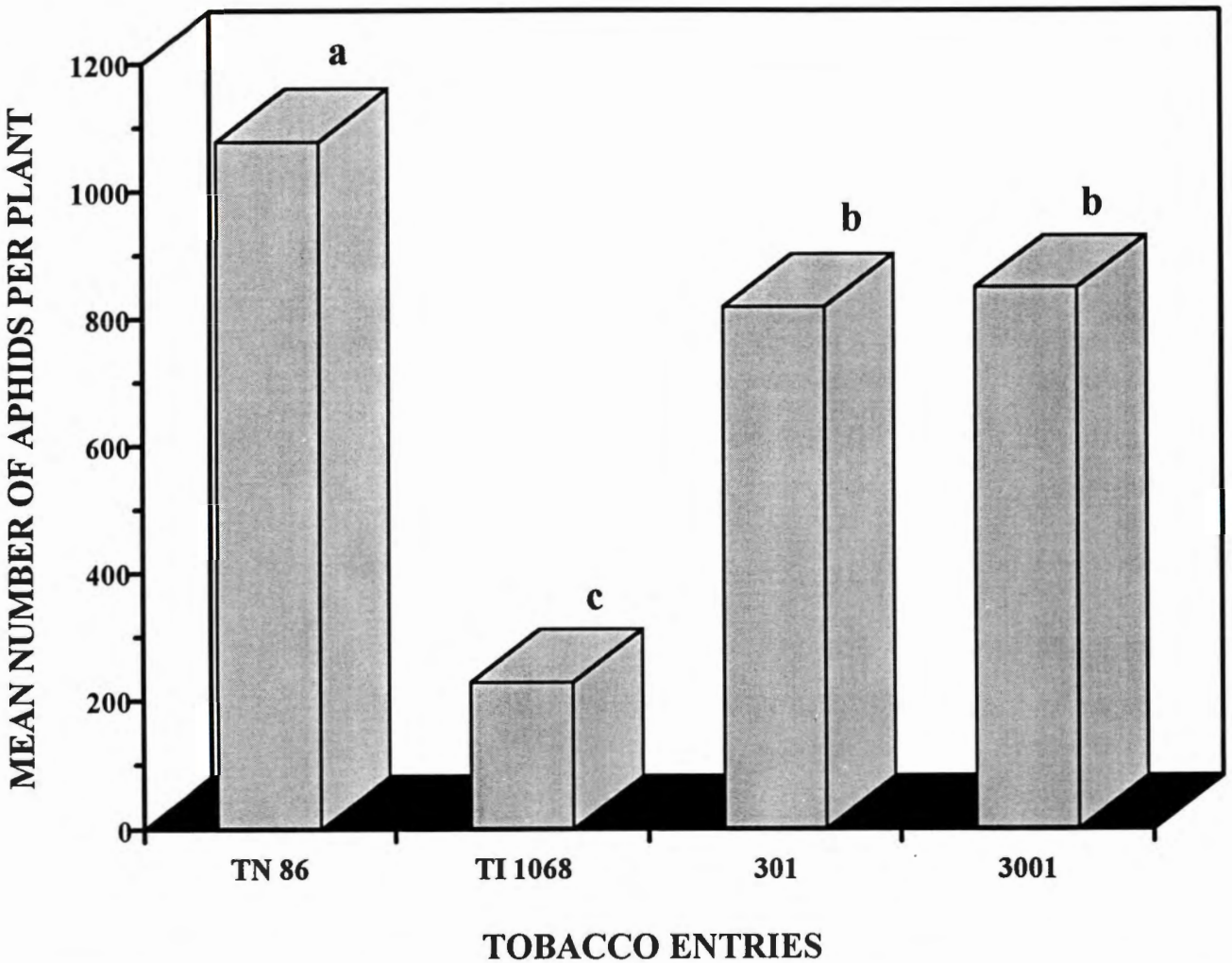

Figure. 3. Populations of tobacco aphids on selected tobacco entries in a greenhouse, 21 days after initial infestation. Means with the same letter are not significantly different (P=0.05; Least Significant Difference) [SAS Institute 1989].

FIELD EXPERIMENT

Alate aphids were first observed in most field plots on 14 July (Fig. 4). On 29 July, large numbers of alates were observed on tobacco plants. Significantly lower numbers ($P<0.05$) of aphids were counted on TN 86 than on TI 1068, 300, and 3001 entries on 29 July (Fig. 4). By 19 August, virtually no alate aphids were observed on all four entries. A possible explanation for more alate aphids accumulating on resistant lines, TI 1068, 300, 3001, than on TN 86, could be the amount of sticky exudates that were produced by the plants. Since TI 1068, 300, and 3000 entries are known to produce high concentrations of exudates, aphids that landed on these plants would be exposed to these sticky exudates. Therefore, higher numbers of alate aphids might accumulate on these plants due to exudates adhering to the wings and legs of aphids preventing their movement. Unable to fly to another plant, aphids stayed on the same plant for several days. Jackson and Severson (1990) also reported that TI 1068 contains antixenotic and antibiotic components which reduced alate establishment.

By 17 July, the 4 entries, TN 86, TI 1068, 301, and 3001, were colonized by apterous aphids (Fig. 5). Between July and August fewer apterous aphids were observed on TI 1068, 301 and 3001 than on TN 86. Apterous aphid populations were highest on TN 86, while lower numbers occurred on TI 1068, 301 and 3001. On the last aphid count date, 19 August, aphid numbers on TI 1068, 301 and 3001 were significantly lower ($P<0.05$) than those on TN 86 (Fig. 6).

Different results occurred when evaluating resistance in growth room, greenhouse, and field experiments. However, results from a particular experiment can change

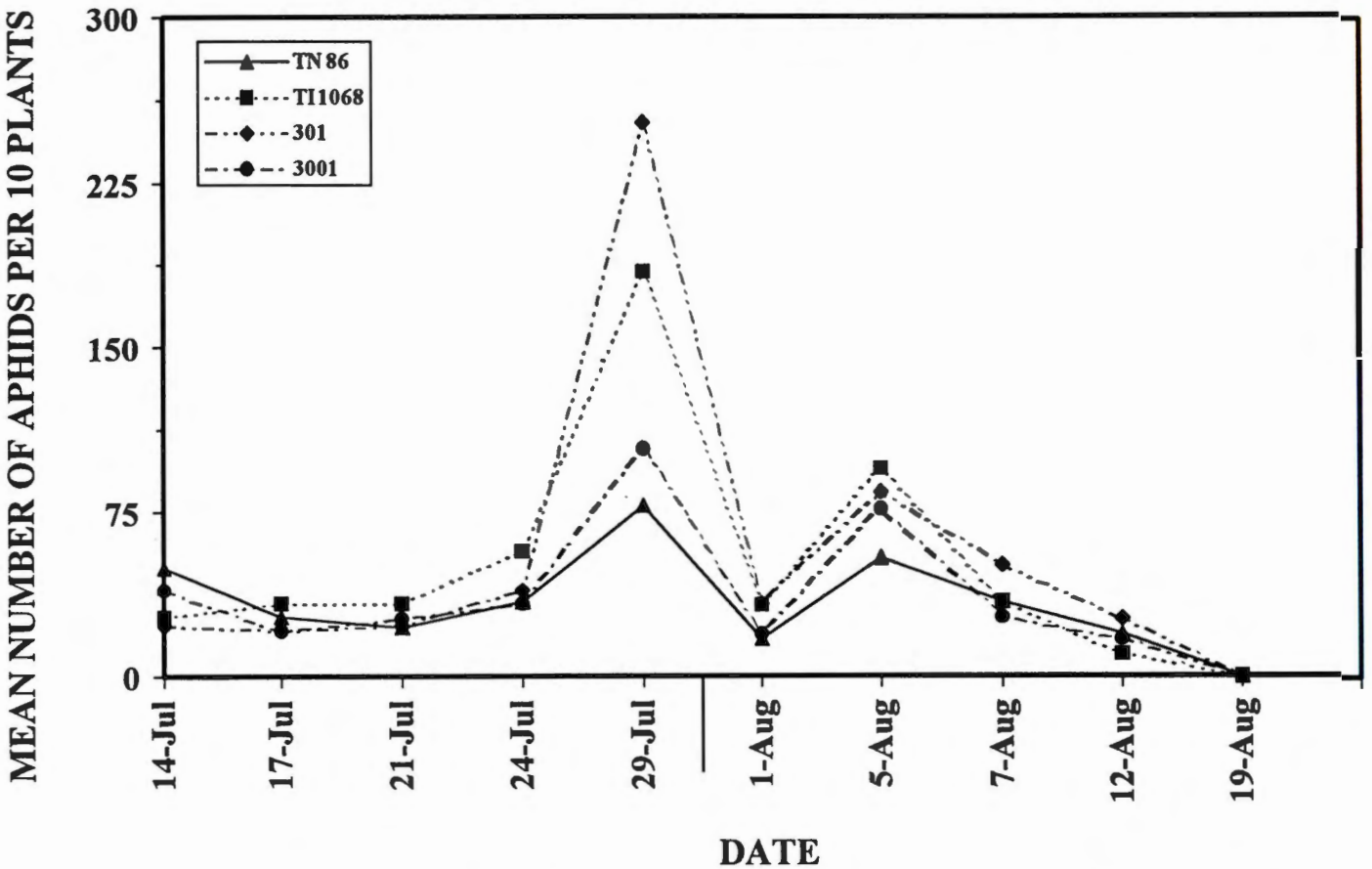

Figure. 4. Seasonal field populations of alate tobacco aphids on selected entries at the Tobacco Experiment Station, Greeneville, TN, 1997.

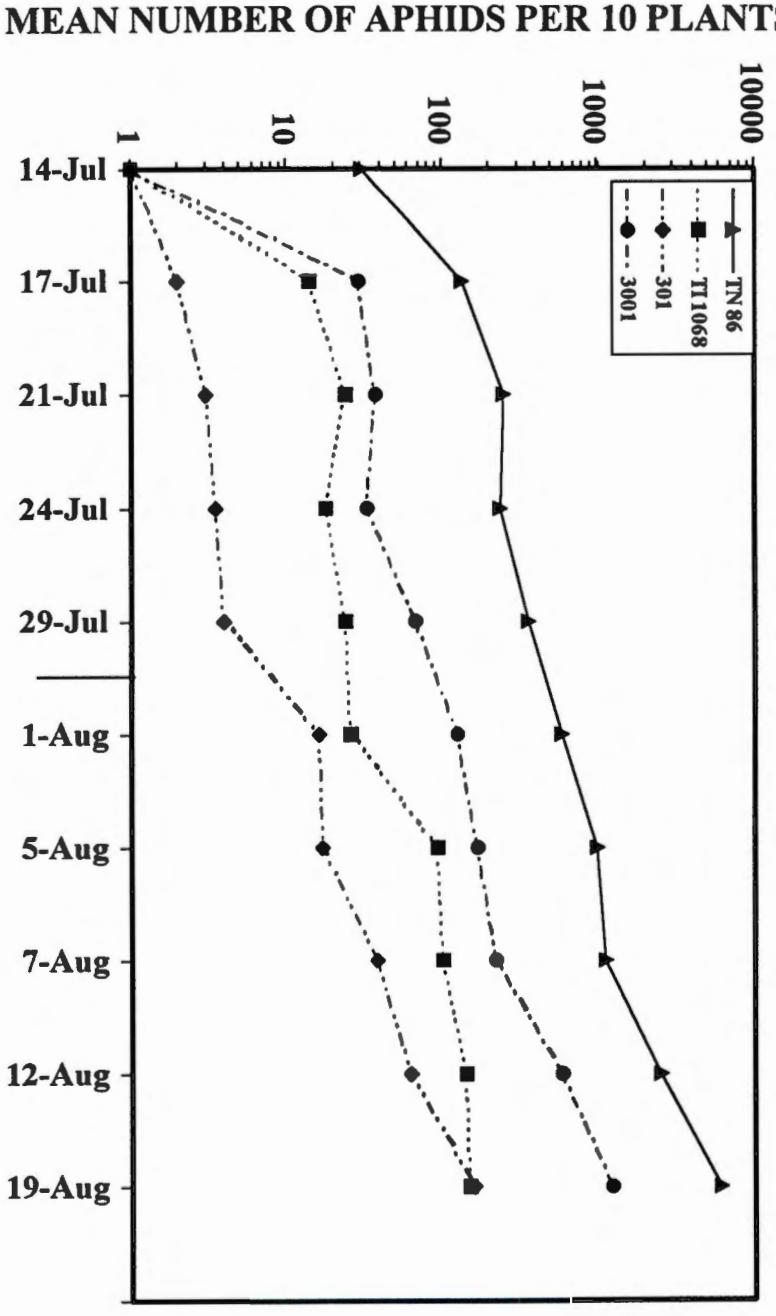

Figure. 5. Seasonal field populations of apterous tobacco aphids on selected entries at the Tobacco Experiment Station, Greeneville, TN, 1997.

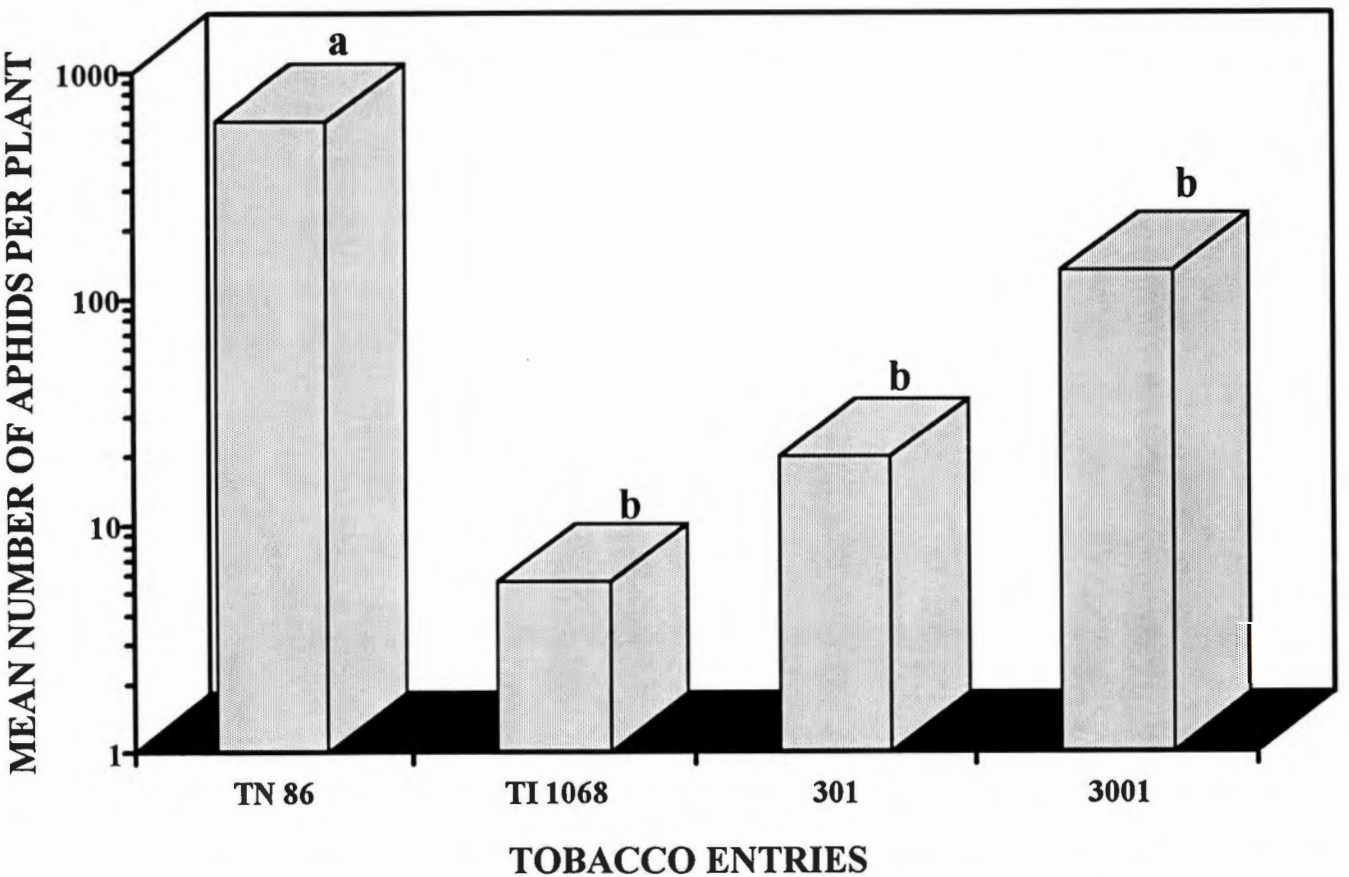

Figure. 6. Field populations of apterous tobacco aphids on selected tobacco entries at the Tobacco Experiment Station,Greeneville, TN, August 19, 1997. Means with the same letter are not significantly different (P=0.05; Least Significant Difference) [SAS Institute 1989].

dramatically when a experiment is conducted in a different environment. Nielsen et. al. (1982) reported disparities between field, laboratory or greenhouse tests when breeding tobacco for insect resistance. Aphid population densities on TI 1068 were significantly lower ($P<0.05$) than on TN 86 in both greenhouse and field experiments. Significant resistance was also observed in selfed-line 301 and backcrossed line 3001 in the field experiment.

Summary

In 1996 & 1997 several studies were conducted to reevaluate degrees of resistance of tobacco breeding lines to the tobacco aphid. Aphid population densities were evaluated to determine the most resistant tobacco line to the tobacco aphid. Originally 10 entries were evaluated in a growth room to determine levels of aphid colonization in the absence of predators. Four entries were chosen from the original 10 entries and were evaluated in a greenhouse for aphid colonization in the absence of predators. A field experiment using the same four entries was also conducted to determine aphid colonization under natural field conditions.

Under all conditions tested, TN 86 became heavily infested by aphids. Aphid resistance was observed on 301 and 3001 from only in field experiments. The tobacco introduction, TI 1068, was resistant to aphids in the greenhouse and field experiments. It was determined that TI 1068 would be the best tobacco line to use to determine the mechanisms of resistance of the tobacco aphid to resistant tobacco. The mechanisms of resistance are discussed in chapter VI.

CHAPTER IV

LIFE CYCLE, FECUNDITY, REPRODUCTIVE LONGEVITY, AND SURVIVAL OF *MYZUS NICOTIANAE* ON SELECTED LINES OF OF RESISTANT AND NON-RESISTANT *NICOTIANA TABACUM*

Introduction

The tobacco aphid, *Myzus nicotianae* Blackman, is an important insect pest on tobacco, *Nicotiana tabacum* L. Heavy infestations can severely stunt the growth of young tobacco plants (Chamberlin 1958). Chemical analyses show that tobacco leaves with heavy aphid infestations also have reduced amounts of nicotine, alkaloids, sugars, and most phenolic compounds (Mistric & Clark 1979). Feeding by aphids can lead to production of large quantities of honeydew, which serves as a substrate for the growth of sooty molds, greatly reducing the value of leaves. Recent studies have dealt with developing cultivars that are resistant to tobacco aphids. Some species of *Nicotiana* are known to produce alkaloids that have a negative effect on aphids (Thurston et al. 1966). A tobacco introduction, TI 1068, has been shown to be resistant to the tobacco aphid at least partially because it contains antixenotic and antibiotic components that reduce alate establishment (Jackson and Severson 1990).

Resistance mechanisms in aphid-resistant cultivars are not well understood (Campbell & Dreyer 1990). Hilderbrand et al. (1993) reported that aldehydes and alcohols from tomato leaves affected the fecundity of aphids. However, leaf surface

chemicals are not the only factors that affect aphids. Environmental factors may also affect an aphid population. Temperature, light, and population overcrowding are mechanisms that are known to stimulate the production of alate aphids (Shaw 1970). Reed & Semtner (1990) reported that temperature played an important role on the life cycle and fecundity of the tobacco aphid. Environmental factors may also influence the production of leaf surface chemicals. Recent research has shown that leaf surface moisture and levels of relative humidity can significantly enhance the negative effects of sugar esters on tobacco aphids (Xia & Johnson 1997).

Studies in 1994 at the Tobacco Experiment Station (TES), Greeneville, TN, indicated that several lines of burley tobacco were resistant to the tobacco aphid. The goal my study was to investigate the biological mechanisms that affect population growth of the tobacco aphid on resistant tobacco. The parameters evaluated included aphid life cycle, fecundity, reproductive longevity, and survival. Results from these studies were incorporated into a computer generated simulation that was used to predict aphid population growth over time.

Materials and Methods

TOBACCO APHIDS

Red color morph tobacco aphids were collected from a colony maintained on tobacco plants in a greenhouse at The University of Tennessee (UTK), Knoxville, TN. The colony was started with aphids collected from field plants at TES in August 1996 and

reared on potted 'TN 86' tobacco plants at temperatures ranging from 21 to 30° C and a controlled photoperiod of 16:8 (L:D) h. Special care was taken to ensure that field collected aphids were not infected with the fungus *Pandora neoaphidis* that causes epizootics in field populations of the tobacco aphid.

PLANT MATERIALS

Plants used in aphid colony maintenance and laboratory or greenhouse experiments were grown in 7.5 cm pots in a greenhouse at UTK at temperatures ranging from 21 to 30° C under a controlled photoperiod of 16:8 (L:D) h. Excised leaves were collected from field plants which were started in float-beds in a greenhouse at the TES.

OBSERVATION & REARING OF APHIDS

The tobacco aphid life cycle, fecundity, reproductive longevity, and survival, were observed on: 1) excised leaves placed in a insect rearing container, 2) leaf discs embedded in agar in a petri dish, 3) clip-on leaf cages on whole plants. Two neonatal nymphs (<12 h old) were placed onto leaves or leaf discs. The duration of the life cycle was determined by counting exuviae produced until the aphids became adults. Fecundity was determined by counting the number of offspring produced by an aphid over time. Survival time was determined by counting the number of days aphids survived. Reproductive longevity was determined by counting the number of days aphids produced offspring. Exuviae and offspring were counted using a 3X hand lens.

Aphids were reared on excised leaves by the following methods. Leaves were excised, and the base of the petiole was placed into a 9 ml plastic vile (floral water pic) containing 1% solution of Miracle-Gro® tomato fertilizer (18-18-21) secured with a soft rubber cap. The fertilizer solution was replenished during the experiment by inserting a syringe filled with the solution through the rubber cap. The water pic and leaf were placed into a clear round plastic insect rearing container measuring 20.7 cm in diameter X 7.5 cm in depth (Fig. 7).

Clip-on cages were constructed in the following manner. Balsa wood (30 X 30 mm) and plastic tubing (27 mm in diameter X 12 mm in length) were attached to the opposite arms of a metal hair clip using glue. Aphids were placed onto a leaf and cages were placed over them (Fig. 8). The balsa wood served to secure the cage to the leaf with minimum damage to the plant.

Petri dishes (100 X 15 mm) were filled with 1.5% water/agar medium. Four wells, 9 mm in depth, were formed in the agar using a 14 mm cork borer . Leaf discs were extracted from tobacco leaves using a 15 mm cork borer and were embedded at the bottom of the wells using fine tip forceps. After aphids were added onto the leaf discs, a 20 X 20 mm no-thrips insect screen (81 X 81 mesh) was placed over each well to prevent aphids from escaping (Fig. 9).

STATISTICAL ANALYSIS

Aphid data were analyzed using least significant difference for mean separation (P=0.05) (SAS Institute Incorporated 1989).

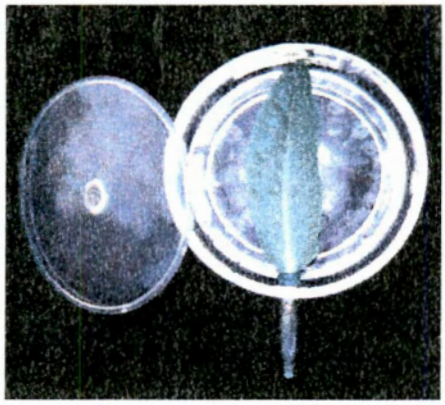

Figure. 7. Insect rearing container containing an excised leaf and water pic.

Figure. 8. Clip-on cage attached to the leaf of a tobacco plant.

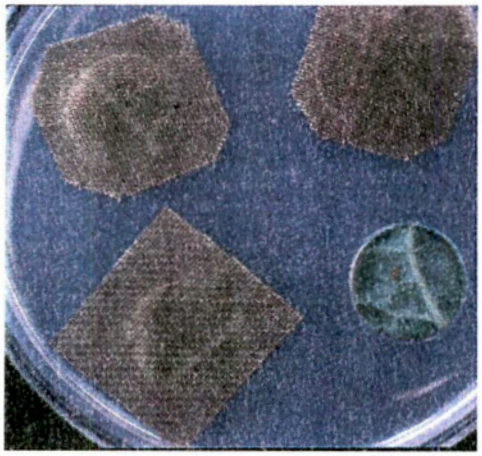

Figure. 9. Tobacco leaf discs embedded in agar.

1996 GROWTH ROOM EXPERIMENT

Preliminary evaluations of the tobacco aphid life cycle, longevity, and fecundity were conducted on excised leaves from TN 86, a non-resistant tobacco cultivar. The experiment was conducted in a growth room at The University of Tennessee, Agricultural Experiment Station, Plant Sciences Unit, Knoxville, TN. The growth room temperature was maintained at 23 ± 2° C with a photoperiod of 16:8 (L:D) h.

One tobacco leaf averaging 14 cm in length was excised from each of 10 plants. One adult aphid was placed onto each leaf. Containers were arranged in a completely randomized design on a table in the growth room. Twenty-four hours after infestation, parental aphids and all but 1 newborn nymph were removed from the leaf. Exuviae were counted daily for 6 d and offspring were counted every 2 d for 13 d starting on the 7th day after infestation (29 October). The experiment was repeated in November. Data were analyzed by calculating the mean number of days nymphs remained immature (development), mean number of nymphs produced per reproductive adult (fecundity) and mean number of days aphids survived (survival).

1997 GROWTH ROOM EXPERIMENT

In May 1997 evaluations were made to determine the tobacco aphid life cycle fecundity, and longevity on excised leaves of resistant and non-resistant tobacco plants. Two tobacco plants, a resistant tobacco line 94-INS-301 (301) and a non-resistant tobacco cultivar (TN 86) were used in the experiment. Similar methods were followed as in the

1996 experiment, except exuviae were counted every 12 h for 7 d starting 24 h after infestation (18 May) and offspring counted daily for 15 d starting 7 d after infestation (25 May).

CLIP-ON CAGE EXPERIMENT

In June 1997, a greenhouse experiment was conducted at UTK to determine the life cycle of the tobacco aphid within clip-on cages on resistant and non-resistant tobacco. The experiment was conducted in a greenhouse at UTK. Four tobacco lines, 2 resistant tobacco lines, 301 & 94-INS-3001 (3001), a resistant tobacco introduction (TI 1068) and a non-resistant tobacco cultivar (TN 86) were tested.

Twenty tobacco plants (5 per treatment) averaging 90 cm in height were completely randomized on a greenhouse table. Two cages were used per plant for a total of 10 replicates of each treatment. The clip-on insect cages were placed over the 2 aphids. Twenty-four hours later the parental aphids and all but 1 newborn nymph were removed from the cage. Exuviae were then counted every 12 h for 6 d starting 24 h after infestation (12 June). Experiment was repeated a month later using the same plants.

PETRI DISH EXPERIMENT

In June 1997, an experiment was conducted to determine the tobacco aphid life cycle on leaf discs from 4 tobacco entries, two resistant tobacco lines (301 & 3001) a resistant tobacco introduction (TI 1068) and a non-resistant tobacco cultivar (TN 86)

were tested. The experiment was conducted in a growth chamber at UTK with a temperature maintained at 22 ± 2° C and a photoperiod of 14:10 (L:D) h.

On 20 June, ten 15 mm leaf disks (per line) were removed from tobacco leaves and embedded into the agar. Two adult apterous aphids were added per leaf disc. Ten petri dishes (replicates), each containing 1 leaf disc from each line, were placed inside a growth chamber in a randomized complete block design. Twenty-four hours later, all aphids except 1 newborn nymph were removed. Exuviae were then counted every 12 h for 6 d starting 24 h later (22 June). The experiment was repeated a using discs from the same plants.

EXPERIMENTS ON EXCISED LEAVES FROM FIELD & GREENHOUSE-GROWN PLANTS

Experiments were conducted to determine the tobacco aphid life cycle, fecundity, reproductive longevity, and survival on excised field and greenhouse leaves from a resistant tobacco introduction (TI 1068) and a non-resistant tobacco cultivar (TN 86). The experiment was conducted in a growth chamber at UTK. Temperature was maintained at 22 ± 2° C, and the photoperiod was 14:10 (L:D) h. Methods for both experiments were similar to those used as in the 1996 growth room experiment.

On 19 August, 20 leaves were randomly chosen and excised from mature field-grown plants at TES. Care was taken to ensure that leaves were healthy and did not contain aphids or other insects. Water pics with the tobacco leaves were placed onto a rack and placed inside cooler, and transported to a growth chamber at UTK. Exuviae

were counted every 12 h for 8 d starting 24 h after removal of parental aphids as described above, and offspring were counted daily for 15 d starting 7 d after infestation (27 August). The experiment was repeated in September. In March of 1997, leaves were excised from greenhouse grown plants that averaged 35 cm in height. Exuviae were counted every 12 h for 6 d and offspring counted daily for 13 d. The experiment was repeated in April.

ESTIMATE OF FIELD APHID POPULATION BY COMPUTER SIMULATION

A deterministic simulation program (Fig. 10) using SAS (1989) was developed by Dr. Arnold Saxton, a statistician at UTK. Four variables (life cycle, fecundity, reproductive longevity, and survival) were used to estimate aphid populations duration over time on field-grown tobacco, using values generated from leaves excised from TN 86 and TI 1068 plants grown in the field. The estimated values were the mean number of hours an aphid remained immature (immhr), the mean number of hours it remained sexually mature (mathr), the mean number of offspring it produced during the mean number of days is remained reproductive (repro), and the percentage of immature aphids that survived to maturity (surv). The simulation was based on the assumption that if the progeny from 1 newborn aphid colonized a plant for 40 d in the absence of predators, parasitoids, pathogens and other limiting factors.

```
options ls=75 ps=50 mprint;
%macro popgrow (dset);
data _null_;
 xx=floor(&immhr+&mathr);
 xxx=compress(xx);
 call symput('lifhr',xxx);
run;
***let lifhr=%eval(&immhr + &mathr);
data &dset;
adult1=&immhr+1;
*** This array ppp contains the number of organisms of
    each age present;
array ppp age1-age&lifhr;
*** Initialize to 1 adult;
do ii=1 to &lifhr; ppp[ii]=0; end;
ppp[adult1]=1;
*** follow population through time;
xx=24*&runday;
do time=1 to xx;
  *** count all adults to get age 1 offspring (newgrp);
  nadults=0;
  do ii=adult1 to &lifhr; nadults=nadults+ppp[ii]; end;
  newgrp=&repro*nadults;
*** age everybody by one period;
  do ii=&lifhr to 2 by -1;  ppp[ii]=ppp[ii-1];  end;
  ppp[1]=newgrp;
****immatures don''t all survive to adults;
  ppp[adult1]= ppp[adult1]*&surv;
**** count all aphids, imm or adult;
  naphids=0;
  do ii=1 to &lifhr;
     naphids=naphids+ppp[ii];
   end;
  output;
end;
run;
%mend;
************************************************************
**  User enter values:                                    *
**  immhr - number of hours of immaturity                 *
**  mathr - number of hours of sexual maturity            *
**  repro - number of offspring per time unit (hours)     *
**  surv  - fraction of juveniles that reach maturity     *
**  runday- number of days simulation should run through
************************************************************;
```

Figure. 10. Program used to predict field aphid populations based on biological mechanisms of resistance of tobacco to the tobacco aphid.

```
***** Population 1 values ***********;
%let immhr =132;
%let mathr =199;
%let repro =.1693;
%let surv  =.9;
%let runday=40;
****************************************;
%popgrow(one);
***** Population 2 values ***********;
%let immhr =171;
%let mathr =91;
%let repro =.0724;
%let surv  =.5;
%let runday=40;
****************************************;
%popgrow(two);
*** following prints raw data and summary plots ***;
data one; set one;
 pop=1;
run;
data two; set two;
 pop=2;
run;
data final; set one two;
 ladult=log10(nadults+1);
 laphids=log10(naphids+1);
 time=time/24;
run;
proc plot;
 title2 'Number of Adults';
 plot nadults*time=pop ladult*time=pop;
proc plot;
 title2 'Number of Adults PLUS Juveniles';
 plot naphids*time=pop laphids*time=pop;
run;
proc gplot;
 symbol1 i=spline l=1 w=3;
 symbol2 i=spline l=3 w=3;
 axis1 w=3 minor=none;
 axis2 w=3 minor=none;
 title2 'Number of Adults';
 legend1 frame;
 plot nadults*time=pop ladult*time=pop/vaxis=axis1 haxis=axis2 frame
                   legend=legend1;proc gplot;
 title2 'Number of Adults PLUS Juveniles';
 plot naphids*time=pop laphids*time=pop/vaxis=axis1 haxis=axis2
frame
                   legend=legend1;
run;
```

Figure. 10. Continued.

Results and Discussion

1996 GROWTH ROOM EXPERIMENT

Preliminary evaluations in the growth room confirmed that under those conditions the tobacco aphid has a relatively short life cycle and high fecundity on TN 86 tobacco. Adults produced 1st instars within 24 h after being placed onto plants. Within 24 h after birth, 80% of nymphs developed into 2nd instars. All nymphs reached the 3rd instar within 72 h after birth (Fig. 11). After 144 h (6 d), 80% of all aphids had become adults. A possible explanation why 100% did not develop into adults within 144 h could be slight differences in time of birth. Mean development time (birth to adult) for all aphids was 132 h (7 d) (Fig. 11). All aphids produced offspring within 24 h after developing into adults. Aphids produced the maximum daily number of offspring on the 6th and 8th day of maturity, at which time they had produced an average of 5.5 aphids per day (Fig. 12). All aphids ceased production of offspring by the 14th day. The mean number of offspring per adult was 59. The reproductive rate was 4.2 aphids per adult per day. Aphids survived an average of 22 d. Repeated experiments in November produced similar results.

1997 GROWTH ROOM EXPERIMENT

Aphids on both entries (TN 86 & 301) developed into 2nd instars within 36 h. Maximum development time for aphids on both entries was 144 h (6 d) (Fig. 13). Aphids on both entries produced offspring within 24 h after becoming adults. Increase in aphid

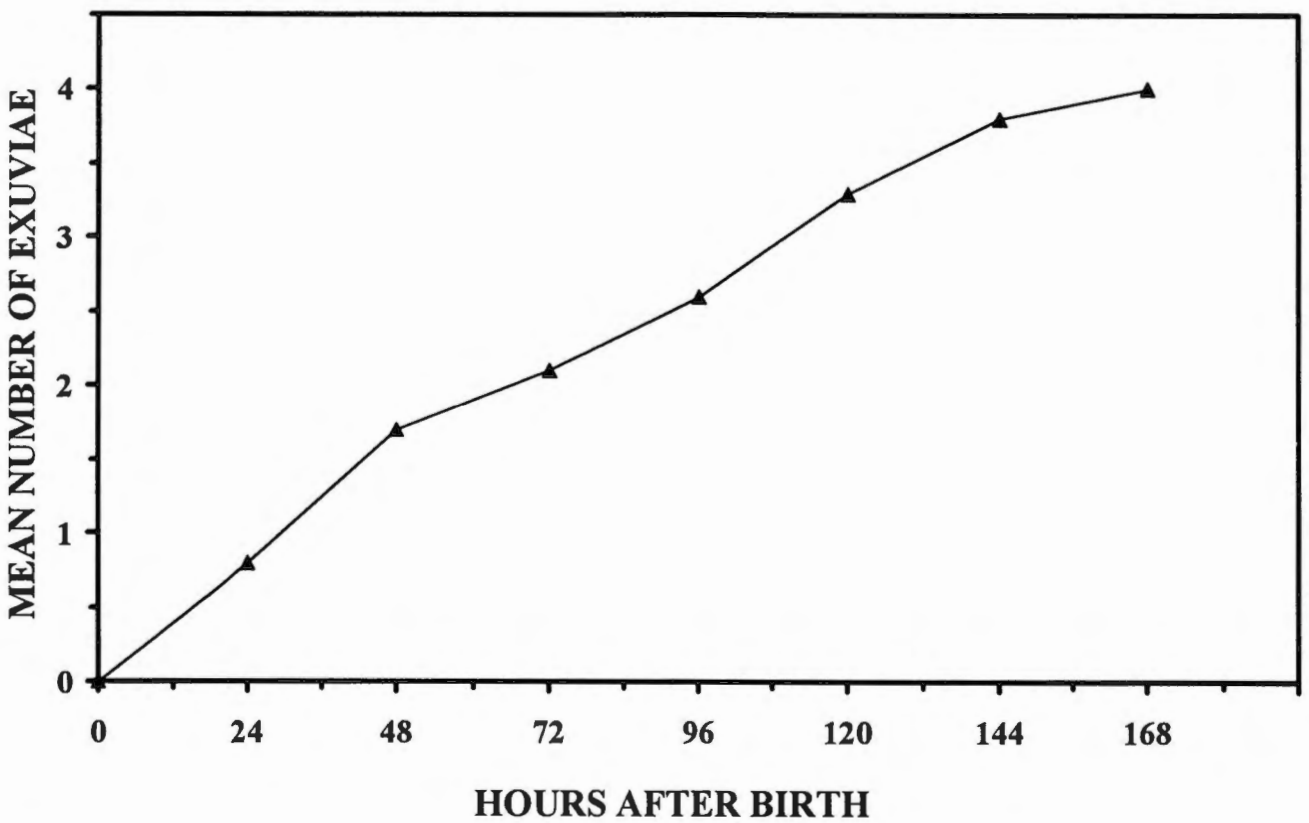

Figure. 11. Life cycle of tobacco aphid on excised leaf of TN 86, October 1996.

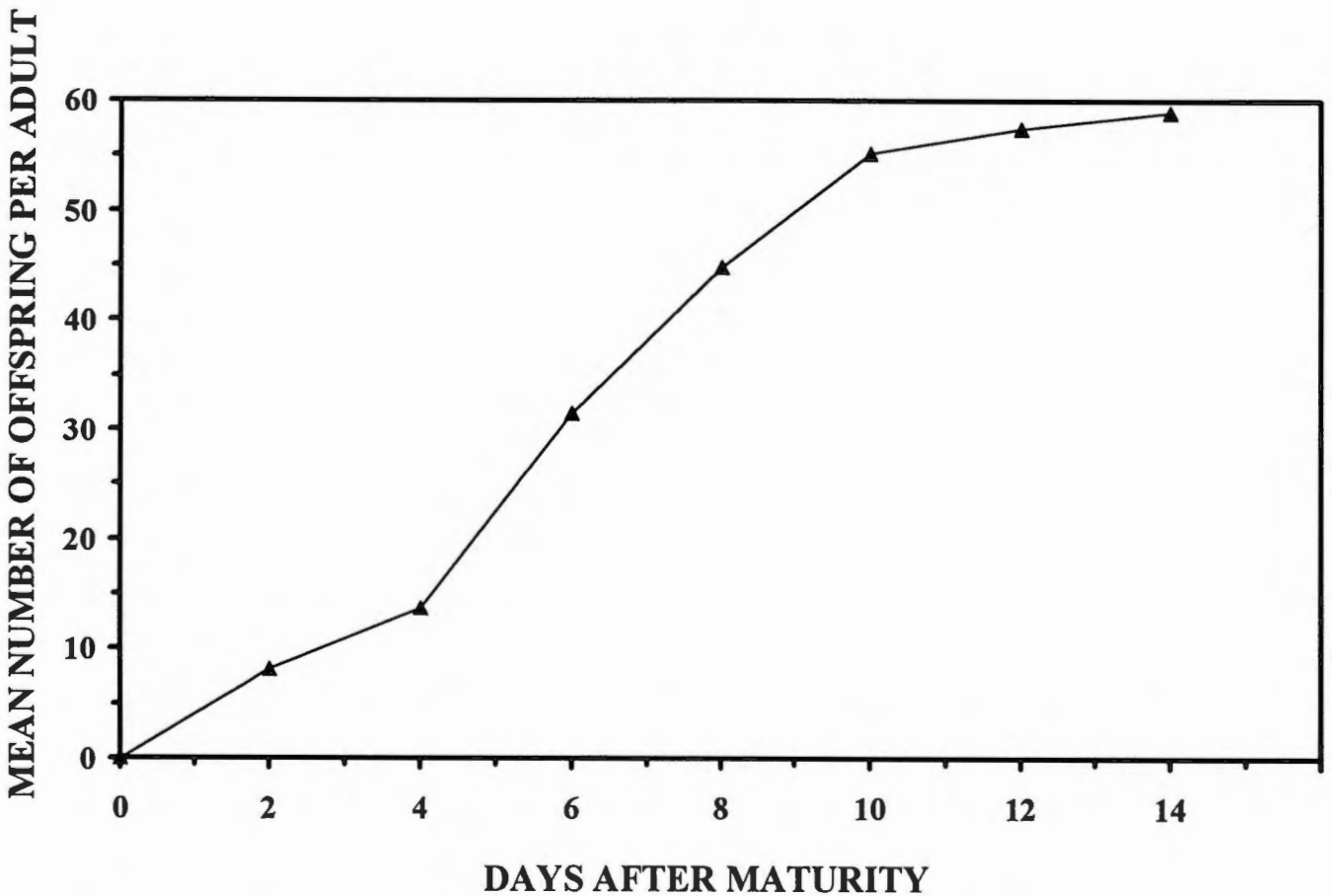

Figure. 12. Fecundity of tobacco aphid on excised leaf of TN 86, Novermber 1996.

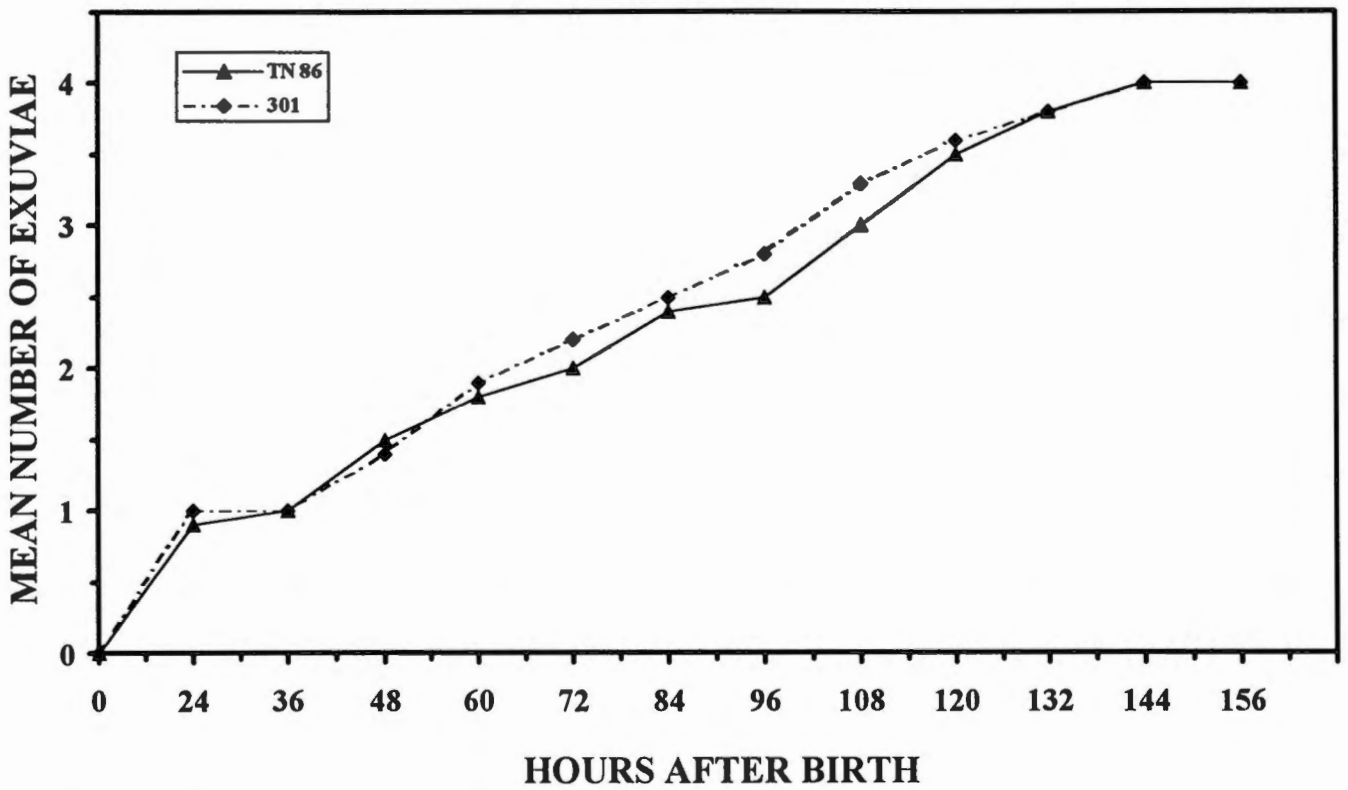

Figure. 13. Life cycle of the tobacco aphid on excised leaves of resistant (301) and non-resistant (TN 86) tobacco grown in a greenhouse, May 1997.

reproductive rate on both entries was observed by the 4th day of maturity (Fig. 14). Both entries reached maximum number of daily offspring on the 6th day of maturity. All aphids on both entries ceased producing offspring by the 12th day of maturity. The mean number of offspring for both tobacco entries was approximately 50 aphids per adult. The reproductive rate on both tobacco entries was 4.2 aphids per adult per day. No significant differences ($P>0.05$) in aphid development or fecundity were observed. Aphids on both entries survived an average of 21 d. No significant differences ($P>0.05$) in survival time were observed. Aphids on resistant (301) plants survived and reproduced at the same rate as those on TN 86. Lack of differences in the life cycle, fecundity, and survival for aphids TN 86 and 94-INS-301, may have been due to the amount of leaf surface exudates produced. Although excised leaves from older plants were used, the concentration of exudates on 301 plants was probably too low to cause any negative effects on an aphid. All of the plants were maintained in the growth room, and therefore environmental conditions inside the growth room may have played a role in the production of exudates on 301 plants. Chemical analyses of the leaf surface exudates of plants grown under these conditions has not been conducted, so the amount of exudates produced by each entry is unknown.

CLIP-ON CAGE EXPERIMENT

Within 36 h from birth, 90% of aphids on all tobacco entries had developed to the 2nd instar. After 108 h, aphids on all entries were either 3rd and 4th instars. (Fig. 15). All aphids on TN 86, TI 1068, and 301 became adults by 168 h (7d); whereas, all aphids

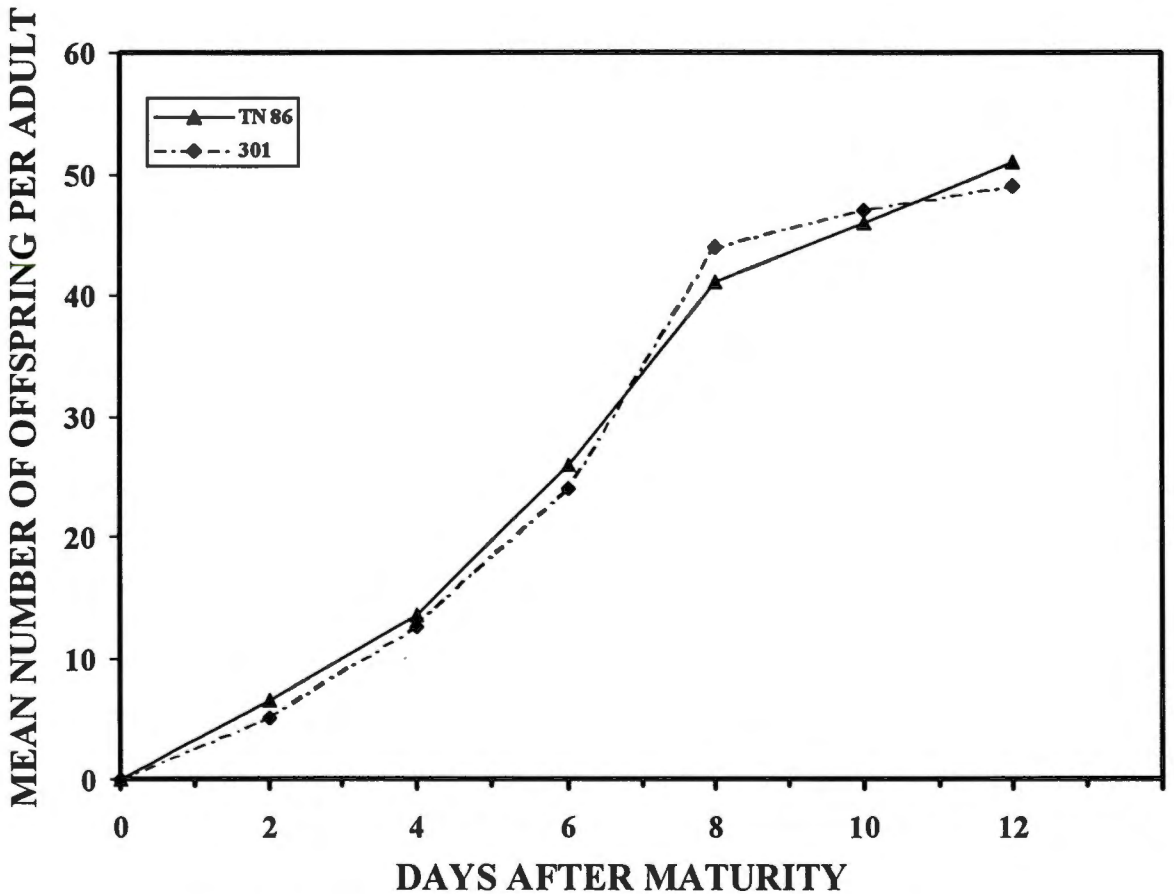

Figure. 14. Fecundity of the tobacco aphid on excised leaves of resistant (301) and non-resistant (TN 86) tobacco grown in a greenhouse, May 1997.

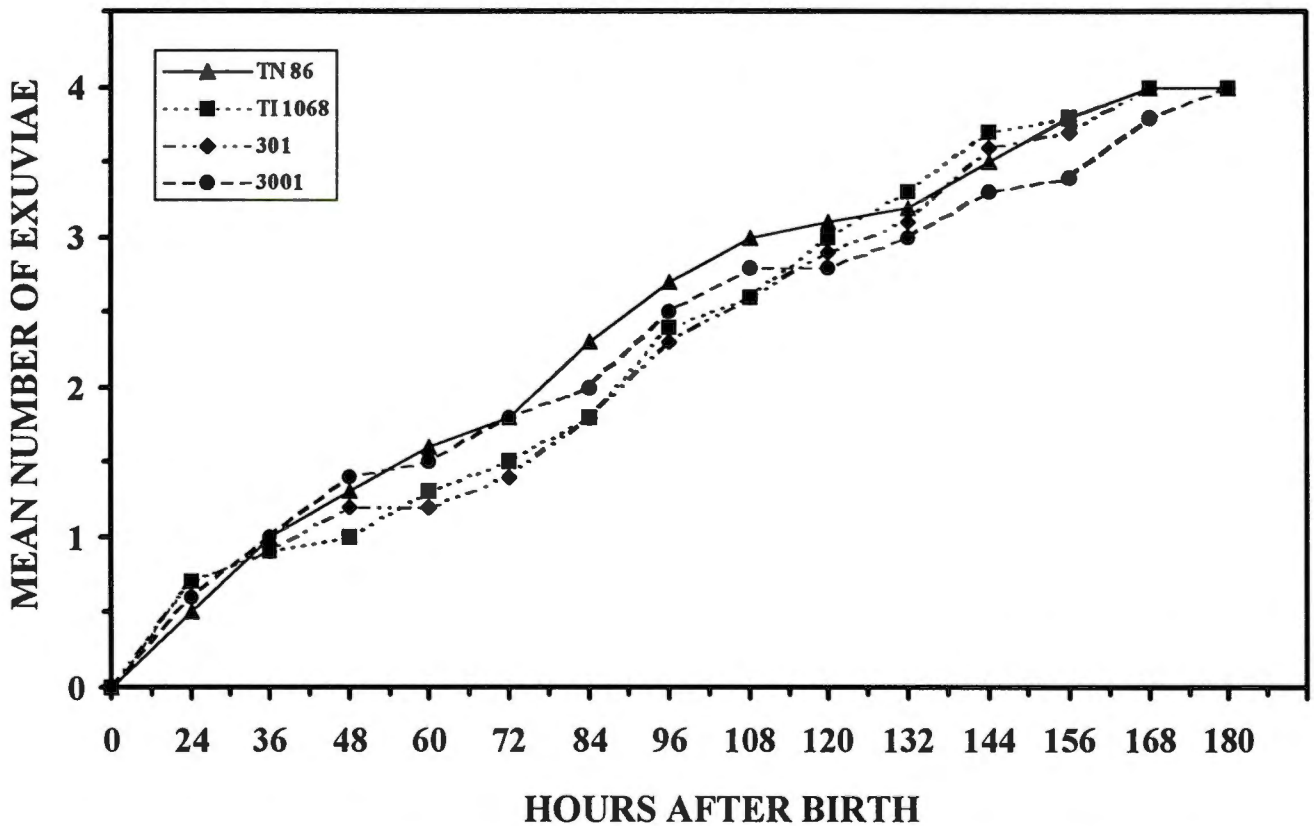

Figure. 15. Life cycle of the tobacco aphid on resistant (TI 1068, 301, 3001) and non-resistant tobacco (TN 86) grown in a greenhouse, June 1997

on 3001 had developed to adults by 180 h. No significant differences in aphid development time ($P>0.05$) on all the tobacco entries were observed. Repeated experiments in July produced similar results. It is suspected that TI 1068, 301, and 3001 tobacco plants, were not producing adequate amounts of sucrose esters to affect the life cycle of the tobacco aphid. Therefore, aphids on TI 1068, 301, and 3001 would complete their life cycle in the same amount of time as those aphids on TN 86. Environmental changes such as temperature and humidity are believed to be factors affecting the concentration of sucrose esters on leaves (Xia & Johnson 1997).

PETRI DISH EXPERIMENT

Within 24 h from birth, 90% of aphids on TN 86 leaf discs had developed to the 2nd instar, but less than 50% on TI 1068, 301, and 3001 had reached 2nd instar (Fig. 16). After 108 h, some aphids on TI 1068 had failed to reach the 2nd instar. Maximum developmental time to the 2nd instar ranged from 36 h for aphids on TN 86 to 132 h for aphids on TI 1068. All adults developed on TN 86 after 144 h (6 d), while mostly 4th instars were observed on 301, mostly 3rd instars on 3001, and mostly 2nd instars on TI 1068. On the last day of the experiment, 29 June (156 h after birth), significant differences ($P<0.05$) in development among all entries were determined (Fig. 16). Repeated experiments in July produced similar results.

Development time for aphids on 301, 3001, and TI 1068 was probably influenced by toxic exudates emitted from the leaf discs. In contrast, in other experiments in which excised leaves or whole plants from 301, 3001, and TI 1068 were used, those lines had

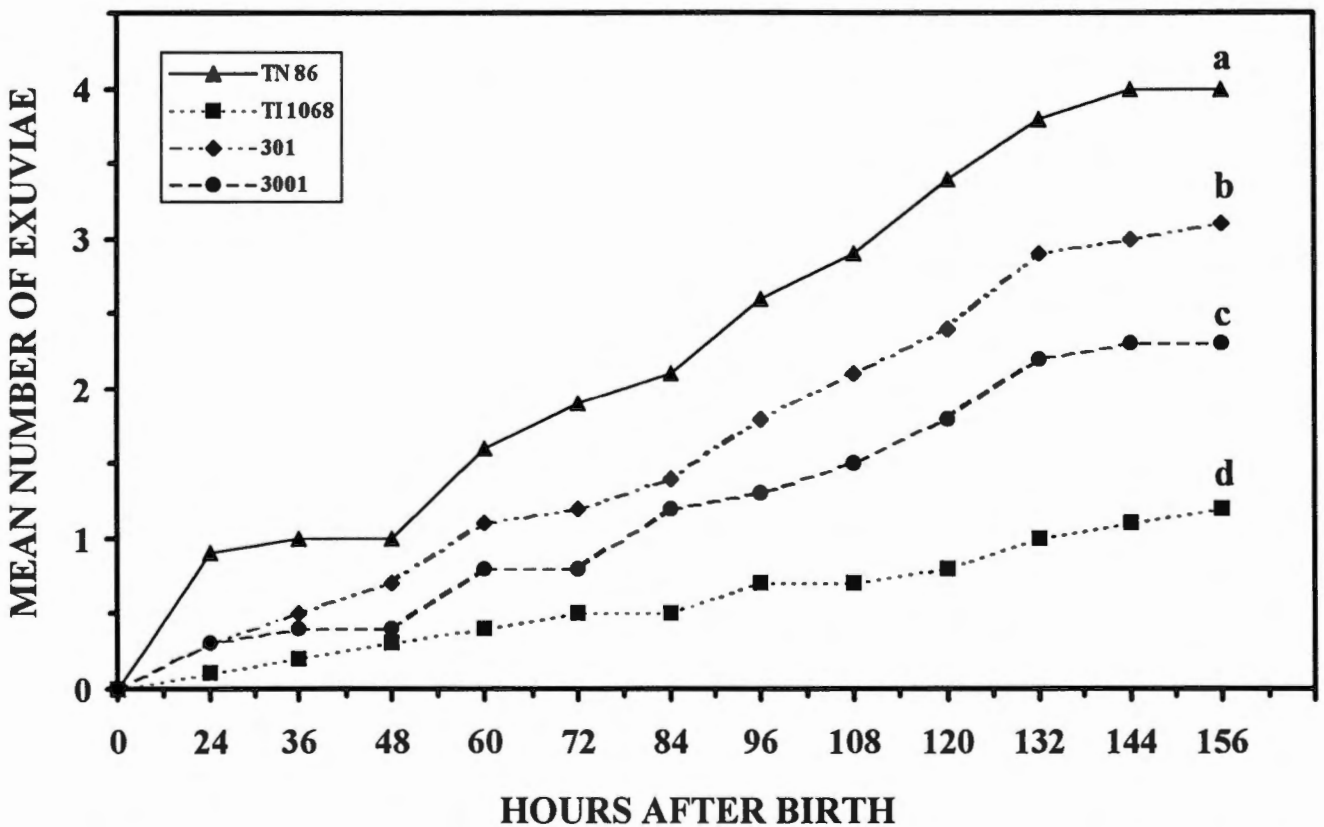

Figure. 16. Life cycle of the tobacco aphid on resistant and non-resistant tobacco leaf discs from plants grown in a greenhouse, June 1997. Means with the same letter are not significantly different at 156 hours (P=0.05; Least Significant Difference) [SAS Institute 1989].

little or no effect on aphid development when compared to the control (TN 86). One possible explanation for the different results between experiments dealing with leaf discs, excised leaves, and whole plant experiments was the unique environments to which leaves or leaf discs were exposed. Temperature and humidity might have affected the amount of exudates produced in the closed environments. Recent research has shown that the toxicity of sugar esters to tobacco aphids is enhanced under high relative humidity or moist conditions (Xia & Johnson 1997). Hence, a moist leaf surface inside a petri dish with agar, would probably have a higher relative humidity than that of a whole plant in a greenhouse or an excised leaf in a container.

EXCISED LEAVES FROM FIELD-GROWN PLANTS

Aphids on both tobacco entries completed the 2nd instar within 48 h after birth (Fig. 17). The life cycle was very similar for aphids on both tobacco entries until 84 h. Aphids on TI 1068 developed very slowly between 84 and 120 h. By 108 h, aphids on TN 86 developed into 4th instars, while only 3rd instars were observed on TI 1068. After 156 h, 100% of aphids on TN 86 had developed into adults, while only 20% of aphids had reached maturity on TI 1068. Mean development time (birth to adult) ranged from 132 h (5.5 d) for aphids on TN 86 to 171 h (7.1 d) for aphids on TI 1068. Aphid development was significantly faster ($P<0.05$) on TN 86 than on TI 1068 (Fig. 18).

Aphids on TN 86 produced progeny within 24 h from developing into adults. On the 2nd day, 85% of aphids on TN 86 were producing offspring, while only 10% of aphids on TI 1068 were reproductive. Maximum rate of reproduction was reached

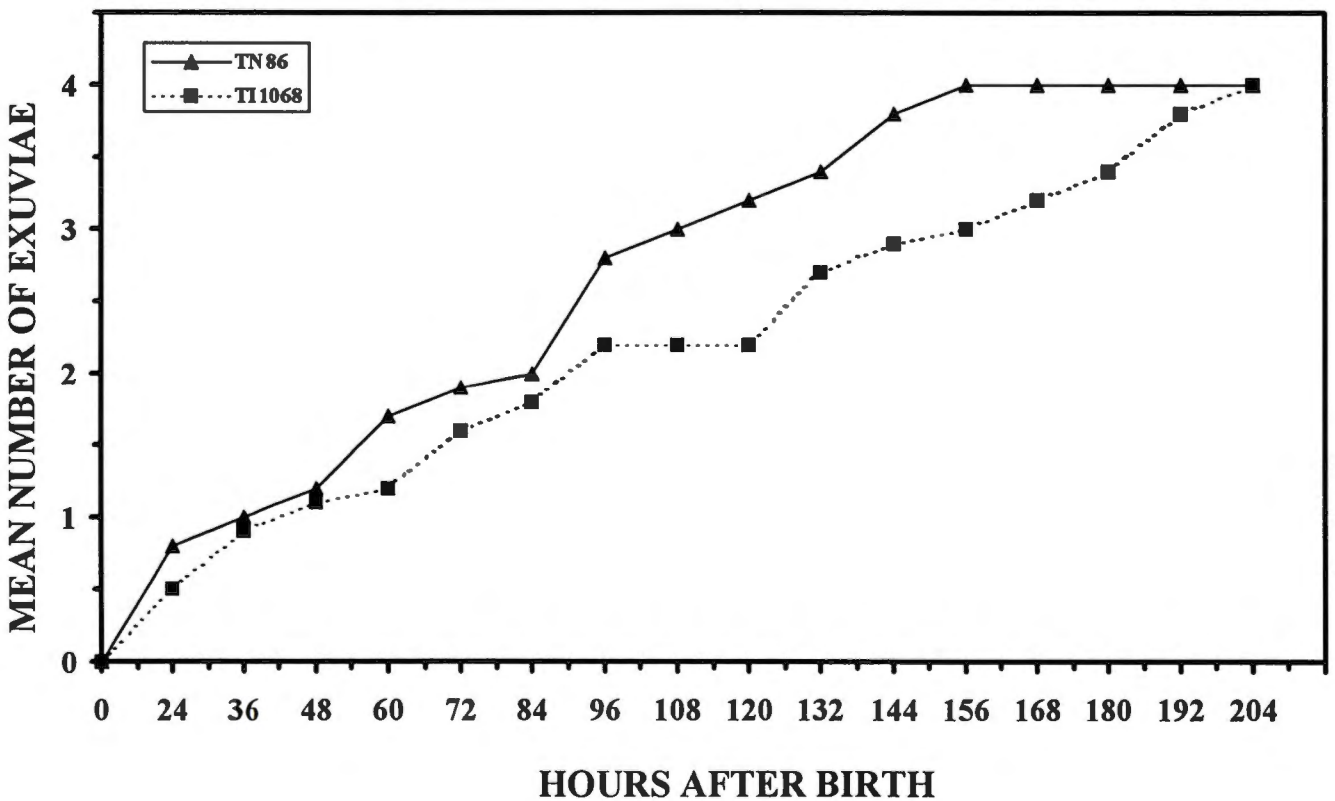

Figure. 17. Life cycle of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from the field, September 1997.

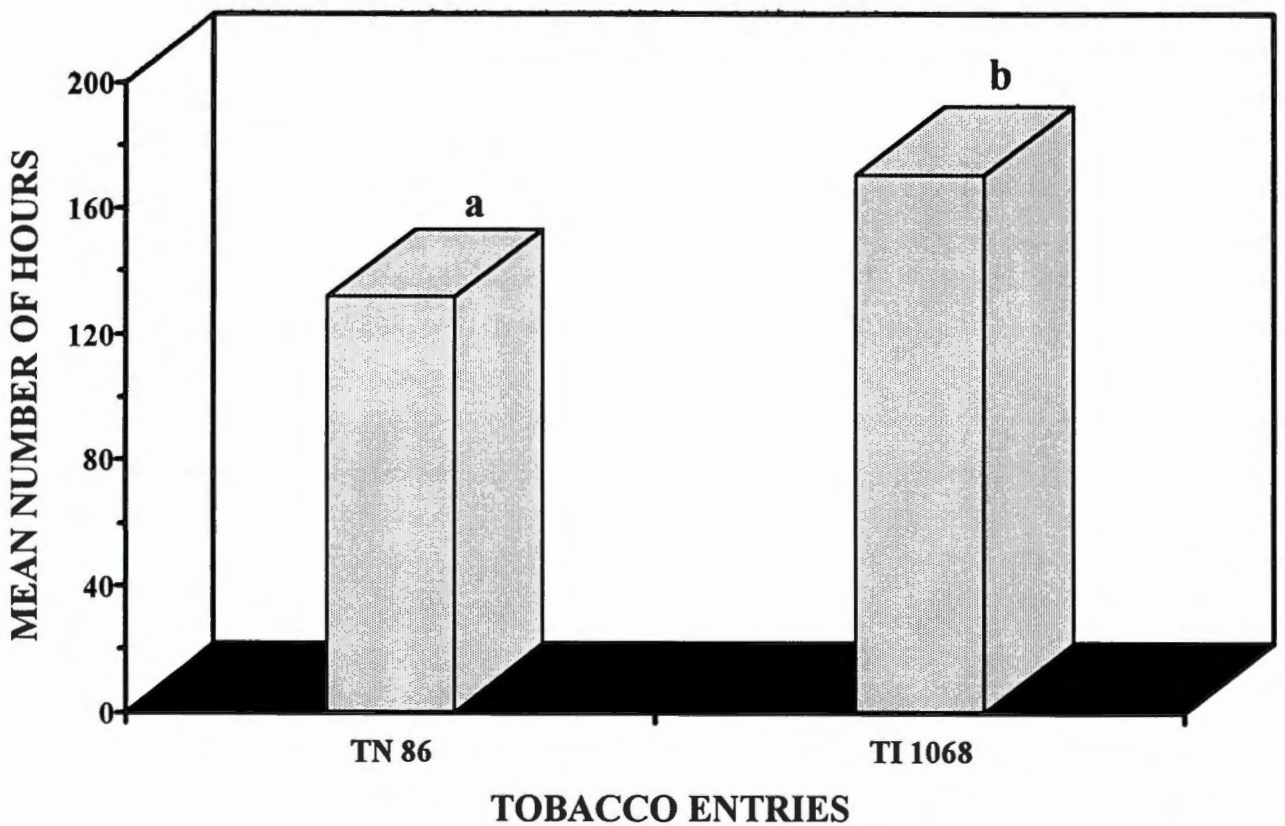

Figure. 18. Time for aphid development, from birth to adult, on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997. Means with the same letter are not significantly different (P=0.05; Least Significant Difference) [SAS Institute 1989].

between the 6th and 10th day of maturity for aphids on TN 86. Fourteen days after reaching maturity, all aphids on both entries had ceased producing progeny (Fig. 19). The reproductive rate for aphids on TI 1068 averaged 1.7 nymphs per adult per day, while on TN 86 the average was 4.0 aphids per adult per day. Significantly higher numbers of aphids ($P<0.05$) were produced on TN 86 than on TI 1068 (Fig. 20). Aphids on TN 86 were reproductive for a significantly longer ($P<0.05$) period of time (8.3 d) than aphids on TI 1068 (3.8 d) (Fig. 21). Only 50% of aphids on TI 1068 survived to the adult stage; whereas, 90% of aphids on TN 86 survived to the adult stage. Significantly fewer aphids survived ($P<0.05$) on TI 1068 than aphids on TN 86 (Fig. 22). Repeated experiments in September produced similar results (Appendix B).

EXCISED LEAVES FROM GREENHOUSE-GROWN PLANTS

Within 72 h after birth, all aphids developed to the 3rd instar on TN 86 and TI 1068 (Fig. 23). Aphids on TN 86 and TI 1068 developed at the same rate until adults. Aphids on TN 86 and TI 1068 produced offspring within 24 h after reaching maturity (Fig. 24). Aphids on both tobacco entries produced maximum daily number of offspring between the 6th and 8th day of maturity. After the 8th day of maturity, the reproductive rate decreased for aphids on both tobacco entries. Fourteen days after reaching maturity, all aphids on both entries had ceased to produce progeny (Fig. 24). The reproductive rate for aphids on TI 1068 was 3.8 nymphs per day, while TN 86 averaged 4 nymphs per day. There were no significant differences ($P>0.05$) in development time or fecundity between aphids on TN 86 and TI 1068. Aphids on both entries survived an average of 23

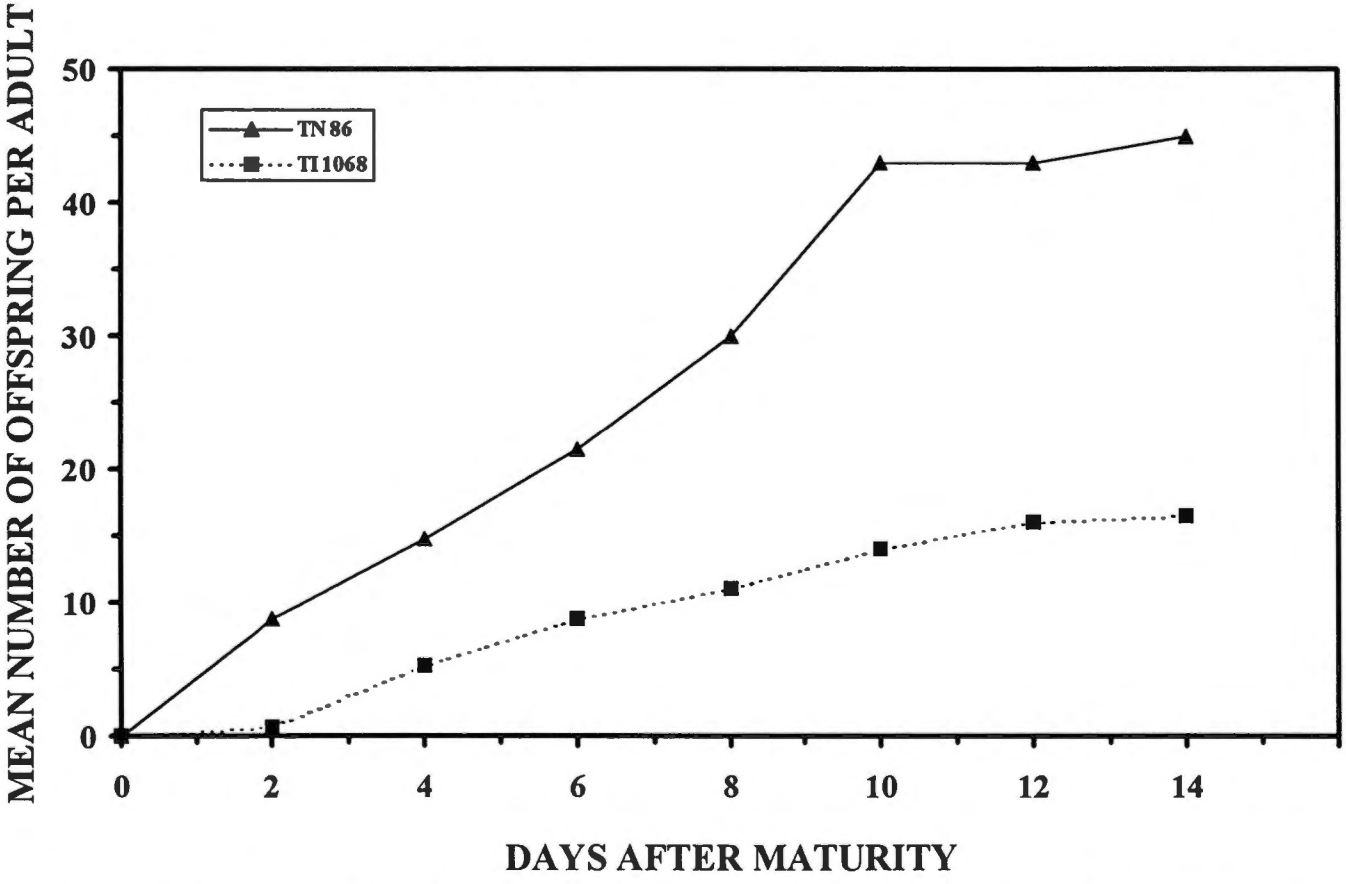

Figure. 19. Fecundity of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997.

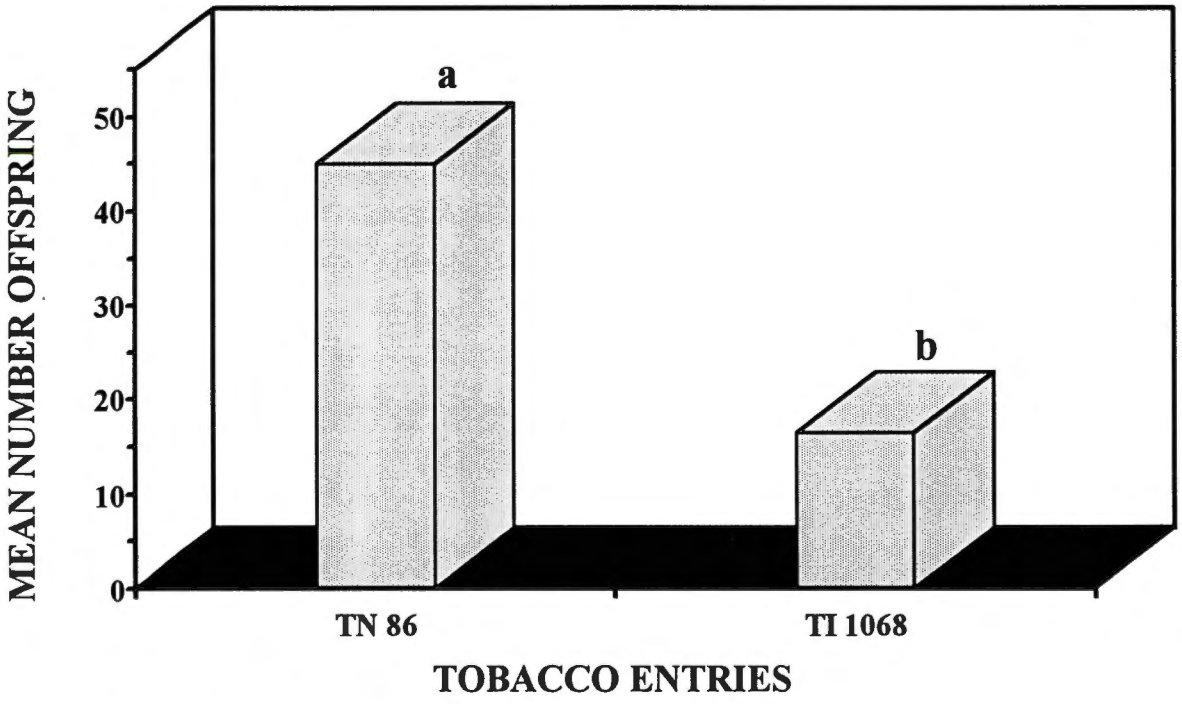

Figure. 20. Number of progeny per female aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco from the field, September 1997. Means with the same letter are not significantly different (P=0.05; Least Significant Difference) [SAS Institute 1989].

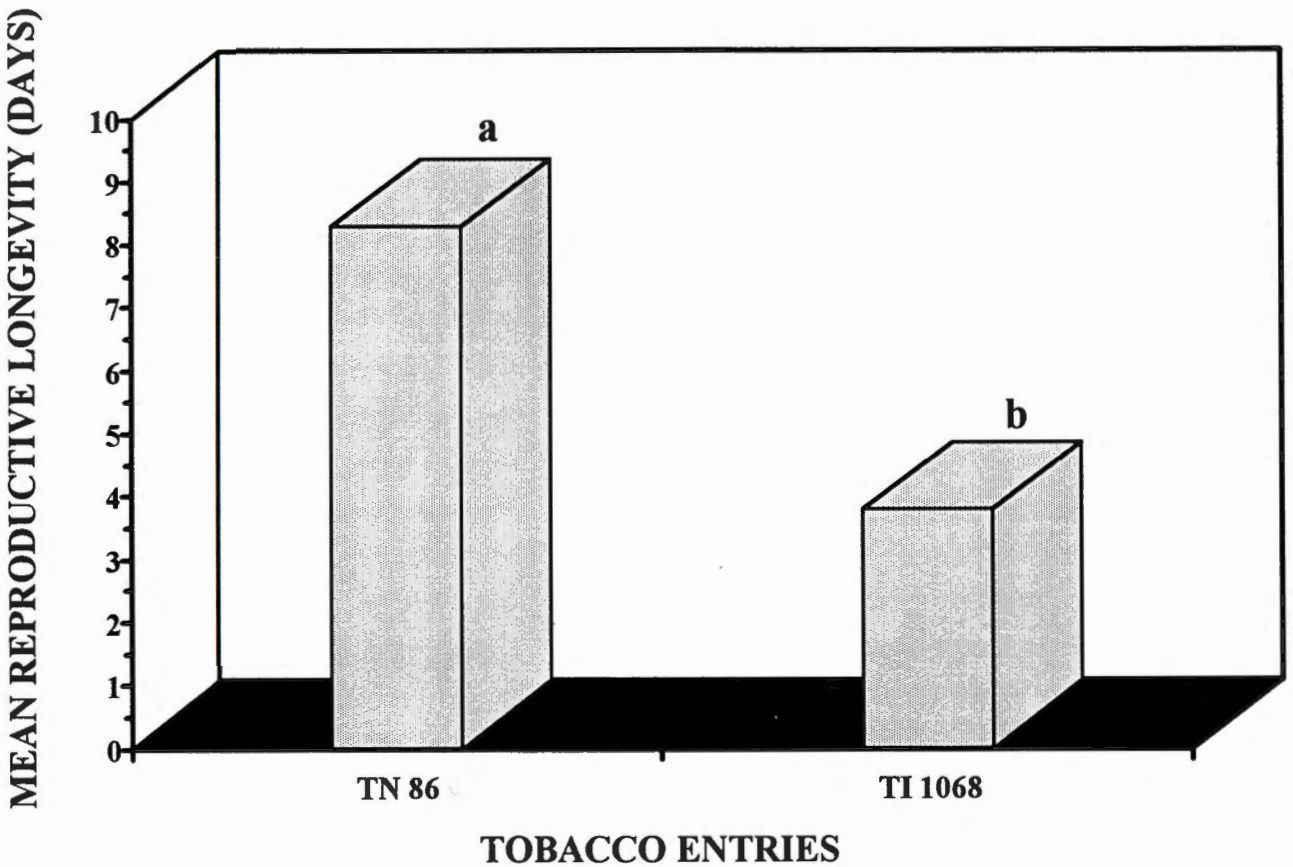

Figure. 21. Reproductive longevity of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from field, September 1997. Means withthe same letter are not significantly different (P=0.05; Least Significant Difference) [SAS Institute 1989].

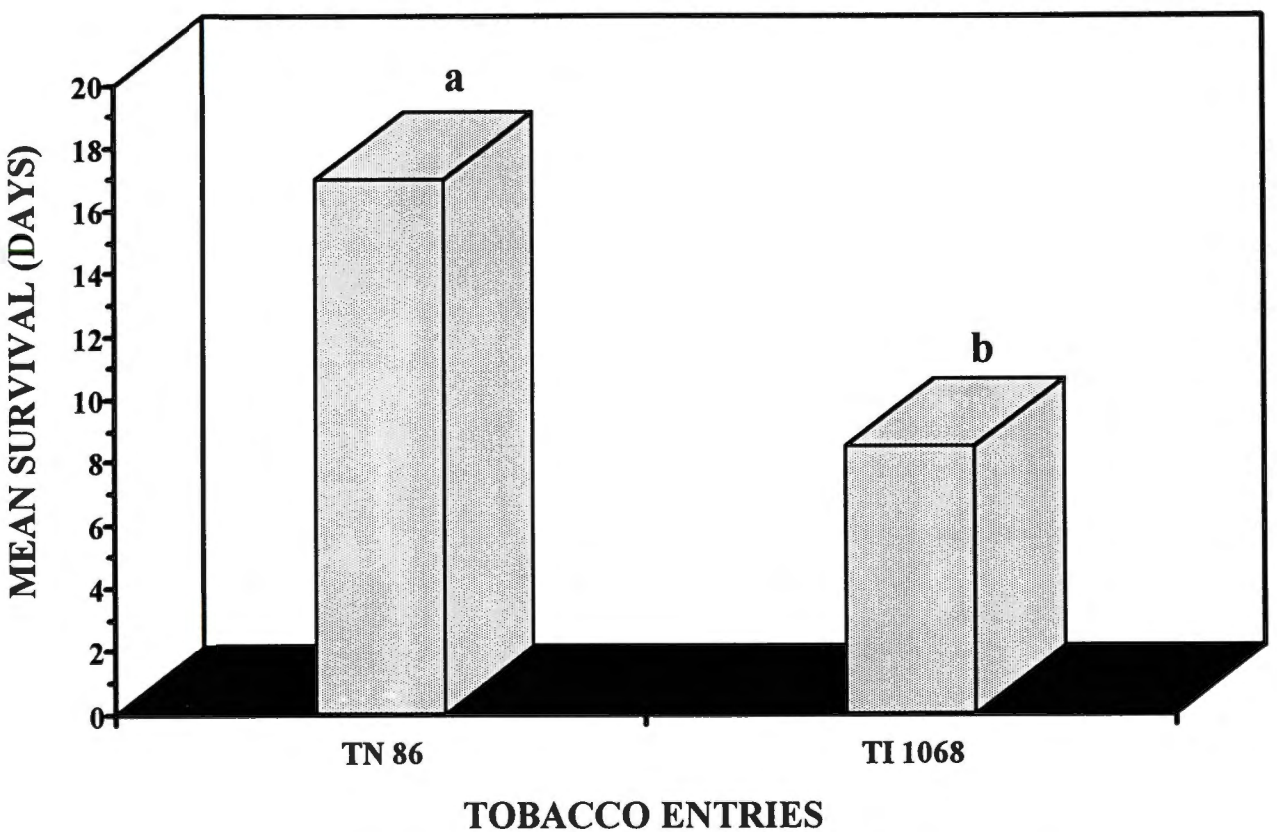

Figure. 22. Survival of the tobacco aphid on resistant (TI 1068) and non-resistant (TN 86) excised tobacco leaves from field, September 1997. Means with the same letter are not significantly different (P=0.05; Least Significant Difference)[SAS Institute 1989].

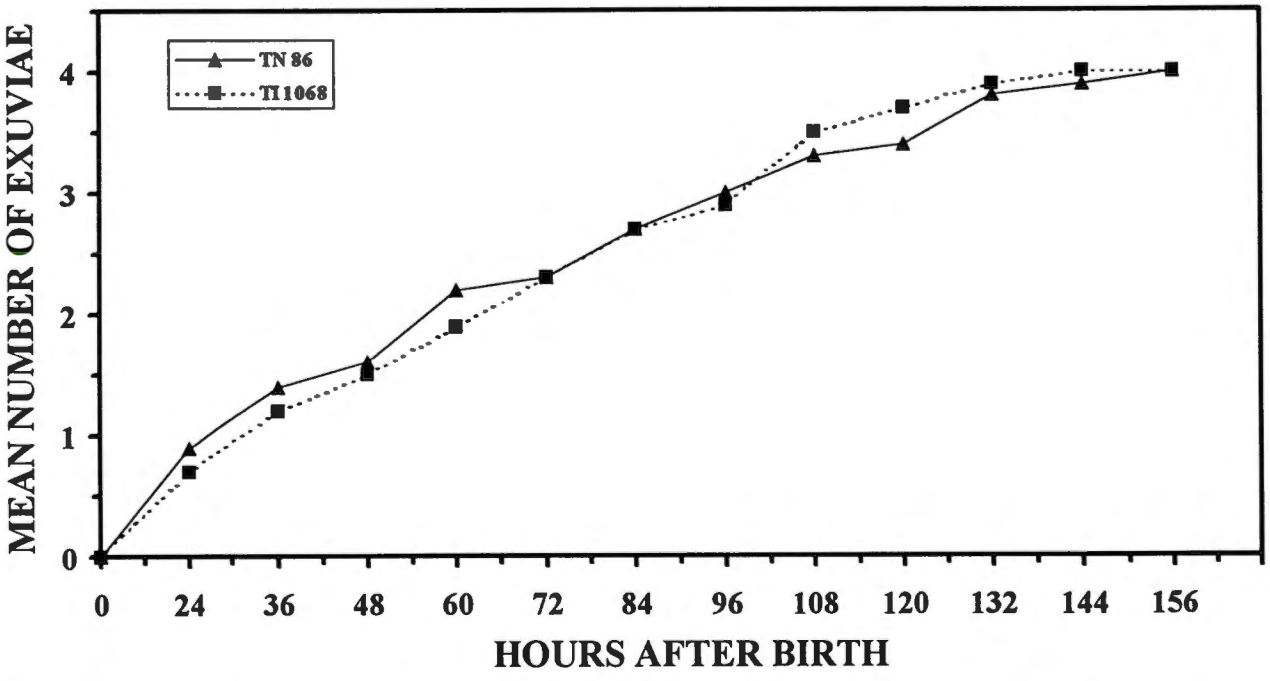

Figure. 23. Life cycle of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco grown in a greenhouse, March 1998.

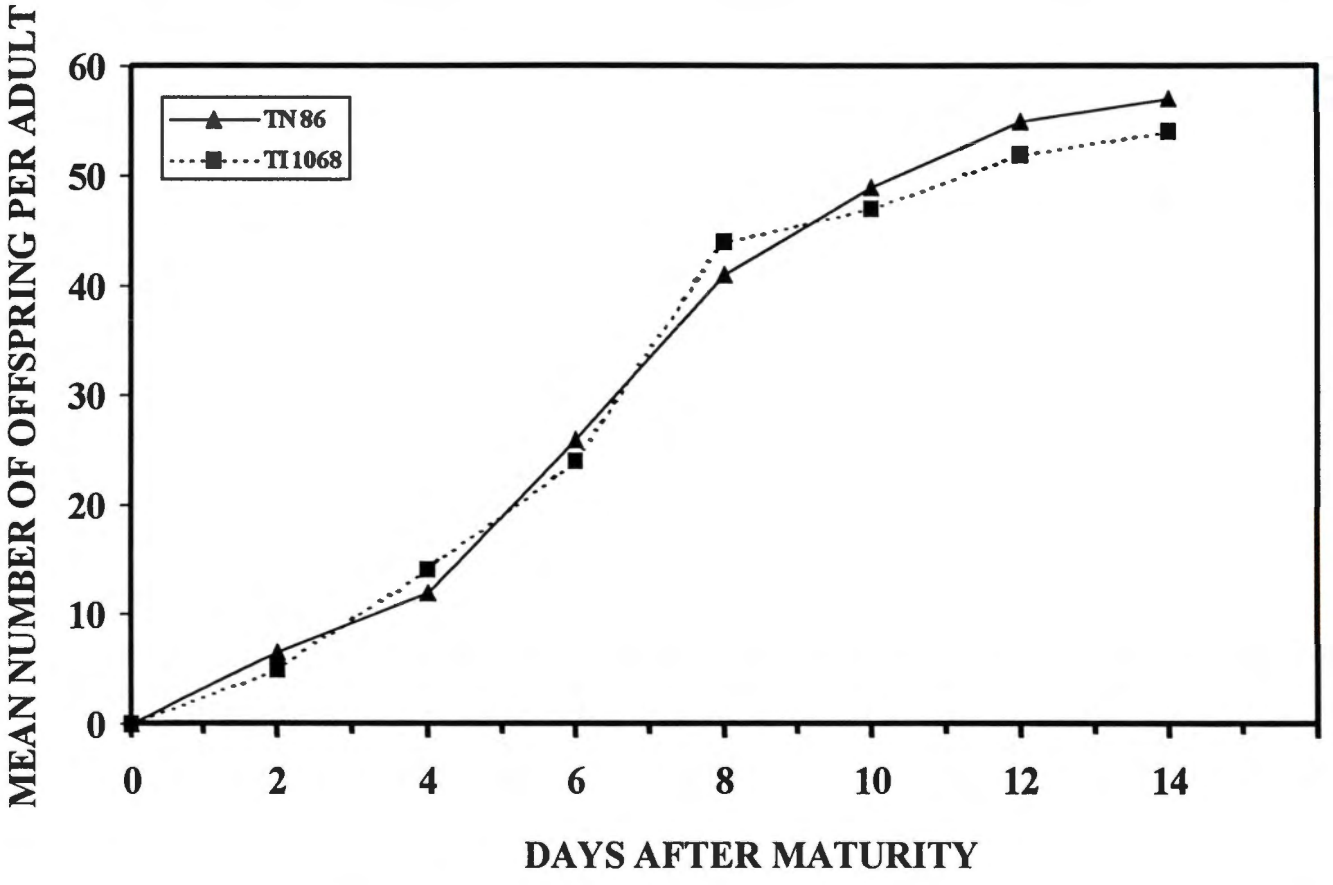

Figure. 24. Fecundity of the tobacco aphid on excised leaves of resistant (TI 1068) and non-resistant (TN 86) tobacco grown in a greenhouse, March 1998.

d. No significant differences ($P>0.05$) in reproductive longevity or aphid survival were observed among aphids on TN 86 and TI 1068. Repeated experiments in April produced similar results.

These experiments confirmed that environmental conditions might play a role in the varying degrees of resistance of tobacco plants to the tobacco aphid. Results from field experiments indicated that development time, fecundity, reproductive longevity, and survival of aphids were negatively affected by TI 1068 plants. An explanation for the lack of differences in the aphid life cycle, fecundity, reproductive longevity and survival on leaves from greenhouse-grown plants may have been the small amount of leaf surface exudates produced under greenhouse conditions. Excised leaves from the field were extracted from plants older than those from the greenhouse. Lower amounts of sucrose esters on greenhouse-grown plants might have been the reason that aphids on TI 1068 were able to develop, reproduce, and survive as those on TN 86. Abernathy & Thurston (1969) determined that as tobacco plants matures an increase in the level of leaf exudates is expected. Higher temperatures in the field might have also increased the amount of exudates produced by TI 1068 plants. There were no chemical analyses of the leaf surfaces of field-grown or greenhouse-grown plants used in my experiments, so the amount of exudates produced by each plant is unknown. Variability in the expression of resistance under different environmental conditions could be a reason that resistance screening with greenhouse-grown plants often produces different results than when field-grown plants are used.

ESTIMATE OF FIELD APHID POPULATION BY COMPUTER SIMULATION

Population 1 designated aphids on TN 86, while aphids on TI 1068 were designated as population 2. Parameters used in this experiment are presented in (Table. 2). According to the simulation 10 adult aphids would appear on TN 86 by the 7th d, while no adult aphids would appear on TI 1068 until the 12th d (Fig. 25). Approximately 100,000 aphids would accumulate on TN 86 by the 33rd d, disregarding migration and other limiting factors such as predators, parasitoids, overcrowding, and other environmental factors. By 40th d, approximately 1,000,000 adult aphids would have accumulated on TN 86, while 40 aphids would occur on TI 1068 (Fig. 25). After adult aphids appeared, approximately 10 immature aphids would appear on TN 86 by the 4th d (Fig. 26). On the 40th d, 10,000,000 would have accumulated on TN 86, while only about 600 aphids would be on TI 1068.

The population growth rate was slower on aphids reared on TI 1068 due to the fact that the number of hours adults remained sexually mature was less than the numbers of hours required for immature aphids to develop to adulthood. Although the population of aphids was considerably smaller on TI 1068 than on TN 86, the aphid population on TI 1068 would probably be less susceptible to a population crash. The laboratory collected data and the computer simulation are attempts to estimate potential aphid population in the field in the absence of limiting factors such as predators, parasitoids, and disease. A population of 10,000,000 immature aphids was never recorded in our experiments on 1 TN 86 tobacco plant in the field. However, predators, parasitoids, diseases, and other limiting factors would normally keep a population below 10,000. A greater number of

Table 2. Parameter values derived from data on excised leaves from field and used in computer simulation of field populations of tobacco aphids, Tobacco Experiment Station, Greeneville, TN, 1997.

	PARAMETERS*			
ENTRY	**IMMHR**	**MATHR**	**REPRO**	**SURV**
TN 86	132	199	.1693	.9
TI 1068	171	91	.0724	.5

* Parameter values: immhr = numbers of hours aphids remained immature, mathr = number of hours aphids remained sexually mature, repro = number of offsprings per time unit (hours), and surv = percentage of immatures that reached maturity.

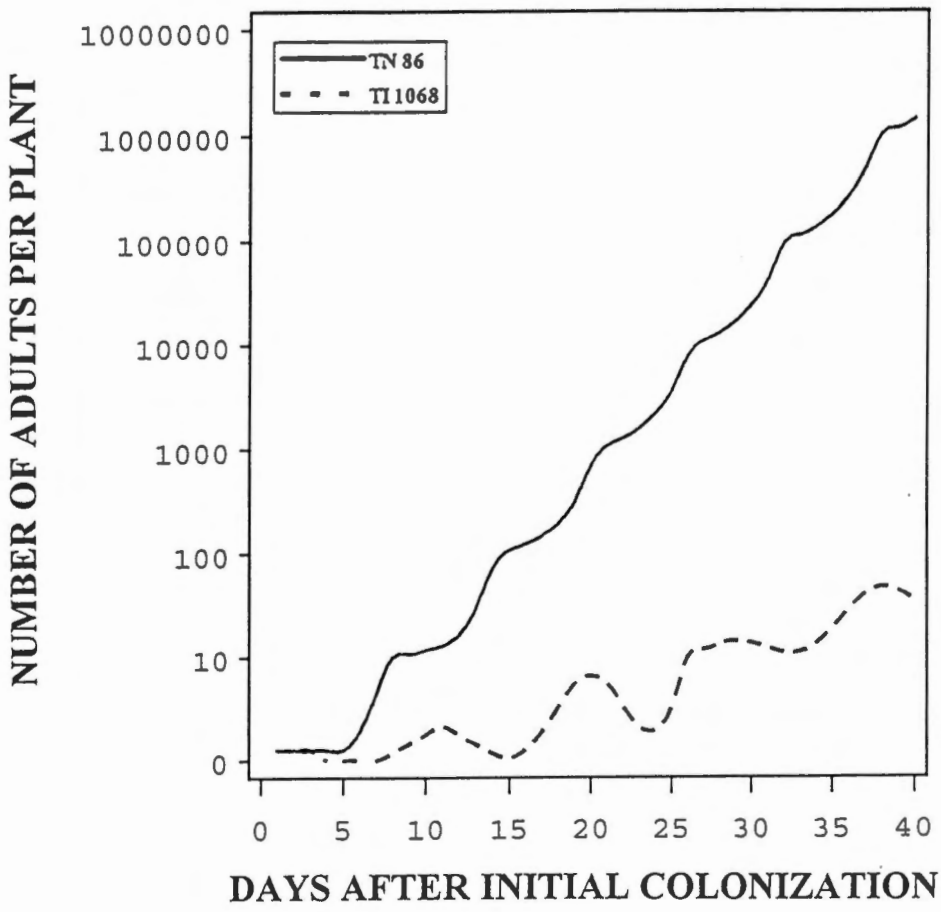

Figure. 25. Computer-generated deterministic simulation of adult tobacco aphid populations, beginning with one 1st instar. Simulation is based on laboratory collected data on the life cycle, fecundity, reproductive longevity, and survival of that insect reared on resistant (TI 1068) or non-resistant (TN 86) tobacco [SAS Institute 1989].

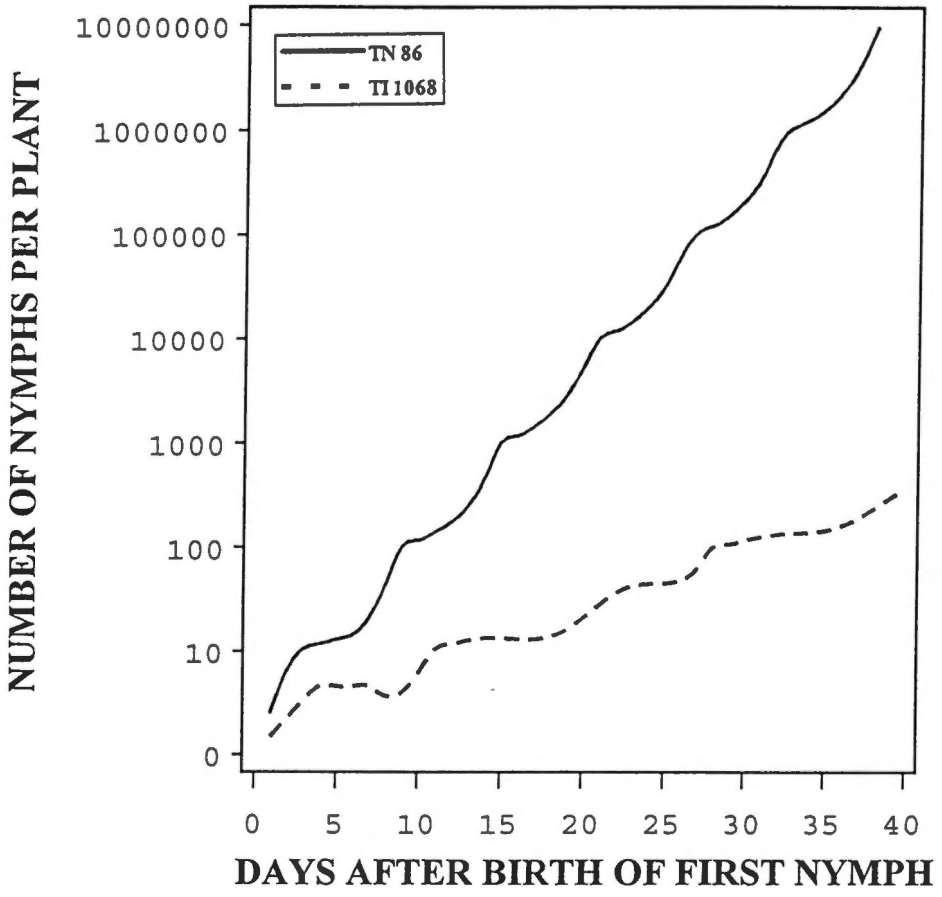

Figure. 26. Computer-generated deterministic simulation of immature tobacco aphid populations, beginning with one 1st instar. Simulation is based on laboratory collected data on the life cycle, fecundity, reproductive longevity, and survival of that insect reared on resistant (TI 1068) or non-resistant (TN 86) tobacco [SAS Institute 1989].

aphids is often seen on TI 1068 than in the laboratory. However, this computer simulation is based on 1 migrant aphid landing on 1 tobacco plant, whereas most plants in the field are colonized by several migrants. Comparisons between estimated values from the computer and actual data obtained from the field showed varying differences. By the 40th day after infestation, the computer estimated that 10,000,000 aphids would develop on TN 86; whereas, only an average of 1,000 aphids were actually observed in the field. Estimated values for aphids on TI 1068 were fairly similar to actually field counts. The computer estimated 600 aphids would develop on TI 1068 in 40 d, and the actual field counts were 150 aphids per plant. Using other variables such as predation, parasitism, and disease in the simulation program should increase the accuracy of predicting aphid populations under field conditions.

Summary

Between 1996 and 1998 studies were conducted to determine life cycle, fecundity, reproductive longevity, and survival of the tobacco aphid on aphid resistant tobacco lines. Three methods were tested to rear aphids on selected lines of tobacco: excised leaves, clip-on leaf cages, and leaf discs. Initially, excised leaves from 2 entries, 94-INS-301 and TN 86, were used to determine biological mechanisms of resistance. Four entries, 94-INS-301, 94-INS-3001, TI 1068, TN 86, were later evaluated using clip-on leaf cages. The same entries were also used in experiments with leaf discs. Experiments were conducted with aphids reared on leaves excised from resistant and non-resistant

greenhouse- and field-grown plants. Two entries, TI 1068 and TN 86, were chosen from the original 4 entries for further evaluation of the biological mechanisms of resistance.

Under all conditions tested on average aphids on TN 86 became mature in 5.5-7 d, produced 53 nymphs within 14 d, were reproductive for 8 d, and lived for 18 d. There were no significant differences ($P>0.05$) in development time among the entries when clip-on leaf cages were used on whole plants. Aphid development was significantly slower ($P<0.05$) on leaf discs from resistant 301, 3001, and TI 1068 leaf discs, than on TN 86 leaf discs. Aphid development, fecundity, reproductive longevity, and survival were significantly ($P<0.05$) different between leaves excised from TI 1068 field-grown plants, and from leaves excised from TN 86 field-grown plants. There were no significant differences ($P>0.05$) in development, fecundity, reproductive longevity, and survival on leaves excised between TI 1068 and TN 86 greenhouse-grown plants. From data collected using excised leaves from the field, a deterministic simulation program using SAS (1989) was developed to estimate potential aphid population growth under field conditions in the absence of limiting factors, such as predation, parasitoids, diseases, and weather for up to 40 d, or approximately 5 generations of the aphid. Results from the simulation demonstrated that an aphid population on TN 86 would develop rapidly within a few days; whereas, aphids on TI 1068 would develop at a relatively slow rate.

CHAPTER V

CONCLUSIONS

Although there is much debate about moral and health issues associated with the production and use of tobacco, it still remains an important cash crop in many parts of the Southeast. The tobacco aphid, *Myzus nicotianae* Blackman, is an important insect pest on tobacco, *Nicotiana tabacum* L. Large numbers of tobacco aphids threaten to reduce tobacco crop value by preventing plant maturity, reducing the value of tobacco leaves due to honeydew accumulation, and inoculating tobacco plants with various viral diseases. For the past 40 years, the tobacco aphid has been primarily controlled by the use of chemical insecticides. However, within the last ten years the tobacco aphid has been increasingly harder to control and is now resistant to many insecticides. Recent studies have dealt with developing breeding lines that are resistant to tobacco aphids. Crossing aphid resistant tobacco lines with aphid susceptible burley tobacco might facilitate development of an aphid-resistant burley cultivar which may result in a potential decrease in the amount of chemical insecticides used on the crop.

At the Tobacco Experiment Station, Greeneville, TN, 4 unique chemical combinations of cis-abienol and sucrose esters in tobacco leaf surface exudates resulted from crosses between TI 1068 and 'TN 86' or 'KY 17' to incorporate aphid resistance into burley tobacco genotypes. As a continuation of research initiated in 1993, a two-year research project was begun in 1996 to determine degrees of resistance among several

lines of tobacco to the tobacco aphid. A major objective of this study was to investigate the population growth parameters of the tobacco aphid that are affected by resistant tobacco. The parameters evaluated in the aphid population included: life cycle, fecundity, reproductive longevity, and survival. A third aspect of the study was to use a computer generated simulation to estimate aphid population growth on resistant and non-resistant tobacco lines based on data collected from laboratory experiments

Ten entries were evaluated in a growth room to determine levels of aphid colonization in the absence of predators. Three weeks after the initial infestation, all tobacco entries became heavily infested with aphids. No significant differences ($P>0.05$) were detected among aphid population densities among the experimental lines. Significant differences ($P>0.05$) were detected between 94-INS-201 and the standard TN86. Lack of leaf exudates, due to the size of the tobacco plants, is thought to be the reason for the lack of differences among the experimental lines and the standard. Four entries were selected from the original 10 entries and were evaluated in a greenhouse and field for aphid colonization. In both greenhouse and field experiments, a significantly larger population ($P<0.05$) was observed on TN 86 than on TI 1068. Significant aphid resistance was also observed in the selfed line (301) and the backcrossed line (3001) in the field experiment.

Studies of the biological mechanisms of resistance to the tobacco aphid were carried out on 4 different lines of resistant and non-resistant tobacco. Different methods using excised leaves, leaf discs, and whole plants were used to determine aphid parameters. No significant differences ($P>0.05$) were detected for aphid development on

the four tobacco entries when using whole plants. A possible explanation for this lack of differences in aphid development is a lack of sucrose esters produced among the 3 resistant entries. However, a significant difference ($P<0.05$) in aphid development between the standard non-resistant line, TN 86, and resistant the resistant lines, TI 1068, 301, and 3001 was observed on leaf discs that were embedded in agar in a petri dish. Aphids developed 3X faster on TN 86 than on TI 1068. High humidity possibly contributed to enhancing the toxicity of sugar esters on the tobacco (Xia & Johnson 1997). An increase in the toxicity of the sugar esters could possibly affect the growth of the tobacco aphid.

A study comparing excised leaves of TI 1068 and TN 86, from the field and greenhouse-grown tobacco plants, indicated that environmental factors might play a role in the amount of leaf exudates produced. Results from experiments using excised leaves from the field-grown plants demonstrated that aphids reared on TN 86 had a significantly ($P<0.05$) shorter life cycle, greater fecundity, greater reproductive longevity, and a greater survival rate than aphids reared on TI 1068. Studies using excised leaves from greenhouse-grown plants indicated no significant differences ($P>0.05$) in life cycle, fecundity, reproductive longevity, or survival of between aphids that were reared on TI 1068 or TN 86. Higher amounts of sucrose esters on field-grown TI 1068 plants are suspected to be the cause of resistance by lengthening the life cycle, reducing fecundity, shortening the reproductive period, and shortening the life span of the tobacco aphid. According to these results, excised leaves from greenhouse-grown plants should not be used to determine other growth parameters in future experiments. Chemical analyses of

the leaf surface in previous research has revealed the presence of sucrose esters on TI 1068, 301, and 3001, while no sucrose esters were detected on TN 86 (Shumate 1995). In both studies, degrees of resistance varied between laboratory, greenhouse, and field experiments. Insect resistance in tobacco breeding lines has shown to fluctuate between field, laboratory or greenhouse tests (Nielsen et. al. 1982). Therefore, the production of sucrose esters by the same plant may vary depending on the environmental conditions in which the plant is grown. Chemical analyses performed in 1994 revealed different levels of sucrose esters were produced in field and cage experiments by the same plant (Shumate 1995).

A computer simulation based on the life cycle, fecundity, reproductive longevity, and survival of the tobacco aphid, was developed to predict aphid population development on TN 86 and TI 1068 in the field. Predictions were based on the assumption that aphid populations would not be influenced by predators, parasitoids, or other factors, such as overcrowding and environmental conditions. The program predicted a population growth rate from one aphid to several thousand aphids within 20 days on TN 86, while a much slower growth rate was predicted for aphids on TI 1068. Comparisons between estimated values from the computer and actual data obtained from the field showed varying differences. Estimated values and actually aphid counts were very different for TN 86, however results were fairly similar for TI 1068. Future work on the effects of predators and parasitoids on an aphid populations could prove useful in accurately determining the number of aphids in a typical tobacco field.

Future work on aphid-resistant tobacco could include the use of electrophysiological and video-optical methods to observe the behavior of aphids. According to Nauen (1995) the use of electrical penetration graphs and honeydew excretion measurements are accurate methods to determine aphid feeding responses. Information on when and how much aphids are feeding could prove useful in determining other biological mechanisms that could influence resistance. Additional selection and evaluation of burley lines having a high sucrose ester content should continue. Aphid-resistant tobacco cultivars would allow farmers to use less insecticide, thus increase profits. Aphid resistance in other plants, such as tomatoes or potatoes, should also be evaluated to determine other possible resistance mechanisms.

REFERENCES CITED

Abernathy, C.O. & R. Thurston. 1969. Plant age in relation to the resistance of *Nicotiana* to the green peach aphid. J. Econ Entomol. 62: 1356-1359.

Akehurst, B.C. 1981. Tobacco. (2nd edition) p. 499. Longman Inc, New York.

Belcher, D. W. & R. Thurston. 1983. Effects of various tobacco cultivars on coccinellid oviposition and survival. Tob. Sci. 27: 7-9.

Blackman, R. L. 1974. Life-cycle variation of *Myzus persicae* (Sulz.) (Hom., Aphididae) in different parts of the world, in relation to genotype and environment. Bull. Ent. Res. 63: 595-607.

Blackman, R. L. 1987. Morphological discrimination of a tobacco-feeding form form *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and a key to New World *Myzus* (Nectarosiphon) species. Bull. Entomol. Res. 77: 713-730.

Blackman, R. L. & V. F. Eastop. 1984. Aphids on the World's Crops, An Identification Guide, pp. 310-315. John Wiley & Sons, New York.

Boiteau, G. & D. T. Lowery. 1989. Comparison of a yellow form of the green peach aphid, *Myzus persicae* (Sulzer), and a green form of the tobacco aphid, *Myzus nicotianae* Blackman, coexisting on greenhouse potato in New Brunswick. The Canadian Entomologist. 121(12): 1029-1035.

Burk, L. G. & P.A. Stewart. 1969. Resistance of *Nicotiana* species to the green peach aphid. J. Econ. Entomol. 62: 115-116.

Campbell, B. & D. Dreyer. 1990. The role of plant matrix polysaccharides in aphid-plant interactions. pp. 149-150. In R. K. Campbell and R. D. Eikenbary [eds.], Aphid-Plant Genotype Interactions. Elservier, New York.

Chamberlain, F. S. 1958. History and status of the green peach aphid as a pest of tobacco in the United States. USDA Tech. Bull. 1175, 12pp.

Dent, D. 1991. Insect Pest Management. pp. 213-228. Redwood Press Ltd, UK, Wiltshire.

Devonshire, A. L. 1989. Resistance of aphids to insecticides. pp. 123-135. In Aphids: Their Biology, Natural enemies and Control, A. K. Minks & P. Harrewijn, [eds.]. World Crop Pest 2C, Elsevier, Amsterdam.

Dixon, A. F. G. 1973. Biology of Aphids. pp. 1-55. Edward Arnold Limited. London.

Dixon, A. F. G. 1985. Structure of aphid populations. Ann. Rev. Entomol. 30: 155-74.

Dixon, A. F. G. 1987. Parthenogenetic reproduction and the rate of increase in aphids. pp. 269-285. In Aphids: Their Biology, Natural Enemies and Control, A. K. Minks & P. Harrewijn, (eds.). World Crop Pests 2A, Elsevier, Amsterdam.

Gerstel, D. U. 1961. Essay on the origin of tobacco. Tob. Sci. 5: 15-17.

Harlow, C. D. & E. P. Lampert. 1990. Resistance mechanisms in two color forms of the tobacco aphid (Homoptera: Aphididae). J. Econ. Entomol. 83(6): 2130-2135.

Harrewijn, P., 1986. Vector resistance of potato to aphids with respect to potato leafroll. In: A. G. B. Beekman (Editor), Potato Research of Tommorrow. Pudoc, Wageningen, pp. 85-95.

Harris, M. K. 1979. Biology and breeding for resistance to arthropods and pathogensin agricultural plants. pp. 45-67. In Proceedings of International Short course in Host Plant Resistance, Texas A & M University College, Station.

Hilderbrand, D. F., G. C. Brown, D. M. Jackson, & T. R. Hamilton-Kemp. 1993. Effects of some leaf-emitted volatile compounds on aphid population increase. Journal of Chemical Ecology. 19(9) 1875-1887.

Jackson, D. M., V. A. Sisson, & R. F. Severson. 1990. Mechanisms of resistance in *Nicotiana tabacum* to the tobacco aphid. p. 281. In D. C. Peters, J. A. Webster & C. S. Chlouber [eds.], Proceedings, Aphid-Plant Interactions: Populations to Molecules. USDA / Agricultural Research Service, Oklahoma State University, Oklahoma.

Johnson, A. W. & R. L. Severson. 1982. Physical and chemical leaf surface characteristics of aphid resistant and susceptibility tobacco. Tob. Sci. 26: 98-102.

Johnson, A. W., R. F. Severson, J. Hudson, G. R. Carner, & R. F. Arrendale. 1985. Tobacco leaf trichomes and their exudates. Tob. Sci. 29: 67-70.

Kawada, K. 1987. Polymorphism and morph determination. pp. 255-266. In Aphids: Their Biology, Natural Enemies and Control, A. K. Minks & P. Harrewijn, (eds.). World Crop Pests 2A, Elsevier, Amsterdam.

Klingauf, F. A. 1987. Feeding, adaption, and excretion. pp. 225-248. In Aphids: Their Biology, Natural Enemies and Control, A. K. Minks & P. Harrewijn, (eds.). World Crop Pests 2A, Elsevier, Amsterdam.

Kumar, R. 1984. Insect Pest Control. pp. 88-102. Edward Arnold Ltd, London.

Lees, A. D. 1959. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton - the influence of these factors on apterous viginoparae and their progeny. J. Ins. Physiol. 3: 92-117.

Metcalf, R. L. & W. H. Luckmann. 1994. Introduction to Insect Pest Management. 3rd ed. John Wiley & Sons, Inc, New York.

Miller, R. D. 1987. Registration of 'TN 86' burley tobacco. Crop Sci. 27: 365-366.

Minks, A. K. & P. Harrewijn, eds. 1987. Aphids: Their Biology, Natural Enemies and Control. World Crop Pests 2A, Elsevier, Amsterdam. 450 pp.

Mistric, W. J. & G. B. Clark. 1979. Green peach aphid injury to flue-cured tobacco leaves. Tob. Sci. 13: 23-24.

Nauen, R. 1995. Behaviour modifying effects of low systemic concentrations of imidacloprid on *Myzus persicae* with special reference to an antifeeding response. Pestic. Sci. 44: 145-153.

Nielsen, M. T., G. A. Jones & B. P. Collins. 1982. Inheritance pattern for secreting and nonsecreting glandular trichomes in tobacco. Crop Sci. 22: 1051-1053.

Painter, R. H., S. C. Salmon & J. H. Parker. 1931. Resistance of vanities of winter wheat to hessian fly. Kans. Agric. Exp. St. Bull. 27. pp. 8.

Painter, R. H. 1951. Insect Resistance in Crop Plants. pp. 23-75. The University Press of Kansas, London.

Reed, D. & P. J. Semtner. 1990. Influence of temperature on population development of two color morphs of the tobacco aphid (Homoptera: Aphididae) on flue-cured tobacco. J. Entomolo. Sci. 26(1) 33-38.

Reich, R. 1986. (ed.) Burley Tobacco Field Manual. R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina. p. 82.

SAS Institute Incorporated. 1989. SAS/STAT user's guide, version 6, vls. 1 & 2, Cary, North Carolina, 1789 pp.

Shaw, M. J. P. 1970. Effects of population density on alienicolae of *Aphis fabae* Scop. Ann. Appl. Biol. 65: 191-196.

Shumate, S. 1995. Role of leaf surface chemistry on aphid resistance in burley tobacco. M. S. Thesis, Univ. Tennessee, Knoxville, pp. 78.

Srivastava, P. N. 1987. Nutritional Physiology. pp. 99-121. In Aphids: Their Biology, Natural Enemies and Control, A. K. Minks & P. Harrewijn, (eds.). World Crop Pests 2A, Elsevier, Amsterdam.

Takada, H. 1982. Influence of photoperiod and temperature on the productionof sexual morphs in a green and a red form of *Myzus persicae*. I. In the laboratory. II. Under natural conditions. *Kontyu.* 50: 533-45., 353-64.

Tennessee Agricultural Statistics 1997. Natl. Agric. Stat. Serv., Tennessee Agric. Stat. Serv., Tennessee Dept. Agric., Nashville, 128 pp.

Thurston, R. 1961. Resistance in *Nicotiana* to the green peach aphid and some other tobacco insect pest. J. Econ. Entomol. 54(5): 946-949.

Thurston, R., W. T. Smith & B. P. Cooper. 1966. Alkaloid secretion by trichomes of *Nicotiana* species and resistance to aphids. Ent. Exp. & Appl. 9: 428-432.

Thurston, R., W. Katanyukul. 1973. Response of certain insects to tobacco cultivars TI 1298 and TI 1068. Tob. Sci. 17: 162.

Tso, T. C. 1972. Physiology and Biochemistry of Tobacco Plant. pp. 3-33. Dowden, Hutchinson, & Ross, Inc. Stroudsburg, Pa.

van Emden, H. F. & S. D. Wratten. 1990. Tri-trophic interactions involving plants in the biological control of aphids. pp. 29-43. In D. C. Peters, J. A. Webster & C. S. Chlouber [eds.], Proceedings, Aphid-Plant Interactions: Populations to Molecules. USDA / Agricultural Research Service, Oklahoma State University, Oklahoma.

van Lentern, J. C. 1990. Biological control in a tritrophic system approach; Aphids, their host plants and natural enemies. pp. 3-28. In D. C. Peters, J. A. Webster & C. S. Chlouber [eds.], Proceedings, Aphid-Plant Interactions: Populations to Molecules. USDA / Agricultural Research Service, Oklahoma State University, Oklahoma.

Webster, J. A. 1990. Developing aphid-resistant cultivars. pp. 87-111. In D. C. Peters, J. A. Webster & C. S. Chlouber [eds.], Proceedings, Aphid-Plant Interactions: Populations to Molecules. USDA / Agricultural Research Service, Oklahoma State University, Oklahoma.

Wellings, P. W., S. A. Ward, A. F. G. Dixon & R. Rabbinge. 1989. Crop loss assessment. pp. 49-64. In Aphids: Their Biology, Natural Enemies and Control, A. K. Minks & P. Harrewijn, (eds.). World Crop Pests 2C, Elsevier, Amsterdam.

Xia, Yulu & A. W. Johson. 1997. Effects of leaf surface moisture and relative humidity on the efficacy of sugar esters from *Nicotiana gossei* against the tobacco aphid (Homoptera: Aphididae). 90(4): 1010-1014.

APPENDICES

APPENDIX A

Numerical Values Generated by Computer-generated Simulation.

* OBS	POP	TIME	NADULTS	NAPHIDS	JUVIES
1	1	1	1.00	5.06	4.06
2	1	2	1.00	9.13	8.13
3	1	3	1.00	13.19	12.19
4	1	4	1.00	17.25	16.25
5	1	5	1.00	21.32	20.32
6	1	6	2.68	26.88	24.20
7	1	7	6.33	45.08	38.75
8	1	8	9.99	78.15	68.16
9	1	9	12.65	122.19	109.55
10	1	10	16.30	180.91	164.61
11	1	11	19.96	254.49	234.53
12	1	12	29.49	350.17	320.68
13	1	13	52.37	511.29	458.92
14	1	14	87.41	791.01	703.60
15	1	15	129.72	1222.35	1092.63
16	1	16	185.40	1852.43	1667.02
17	1	17	255.04	2735.79	2480.74
18	1	18	360.04	3958.61	3598.57
19	1	19	547.69	5757.04	5209.34
20	1	20	858.54	8554.49	7695.94
21	1	21	1312.23	12874.45	11562.22
22	1	22	1955.17	19385.71	17430.54
23	1	23	2844.22	28967.78	26123.56
24	1	24	4107.37	42827.00	38719.64
25	1	25	6042.62	63018.60	56975.98
26	1	26	9081.15	93104.17	84023.02
27	1	27	13705.31	138448.58	124743.27
28	1	28	20557.06	206664.47	186107.42
29	1	29	30510.43	308426.31	277915.88
30	1	30	44902.13	458719.52	413817.39
31	1	31	66134.18	679864.82	613730.63
32	1	32	98113.89	1006853.32	908739.44
33	1	33	146377.91	1493549.74	1347171.83
34	1	34	218639.07	2220239.97	2001600.90
35	1	35	325727.51	3304322.98	2978595.46
36	1	36	483266.08	4915783.70	4432517.63
37	1	37	715437.84	7303357.20	6587919.43
38	1	38	1060240.21	10839503.30	9779263.12

39	1	39	1574980.56	16086714.28	14511733.72
40	1	40	2343664.28	23889144.34	21545480.07
41	2	1	1.00	2.74	1.74
42	2	2	1.00	4.48	3.48
43	2	3	1.00	6.21	5.21
44	2	4	0.00	6.59	6.59
45	2	5	0.00	6.59	6.50
46	2	6	0.00	6.58	6.58
47	2	7	0.00	6.58	6.58
48	2	8	0.72	6.37	5.65
49	2	9	1.59	7.55	5.96
50	2	10	2.46	10.23	7.77
51	2	11	3.25	14.42	11.17
52	2	12	2.38	18.43	16.04
53	2	13	1.52	20.93	19.41
54	2	14	0.65	21.91	21.26
55	2	15	0.17	21.58	21.40
56	2	16	1.07	21.66	20.59
57	2	17	2.72	23.23	20.50
58	2	18	5.13	27.60	22.47
59	2	19	7.38	35.90	28.52
60	2	20	8.13	46.77	38.63
61	2	21	7.38	57.59	50.20
62	2	22	5.14	65.72	60.57
63	2	23	3.12	69.84	66.76
64	2	24	2.86	71.66	68.80
65	2	25	5.03	74.20	69.17
66	2	26	10.05	81.58	71.53
67	2	27	16.25	97.31	81.05
68	2	28	21.50	121.92	100.46
69	2	29	23.82	152.64	128.81
70	2	30	21.92	183.46	161.53
71	2	31	17.54	208.35	190.81
72	2	32	13.71	225.21	211.49
73	2	33	14.05	237.20	223.15
74	2	34	21.31	252.98	231.67
75	2	35	34.73	282.95	248.21
76	2	36	51.02	334.78	283.76
77	2	37	64.73	409.53	344.80
78	2	38	70.73	499.17	428.43
79	2	39	67.83	589.84	522.01
80	2	40	59.36	668.59	609.23

*OBS = Observations, POP = Population (1 = TN 86, 2 = TI 1068), TIME = Days NADULTS = Number of adult aphids, NAPHIDS = Total number of aphids, JUVIES = number of aphid nymphs

APPENDIX B

Mean Values for Parameters on the Life Cycle, Fecundity, Reproductive Longevity, and Survival on Excised Leaves from the Field (Repeated Experiment).

Life Cycle

Mean number of exuviae

Hours after birth	TN 86	TI 1068
12	.4	.2
24	.9	.2
36	1.1	.5
48	1.3	.6
60	1.8	.8
72	2.0	1
84	2.5	1.1
96	2.7	1.2
108	3.1	1.4
120	3.3	1.6
132	3.7	1.7
144	3.8	2
156	4.0	2.4
168		2.6
180		2.9
192		3.3
204		3.4
216		3.7
228		3.8
240		4.0

Fecundity

Mean number of offspring

TN 86	TI 1068
51	19

Reproductive Longevity

Mean number of days aphids remained reproductive

TN 86	TI 1068
7.2	4.3

Survival

Percentage of aphids reaching maturity

TN 86	TI 1068
85%	62%

VITA

Peter Joseph Obenauer was born in Pittisfield, Massachusetts, on May 11, 1973. He grew up in Vienna, Virginia and received his high school diploma from Paul VI High School in Fairfax, Virginia, in June of 1992. In May, 1996, he received the Bachelor of Science Degree in Biology and Secondary Education from Longwood College, Farmville, Virginia. In August 1996, he was accepted into the Department of Entomology and Plant Pathology at The University of Tennessee, Knoxville. Under the direction of Dr. Charles D. Pless, he completed the requirements for a Master of Science degree in Entomology and Plant Pathology in August 1998.

The author is a member of the Entomological Society of America, Tennessee Entomological Society, Beta Beta Beta Biological Honor Society, and Gamma Sigma Delta Agricultural Honor Society. In March of 1998, Mr. Obenauer was commissioned as a lieutenant (JG) in the Medical Services Corp of the United States Navy. Mr. Obenauer intends to pursue a career in the United States Navy working as a medical entomologist.