Potential of *Pandora neoaphidis* (Remaudière & Hennebert) Humber as a fungal pathogen for the control of tobacco aphid, *Myzus nicotianae* Blackman, on tobacco

by

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POTENTIAL OF *PANDORA NEOAPHIDIS* (REMAUDIÈRE & HENNEBERT) HUMBER AS A FUNGAL PATHOGEN FOR THE CONTROL OF TOBACCO APHID, *MYZUS NICOTIANAE* BLACKMAN, ON TOBACCO

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(ABSTRACT)

The potential of *Pandora neoaphidis* (Remaudière & Hennebert) Humber as a fungal pathogen for the control of the tobacco aphid, *Myzus nicotianae* Blackman, on tobacco was evaluated in a 4-year study between 1992 and 1995. The objectives of this study included determination of the seasonal incidence of *P. neoaphidis* in populations of tobacco aphid on tobacco and nonsolanaceous host plants, within plant distribution of the pathogen in aphids on tobacco, influence of tobacco cultivars and cultural practices on the incidence of the pathogen, methods of artificially introducing the pathogen into tobacco aphid populations and their potential in controlling aphids relative to chemical control, virulence of the Virginia isolate of the pathogen to tobacco aphid and green peach aphid, *M. persicae* (Sulzer), from different geographic locations in the eastern United States, and influence of temperature and type of substrate on the developmental morphology of the pathogen.

Infections as high as 91% occurred in aphid populations on tobacco under favorable weather. The pathogen survived at moderate levels in the red morph of

tobacco aphid on nonsolanaceous hosts during fall, parts of winter and spring. *P. neoaphidis* infections in aphids tended to increase towards the upper leaf positions. Incidence of the pathogen in aphid populations varied widely on various cultivars and types of tobacco. Planting date, topping of tobacco, and stage at which tobacco was topped did not influence the incidence of *P. neoaphidis* in tobacco aphids. Artificial introduction of *P. neoaphidis* successfully established infections in tobacco aphids, but failed to prevent the build up of aphid populations. *P. neoaphidis* was equally virulent to the red and green morphs of the tobacco aphid, and the green peach aphid. The developmental morphology of *P. neoaphidis* was influenced by temperature and was similar on the surfaces of living substrates, tobacco aphid and tobacco leaf, but different on the inert surface of the coverslip.

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CHAPTER I

Introduction and objectives

Tobacco cultivation in the United States dates back to 1612 when John Rolfe planted his first experimental crop at Jamestown, Virginia. Since then tobacco in Virginia has been closely tied to the state's economic growth and development (Virginia Department of Agriculture and Consumer Services (VDACS) 1992). Though there has been a reduction in per capita consumption of tobacco products in the United States since 1986 (USDA 1995), other potential uses for tobacco are being developed for the future. Tobacco contains extremely high-quality proteins which could be extracted and used as natural additives to increase the nutritional value of some foods (VDACS 1992). Potential uses of tobacco by-products include the manufacture of pharmaceuticals, films, animal feed, coloring agents, cosmetics, paper, and textiles. Genetically engineered products such as anti-viral drugs and human blood protein also could be produced from tobacco using a new technology called 'Geneware' (VDACS 1992).

The tobacco industry contributes to $129.3 billion of the US gross national product (The Golden Leaf 1993). Tobacco is Virginia's leading commercial crop in production and cash receipts accounting to 28 and 9% of state's total crop and agriculture incomes, respectively (USDA 1995). Virginia is fifth in crop

production among 16 tobacco growing states. In 1994, 48 million kilograms of tobacco, valued at $183 million, was grown on 18,800 ha in Virginia (USDA 1995). Virginia is the second largest tobacco manufacturing state in the US and the tobacco industry, directly or indirectly, generates 5% of state's total employment (VDACS 1992).

The tobacco aphid, *Myzus nicotianae* Blackman, is the most important insect pest of tobacco in North America and causes serious reductions in both yield and quality of tobacco leaf (Cheng and Court 1977, Mistric and Clark 1979, Cheng and Hanlon 1985, Lampert 1989, Reed and Semtner 1992). The tobacco aphid can reduce tobacco yields by 10-30% (Reed and Semtner 1992). Its damage also reduces the value of the cured leaves, especially those from the lower one-half of the plant (Mistric and Clark 1979, Reed and Semtner 1992). The tobacco aphid damages the crop by sucking the plant sap and depositing honeydew on the leaves (Kulash 1949, Feinstein and Hannan 1951, Mistric and Clark 1979). Infested leaves show premature yellowing and necrosis along the margins and at the base of the petiole (Dominick 1949). Sooty mold develops on the honeydew secretions and may cause additional discoloration of the leaves (Kulash 1949, Dominick 1949). In Virginia, the tobacco aphid is a serious annual problem, and is the target of more than 50% of the insecticides applied to tobacco. The tobacco aphid is also an important vector of several tobacco pathogens including tobacco etch virus, tobacco leaf mottle virus, and PVY (Chamberlin 1958, van Emden et al. 1969,

Lucas 1975). Development of resistance of the tobacco aphid to insecticides posed problems with chemical control in the recent years and the number of effective aphicides has considerably decreased (Koziol and Semtner 1984, McPherson and Bass 1990, Harlow and Lampert 1990, Abdel-Aal et al. 1992, Chryssochoou and Bloukidis 1992).

The future availability of effective aphicides is uncertain. In addition, problems associated with insecticide use make it necessary to find alternatives to chemical control. Cultural practices help to reduce aphid problems on tobacco. These practices include adjustments in transplanting date (Semtner 1984, McPherson et al. 1993), selection of early maturing cultivars, proper nitrogen fertilization, and early topping (Semtner unpbl.). However, none of these can prevent economic losses under heavy aphid pressure (Semtner 1984, Semtner unpbl.). Beneficial insects provide some natural control of the tobacco aphid under certain conditions. However, they, too, are ineffective in preventing extensive aphid population buildups, under favorable host plant and environmental conditions (Hamid 1987, Norowi and Semtner 1990).

Fungi of the order Entomophthorales attack insect hosts from more than 32 families in the orders Orthoptera, Hemiptera, Homoptera, Lepidoptera, Coleoptera, and Hymenoptera and often cause epizootics in the host populations (MacLeod and Müller-Kögler 1973). There have been several studies of entomophthorales infecting various aphids on different crops (Wilding 1975, Milner et al. 1980, Feng

et al. 1990, Feng et al. 1991, Elkassabany et al. 1992). The genera of the entomophthorales that infect aphids commonly are *Conidiobolus*, *Entomophthora*, *Erynia*, *Neozygites*, and *Zoophthora* (Latgé and Papierok 1988).

The entomophthoralean, *Pandora* (*Erynia*) *neoaphidis* (Remaudière and Hennebert) Humber causes epizootics late in the tobacco season and can greatly reduce aphid infestations and under conditions of high humidity and high aphid populations, the disease spreads very rapidly and can kill most aphids (Semtner unpbl.). Dean and Wilding (1971) reported that many populations of cereal aphids, *Metapolophium dirhodum* (Walker) and *Sitobion avenae* (F.) were killed by *Entomophthora aphidis* Hoffman in Fresenius, *E. planchoniana* Cornu, and *E. thaxteriana* in barley fields in England. *P. neoaphidis* successfully controlled various aphids (76 to 100%) including *Aphis gossypi* Glover in China (Anonymous, 1976). *P. neoaphidis* appears to be the most common species infecting the tobacco aphid in Virginia (Semtner unpbl.).

The increased concern over pesticides in the environment has resulted in increased research efforts in integrated pest management. The goals of integrated pest management research are to develop ways to reduce the use of chemical insecticides and to learn more about nonchemical control methods that do not pollute the environment. In addition, several forms of the tobacco aphid are highly resistant to several insecticides that provided effective control in the past. The potential is also great for the occurrence of other highly resistant strains of the

tobacco aphid.

Pathogens of the tobacco aphid occur naturally in most tobacco fields each summer. Information on the ecology of these pathogens could be used to develop strategies for reducing aphid infestations on tobacco without the need to apply pretransplant or foliar insecticides for aphid control. This research was conducted to study various aspects of the relationship of *P. neoaphidis* with the environmental factors, season, leaf position within the tobacco plant, tobacco cultivars, cultural practices, and host plants of the tobacco aphid, and to evaluate its the potential for aphid control. To improve the knowledge on *P. neoaphidis* at basic level, virulence of the pathogen to the tobacco aphid and the green peach aphid, *M. persicae* (Sulzer),and the influence of temperature and type of substrate on its developmental morphology were also investigated.

The primary objectives of this research were to,

1. Investigate seasonal incidence of *P. neoaphidis* in *M. nicotianae* on flue-cured tobacco in summer and evaluate the influence of environmental factors,
2. Investigate seasonal incidence of *P. neoaphidis* in the red morph of *M. nicotianae* on various nonsolanaceous host plants during fall, winter, and spring,
3. Investigate seasonal incidence and within-plant distribution of *P. neoaphidis* in the red morph of *M. nicotianae* on flue-cured tobacco,

4. Study the occurrence of *P. neoaphidis* in populations of tobacco aphid on different tobacco types, tobacco introductions, and a breeding line and evaluate the influence of cultural practices,
5. Evaluate the methods of artificially introducing *P. neoaphidis* into the populations of *M. nicotianae* and the potential of the pathogen in controlling aphids in comparison with chemical control,
6. Determine the virulence of the Virginia isolate of *P. neoaphidis* to different biotypes of *M. nicotianae* and a biotype of *M. persicae* from different geographic regions in the eastern United States, and
7. Determine the influence of temperature and the type of substrate on the developmental morphology of *P. neoaphidis*.

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CHAPTER II

Review of literature

***Myzus nicotianae* Blackman.** Blackman (1987) described the tobacco aphid, *Myzus nicotianae* Blackman, as a tobacco-feeding form of *Myzus* closely related to the green peach aphid, *M. persicae* (Sulzer). Both species have 2n=12 chromosome karyotypes and are heterozygous for the same autosomal translocation. Blackman and Spence (1992) electrophoretically distinguished these two species based on the mobility of the enzyme glutamate oxaloacetate transaminase. *M. persicae* is monomorphic, while *M. nicotianae* is polymorphic for this enzyme. Before Blackman's description, *M. nicotianae* was grouped with *M. persicae*. *M. nicotianae* is morphologically similar to, but genetically isolated from *M. persicae*. *M. nicotianae* is permanently parthenogenetic (anholocyclic) in North America. However, sexually reproducing (holocyclic) populations are seen in some parts of the world. *M. nicotianae* is distributed in North and South America, the Mediterranean, the Middle East, Africa, the Indian sub-continent, and South-East Asia (Blackman 1987). Blackman (1987) reported that *M. nicotianae* has occurred as a distinct form on tobacco since the 1920s in Europe, the 1930s in Africa, and the 1970s in North America. He did not include any aphids in his study that were collected from tobacco in North America before

1975. However, he indicated that earlier records on tobacco in North America were also probably *M. nicotianae*. Chamberlin (1958) summarized the early history of *Myzus* on tobacco in the United States. In recent years, the common green morph of the tobacco aphid has been replaced by a red morph (McPherson 1989, Reed and Semtner 1991). This red morph is more tolerant of high temperatures and has higher rates of development, survival, and fecundity than the green morph (Lampert and Dennis 1987, Reed and Semtner 1991). In addition, it has greater resistance to many aphicides (McPherson and Bass 1990, Harlow and Lampert 1990). The number of effective aphicides has decreased considerably since the mid-1980s and chemical control has been difficult where the red forms predominate (Harlow and Lampert 1990). Chryssochoou and Bloukidis (1992) reported that the tobacco aphid has become resistant to acephate in Greece. Koziol and Semtner (1984) found resistance to acephate in the tobacco aphid.

Abdel-Aal et al. (1992) found insecticide resistance in the tobacco aphid in all of the red morphs and in the green morphs with the translocated chromosome karyotype. This organophosphate resistance was attributed to high carboxylesterase activity. Abdel-Aal et al. (1992) also reported that some electrofocusing-detectable esterase isozymes were associated with malathion resistance.

***Pandora neoaphidis* (Remaudière and Hennebert) Humber.** The

entomophthoralean, *P. neoaphidis* (Zygomycotina: Entomophthoraceae) was previously known as *Entomophthora aphidis* Hoffman in Fresenius, *E. aphidis* Hoffman sensu Thaxter, *Erynia aphidis* (Hoffman in Fresenius) Humber & Ben-Ze'ev, and *E. neoaphidis* Remaudière & Hennebert. The life cycle and morphology of *P. neoaphidis* was described by Butt et al. (1990). When the primary conidium becomes attached to the aphid body it either produces a secondary conidium or germinates by means of germ tube (Fig. 2.1). A penetration structure called the appressorium is produced at the end of the germ tube. It breaches the aphid cuticle and penetrates through a circular hole. Protoplasts are produced and invade the haemocoel and tissues within 36-48 h after inoculation. Later rhizoids, which terminate in digitate holdfasts, emerge from the midventral region of the aphid, and attach the insect to the leaf cuticle. Finally pseudocystidia (sterile supporting structures) and conidiophores enzymatically and mechanically break through the aphid cuticle (Butt et al. 1990).

Seasonal incidence of *P. neoaphidis* in *M. nicotianae* on tobacco. There were various studies on *P. neoaphidis* infecting different species of aphids (Milner et al. 1980, Glare et al. 1986a, Feng et al. 1990, Poprawski et al. 1992). Summers and Newton (1989) reported 100% infection of sugarbeet root aphid, *Pemphigus populivenae* Fitch by *P. neoaphidis* in California. *P. neoaphidis* dominated other fungi in populations of *Metopolophium dirhodum* (Walker), *Diuraphis noxia*

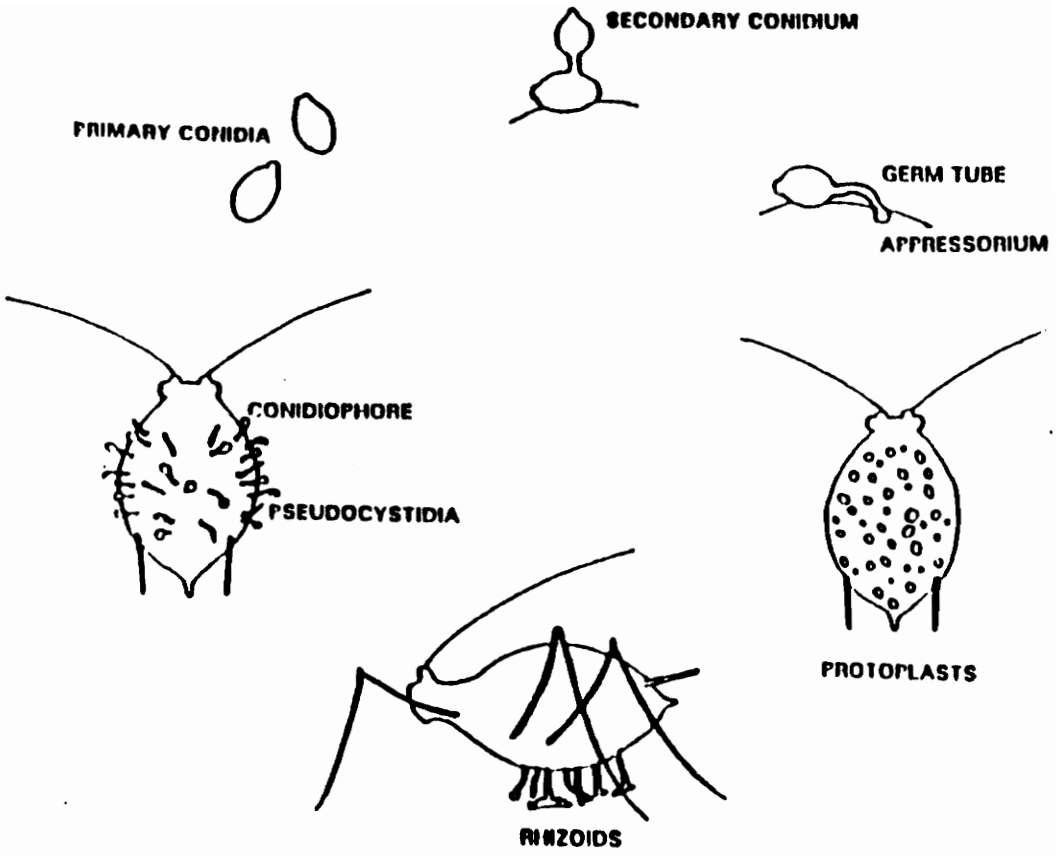

Fig. 2.1. Life cycle of *P. neoaphidis* in the aphid host.

(Mordvilko) in barley, corn, and spring and winter wheat (Feng et al. 1990). *P. neoaphidis* and other entomophthorales require moisture saturated conditions for germination and host invasion (Wilding 1971, Carruthers and Hural 1990). Influence of water potential on conidial germination of *P. neoaphidis* was reported by Morgan et al. (1992). Wilding et al. (1986) found that irrigation greatly increased the proportion of *Aphis fabae* Scopoli killed *by P. neoaphidis* in field beans. *P. neoaphidis* and *Zoophthora radicans* (Brefeld) Batko destroyed a dense population of *Acyrthosiphon pisum* (Harris) (74% infection) in the legume ground cover in an orchard watered with a sprinkle irrigation system (Pickering et al. 1989). No fungus-induced mortality occurred in a nearby plot with drip irrigation. However, Feng et al. (1991) observed a better correlation of infections of *P. neoaphidis* and *Conidiobolus* spp. with cereal aphid population densities than with environmental factors.

Seasonal incidence of *P. neoaphidis* in the red morph of tobacco aphid on nonsolanaceous hosts. Production of resting spores as an overwintering mechanism is not known for *P. neoaphidis* (Rockwood 1950, Ben-Ze'ev and Kenneth 1982, Waterhouse and Brady 1982, Wilding and Brady 1984). There is only one report of in vitro resting spore production (Uziel and Kenneth 1986), but the identity of those resting spores is uncertain. Its conidia are known to survive winter temperatures in soil and on foliage (Latteur 1977, Morgan et al. 1992,

Schofield 1995). Earlier reports revealed that *P. neoaphidis* (syn. *Empusa/ Entomophthora aphidis*) was viable for 3-32 wk in the cadavers of aphid hosts at temperatures ranging between 7 and -196^{0}C (Rockwood 1950, Remaudière and Michel 1971, Wilding 1973, Wilding et al. 1986, Feng et al. 1992). In vitro grown cultures were of *P. neoaphidis* were viable for four months at about 2^{0}C (Rockwood 1950). The pathogen survived in the cadavers of aphid hosts for 32 wk at 0^{0}C and for 3.5 mo at a temperature range between 2 and -196^{0}C and produced infective conidia when the cadavers were moistened (Remaudière and Michel 1971, Wilding 1973). Feng et al. (1992) observed formation of spherical hyphal bodies (SHB) in *P. neoaphidis* during late autumn and suggested that the pathogen may overwinter as SHB. Feng et al. (1992) also reported that the pathogen was viable after 6 mo of storage at 4^{0}C. *P. neoaphidis* also survived in the soil as conidia for 4 to several months at -3 to 5^{0}C (Latteur 1977, Morgan et al. 1992). Schofield et al. (1995) reported survival of conidia of *P. neoaphidis* on the leaf surface for 32 d at 5^{0}C. These reports suggest that the pathogen overwinters in the host body, or in soil, or on foliage. Reports on the incidence of *P. neoaphidis* during parts of winter are available (Feng et al. 1990, Elkassabany et al. 1992), however, information is not available on its seasonal incidence throughout the winter.

Within plant distribution of *P. neoaphidis* in *M. nicotianae* on flue-cured

tobacco. Brobyn et al. (1985) demonstrated in field tests that the infectivity of conidia of P. neoaphidis lasted longer on the leaves near the base of field beans than on leaves near the top. The infectivity also lasted longer on the abaxial surface than on the adaxial surface. They attributed this to the greater protection on abaxial surface from rain and harmful solar radiation. Transpiration, respiration (in maintaining high humidity), and greater protection from wind on lower leaflets than on upper leaflets were also thought to contribute to the differences. The location that an aphid species inhabits the plant may influence the level of infection and its association with weather factors (Feng et al. 1991). Feng et al. (1992) suggested that aphids inhabiting the plant parts like rolled leaves that retain high humidity would have better chances for disease development and less chances of exposure to inocula of pathogens, while those infesting other parts of the plant would have better exposure to inocula, but the infections in those populations largely depend on environmental factors. The level of infections in tobacco aphids by unidentified fungal pathogens found to be variable in upper and lower parts of tobacco plants (Lykouressis and Mentzos 1995). These findings are indicative of possible variation in the infection levels in tobacco aphids inhabiting different leaf positions of a tobacco plant. Lampert (1989) found that the number of tobacco aphids was higher in the middle 1/3 of the tobacco plant, while the density of aphids was higher in the upper 1/3 of the plant. The epizootics of *P. neoaphidis* and another fungal pathogen, *Schizolachnus piniradiatae* (Davidson),

were influenced by level of inoculum and density of aphid populations (Wilding 1975, Soper and MacLeod 1981).

Occurrence of *P. neoaphidis* on different tobacco cultivars and influence of cultural practices. There are no reports available on the influence of host plant, its cultivar, or the cultural practices on *P. neoaphidis* occurrence in aphid populations. Aphid populations vary on different tobacco introductions and cultivars in response to the resistance of the tobacco plant (Thurston 1961, Thurston et al. 1977, Johnson 1978, 1980). The stage of tobacco development and cultural practices also influences the level of aphid infestations (Thurston 1961, Semtner 1984, Lampert 1989, McPherson 1989). Populations of tobacco aphids increase until the tobacco is topped and decline rapidly thereafter (Semtner 1984, Lampert 1989, McPherson 1989). Usually, early transplanted tobacco suffers from early and high aphid infestations (Semtner 1984 and McPherson 1989, McPherson et al. 1993). These variations in aphid populations might influence the level of *P. neoaphidis* infection as it appeared in the infections in the *A. pisum* (Wilding 1975) and also in case of another fungal pathogen, *Schizolachnus piniradiatae* (Soper and MacLeod 1981). Leaf surface chemicals of tobacco are known to be toxic to phytopathogenic fungi (Cruickshank et al. 1977, Severson et al. 1985, Menetrez et al. 1987, Menetrez et al. 1990). Variation of leaf surface chemistry occurs among different tobacco cultivars (Girardeau et al. 1973,

Severson et al. 1984, Johnson et al. 1985, Severson et al. 1985) and this could influence *P. neoaphidis* and its infections in the tobacco aphid, although such influence on entomopathogenic fungi is not known.

Introducing *P. neoaphidis* into populations of tobacco aphids for their control.

Conidiobolus obscurus Remaudière & Keller (=*Entomophthora thaxteriana* (Petch.) Hall & Bell) and *Zoophthora radicans* (Brefeld) Batko (=*Entomophthora sphaerosperma* sensu Thaxter) were the first two species of fungi used against various aphids and tetranychids in the greenhouses and in the field. In vitro grown cultures of these fungi provided good control of peach-potato aphids and tetranychids (Eguina and Tsinovskii 1972, Eguina et al. 1977, Tsinovskii and Eguina 1972). *P. neoaphidis* used as mycelium produced in a fermenter gave satisfactory control of several species of aphids only under heavy aphid densities on lettuce (Dedryver and Rabasse 1982). Field tests of *P. neoaphidis* showed that a homogenate of the fungus grown on agar plates failed to establish infection in *Aphis fabae* populations (Wilding et al. 1986). However, the applications of powder of fungus-killed aphids and release of living laboratory-infected aphids were successful in establishing infection (Wilding et al. 1986). In field experiments, Latteur and Godefroid (1982), obtained poor control of cereal aphids treated with the mycelium of *P. neoaphidis* cultured in nutrient broth. This pathogen was later found to be another species, with low pathogenicity, closely

related to *P. neoaphidis*.

Wilding et al. (1990) were successful in establishing infection in cereal aphid populations by applying dried *P. neoaphidis*-killed aphids. Wilding (1982) reported that application of powder of fungus-killed pea aphids (2.8 mg/plant) initiated infection as effectively as the release of living-infected aphids. The infections were higher in aphids in irrigated than in unirrigated fields. Wilding (1982) also observed a two-fold increase in the yield of beans in plots treated with the pathogen than in untreated plots. However, the pathogen-treated plots only had half of the yield of insecticide-treated plots.

Virulence of *P. neoaphidis* to tobacco and green peach aphids. Biotypes of the pea aphid, *A. pisum* showed differences in their susceptibility to *P. neoaphidis* (Milner 1982 and 1985). Isolates of *P. neoaphidis* also differed in their virulence to resistant and susceptible biotypes of the pea aphid (Milner 1982 and 1985). Two out of 11 isolates of *P. neoaphidis* infected both resistant and susceptible biotypes of the pea aphid on alfalfa leaflets in the laboratory studies in Australia (Milner 1982). The prevalence of *P. neoaphidis* also varied among different species of aphids (Dean and Wilding 1971 and 1973, Cameron and Milner 1981, Feng et al. 1990, Feng et al. 1991). It was not known if *P. neoaphidis* is equally virulent to the tobacco aphid and the green peach aphid, *M. persicae* (Sulzer) and to different biotypes of the tobacco aphid.

Influence of temperature on the developmental morphology of *P. neoaphidis* on tobacco aphid cuticle, tobacco leaf, and coverslip. Milner and Bourne (1983) reported no consistent effect of temperature on infectivity of the primary spores of *P. neoaphidis*. They also observed that increasing the leaf wetness period for 24 h after inoculation resulted in a higher infection in blue-green aphid, *A. kondoi* Shinji in alfalfa. Higher relative humidities near saturation are required for conidial germination and host invasion of *P. neoaphidis* and other entomophthorales (Wilding 1971, Carruthers and Hural 1990, Yu et. al. 1995). Studies of Nordin et al. (1993) indicated that an isolate of *P. neoaphidis* obtained from the tobacco aphid did not produce conidia or infect aphids at temperatures higher than 30^0C or at relative humidities 98%. However, Brobyn et al. (1987) showed that the infectivity of *P. neoaphidis* conidia persisted longest at medium to low relative humidities (for 14 d at 40% and for >21 d at 5% relative humidity) on alfalfa leaves, and for 21 days at 40% relative humidity on coverslips. They also reported that conidia lose their infectivity faster at higher humidities as they produce successive generations of conidia and exhaust their energy reserves. The response of the pathogen also varies on different surfaces. The loss of infectivity *P. neoaphidis* conidia was faster, which means germination was higher, on coverslips than on bean leaves at 70-77% relative humidity (Brobyn et al. 1987). In the case of *Aschersonia aleyrodis*, another fungal pathogen, conidial

germination was higher on the cuticle of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), than on cucumber leaf (Fransen 1995). The mode of germination of the entomophthoralean, *Zoophthora radicans* Batko significantly differed on the three body regions of the potato leafhopper, *Empoasca fabae* at 20^0C and 100% relative humidity (Wraight et al. 1990).

The optimum temperature for germination of *P. neoaphidis* is between 18 and 23^0C (Morgan et al. 1992, Morgan et al. 1995). The germination of primary conidia of another entomophthoralean, *Z. phalloides* Batko, on coverslip was directly proportional to the increase in temperature between 10 and 20^0C (Glare et al. 1986b). Wraight et al. (1992) observed that optimum temperatures for infection and germination of conidia were 23 and 22^0C, respectively for *Z. radicans* on the green leafhopper, *E. kraemeri* Ross & Moore, and germination was affected at 28^0C and above. The infections *Z. phalloides* in nymphs of *M. persicae* was higher at 12, 15 and 18^0C with 56, 44, and 53% mortality, respectively (Glare et al. 1986a). The infections were considerably lower out of this range with 28, 15 and 2% mortality at 10, 20 and 22^0C, respectively.

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CHAPTER III

Seasonal incidence of *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Zygomycotina: Entomophthorales) in *Myzus nicotianae* Blackman (Homoptera: Aphididae) on flue-cured tobacco.

Abstract: Seasonal incidence of *Pandora neoaphidis* (Remaudière & Hennebert) Humber in the tobacco aphid, *Myzus nicotianae* Blackman, was investigated on flue-cured tobacco in Virginia from 1992 to 1995. In 1992 and 1994, *P. neoaphidis* was first observed during the first 10 days of July and epizootics peaked during early August. In 1995, the epizootic started during the first week of July and peaked on 14 July. In 1993, the pathogen was not found in the tobacco fields intended for this study although it occurred in nearby fields during the first week of August. The maximum levels of infection observed in the field populations of aphids were 34, 91, and 15% in 1992, 1994 and 1995, respectively. Aphids without the symptoms of infection were sampled and observed for disease development in the laboratory in 1992. During 1993-95, single leaf samples with aphids were collected for the same purpose. Leaf samples were also collected between late August and early November from suckers grown from cut-back tobacco in the fall, 1993. The maximum levels of infection in the laboratory

observations were 69, 30, 97, and 9% in 1992, 1993, 1994, and 1995, respectively. Topping (removal of terminal floral portion) of tobacco seemed to interfere with disease development as the infection in aphids on topped tobacco did not multiply to the level observed in aphids on tobacco that was not topped. Fields located short distances apart showed wide variation in the timing and extent of incidence of *P. neoaphidis.*

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, seasonal incidence, tobacco.

Introduction:

The tobacco aphid, *Myzus nicotianae* Blackman, is a major pest of tobacco in North America which causes great reductions in the yield and quality of tobacco leaf (Cheng and Court 1977, Mistric and Clark 1979, Cheng and Hanlon 1985, Reed and Semtner 1992). Blackman (1987) described the tobacco feeding form of the green peach aphid, *M. persicae* (Sulzer), as the tobacco aphid, *M. nicotianae*. In recent years, a red morph of the tobacco aphid has replaced the common green morph (McPherson 1989, Reed and Semtner 1991). This red morph is more tolerant of high temperatures and has higher rates of development, survival, and fecundity than the green morph (Lampert and Dennis 1987, Reed and Semtner 1991). In addition, it has greater resistance to many aphicides (McPherson and Bass 1990, Harlow and Lampert 1990). The number of effective aphicides has decreased considerably since the mid-1980s and chemical control has been difficult where the red forms predominate (Harlow and Lampert 1990). This increases the importance of effective alternatives to chemical control.

There have been several studies of entomopathogenic fungi infecting various species of aphids on different crops (Milner et al. 1980, Glare et al. 1986, Feng et al. 1990, Poprawski et al. 1992). The entomophthoralean, *Pandora (Erynia) neoaphidis* (Remaudière & Hennebert) Humber is the most common (Wilding and Brady 1984, Sivcev 1991, Kish et al. 1994) and dominant (Pickering et al. 1989, Feng et al. 1990, Feng et al. 1991, Sivcev 1992) pathogen of various species of aphids on different host plants. Laboratory studies of *P. neoaphidis* infecting *M. nicotianae* were reported by Yu et al. (1995), but field investigations on the occurrence of *P. neoaphidis* in *M. nicotianae* have not been published.

The objective of this study was to investigate the seasonal incidence of *P. neoaphidis* in populations of the tobacco aphid on flue-cured tobacco and evaluate the potential of the pathogen for reducing tobacco aphid populations.

Materials and Methods:

This study was conducted at the Southern Piedmont Agricultural Research and Extension Center (SPAREC), Blackstone, Virginia from 1992 until 1995 on flue-cured tobacco. Standard agronomic practices recommended by the SPAREC for flue-cured tobacco production in Virginia were followed except that plants were not topped (removal of terminal floral portion) in some fields and none were treated with aphicides (Reed et al. 1995). Fenamiphos (Nemacur 3), pebulate (Tillam 6E), chlorpyrifos (Lorsban 4E), and metalaxyl (Ridomil 2E) were applied as preplant treatments for the control of nematodes, weeds, wireworms, and blackshank and blue mold, respectively. Aphid populations usually decrease after topping (Semtner 1984, Lampert 1989) and avoiding that practice helped to maintain high aphid populations.

Ambient temperature, relative humidity, leaf wetness, rainfall, and solar radiation information was collected from a weather station (Omnidata International, Logan, Utah) located about 0.8 km from the field site during 1993-95. In 1992, ambient temperature and rainfall data were obtained from a hygrothermograph and rain gauge at the weather station.

1992: The experiment was conducted in three fields. Fields 1 (0.06 ha) and 2 (0.11 ha) were located about 80 m apart and were transplanted into flue-cured tobacco 'NC 567' on 12 and 13 May, respectively. Field 3 (0.1 ha) was located about 0.5 km from the first two fields and was transplanted into 'K-326' on 28 May. Fields were topped during the second half of July. Incidence of *P. neoaphidis* infection was monitored during July and August. The fields were checked once a week during the first 3 wk of July and twice a week during rest of the observation period. On each observation date in July, 150 plants in each field were randomly selected and aphids were counted on one of the upper four leaves

(at least 15 cm long). The number of sample plants was limited to 50 for observations made during August. Fifty aphids were sampled between the fourth and sixth lateral veins on the right abaxial side of the midvein and the number of healthy and *P. neoaphidis*-infected aphids were counted. If necessary, the sample area was expanded until 50 aphids were counted. The proportion of fungus-killed aphids in each sample was calculated.

In addition to the field observations, 50 aphids that were apparently healthy were collected from each of 25 randomly selected plants, in each field, between 28 July to 19 August, to observe the disease development in the laboratory. Aphids were placed on tobacco leaf discs (90 mm dia) in a Petri plate (100 by 15 mm) with a moist filter paper (Whatman No. 4, 90 mm circle) in the bottom. Moist filter paper maintained high relative humidity in the Petri plate. These aphids were incubated at 16L:8D photophase and 22-24^{0}C. Aphids were checked daily for 2-3 d and proportion of aphids killed by *P. neoaphidis* during this period was calculated.

1993: The pathogen was not seen in any of the three fields intended for this study. However, the was observed in another field in early August. The incidence of *P. neoaphidis* was monitored from 30 August to 1 November on suckers grown from tobacco that had been cut-back. This field (0.26 ha) was planted on 12 May in flue-cured tobacco 'K-326'. Plants in this field were cut about 15 cm above the ground on 26 July and suckers were allowed to grow. Since the growing season was about to end at that time, field observations were not taken. However, single leaf samples were collected from 25 randomly selected plants. Leaves, 15-20 cm long, were collected at 1 to 5 d intervals and placed separately in plastic bags after the total number of aphids on each leaf had been counted. These plastic bags were sealed and aphids were incubated at 16L:8D photophase and 22-24^{0}C. The number of aphids infected by *P. neoaphidis* from each leaf sample were counted

the next day and the percent infection was calculated.

1994: The incidence of *P. neoaphidis* was monitored in three fields. These fields were transplanted into flue-cured tobacco 'K-326' on 10 May in Fields 1 (0.2 ha) and 3 (0.08 ha), and on 17 May in Field 2 (0.08 ha). The three fields were no more than 170 m apart. About 75 plants each in Fields 1 and 3, and 35 plants in Field 2 were randomly selected and marked with flags. Disease incidence was monitored on these plants. Sample plants were checked twice a week from the middle of June to the third week of August in Fields 1 and 3, and from the end of July to the third week of August in Field 2. Aphids numbers were estimated on all leaves of each sample plant on each observation date. Once the pathogen appeared in aphid populations, the number of infected aphids was also counted. Both living-infected (pale colored) and fungus-killed (golden brown cadavers) aphids were counted as infected aphids. Plants in Fields 1 and 3 were topped on 26 July. Observations were started in Field 2 after the other two fields had been topped.

Single leaf samples were also collected, following the procedure used in 1993, to observe disease development in the laboratory. Samples were collected twice a week from 18 July to 28 August on 10 dates. On the first three dates (on 18, 22, and 25 July), leaves were sampled from Field 1. Since that field had been topped on 26 July, samples were taken from an untopped field adjacent to Field 1, for the next four observation dates (on 29 July, 2, 3, and 9 August). Aphid and infection levels started declining in this second field after 9 August, while the pathogen was at higher levels in an adjacent field on suckers grown from cut-back tobacco. The samples for the last four observation dates (on 18, 24, and 28 August, and 3 September) were collected from suckers on cut-back tobacco near the second field.

1995: The study was conducted in two fields (each 0.1 ha) located about 200 m apart. These fields were transplanted into flue-cured tobacco 'K-326' on 17 May.

Fifty plants in each field were randomly selected and marked with flags. One half of each field was topped on 25 July and the other half was left untopped. Each of the topped and untopped sections had 25 sample plants. Marked plants were checked for aphids twice a week between 5 July and 8 August. The number of healthy and *P. neoaphidis*-infected aphids were counted for all leaves of each sample plant and the percentage of infected aphids was calculated on each observation date.

Leaf samples were collected twice a week from Field 1 between 10 July and 3 August. The proportion of diseased aphids was determined in the manner described for previous years.

Identification of *P. neoaphidis*: On each sampling date, conidia collected on coverslips from 5-10 cadavers were examined in lactophenol under phase contrast microscope. The identity of *P. neoaphidis* was confirmed based on the descriptions of Remaudière and Hennebert (1980), Wilding and Brady (1984), and Humber (1989).

Statistical analysis: The data were analyzed using PROC MEANS statement of SAS software (SAS Institute 1987). Means for total number of aphids and percent infection per plant (for field observations) and per leaf (for laboratory observation) were obtained.

Results:

1992:

Field observations: Infection of *P. neoaphidis* in tobacco aphids was first observed on 8 July (Fig. 3.1A). Afterwards there was a decrease in infection levels in all three fields, probably due to increase in ambient temperature and a brief period of dry weather (Fig. 3.1). The average daily temperatures increased from about 23^0C on 8 July to about 31^0C on 13 July, while the daily maxima increased from about 30 to 38^0C during the corresponding period (Fig. 3.1B).

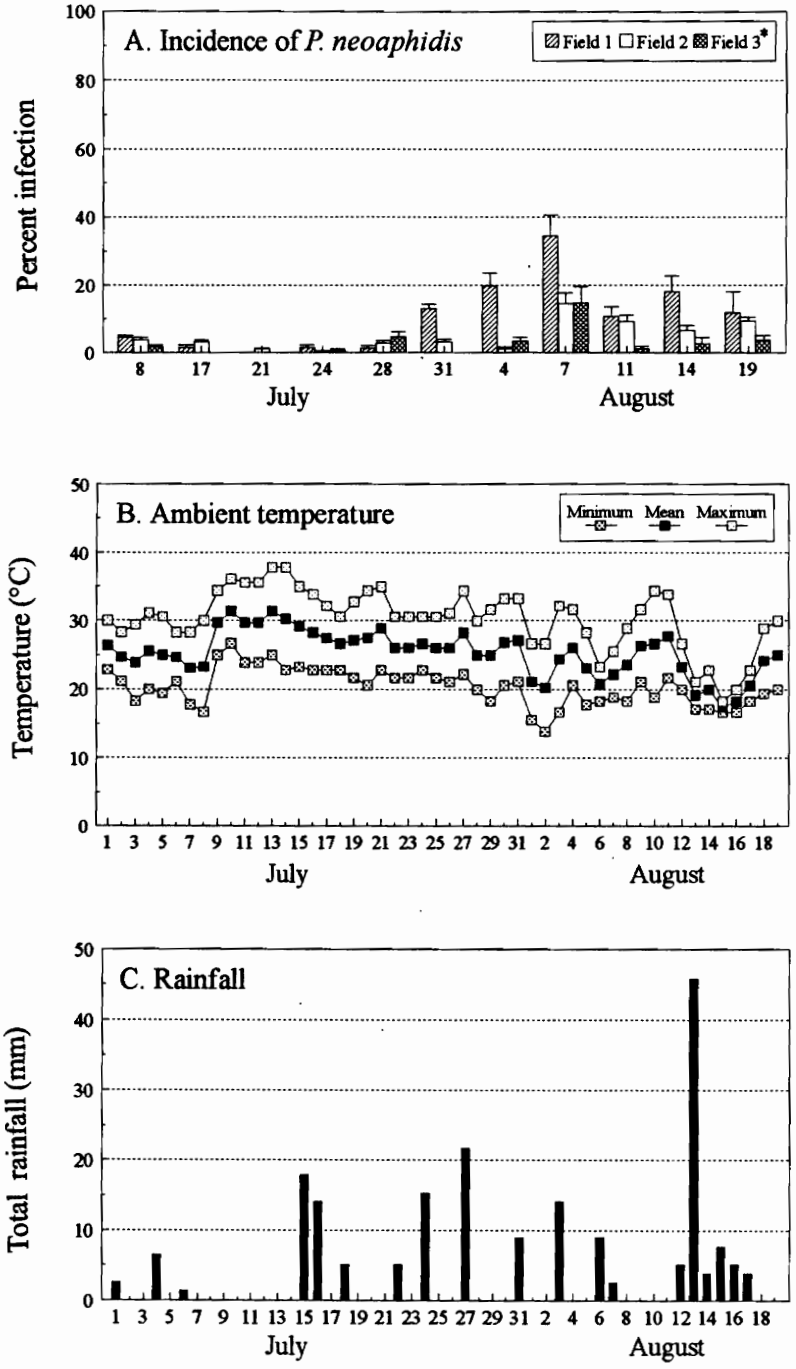

Fig. 3. 1. Seasonal incidence of *P. neoaphidis* in populations of *M. nicotianae* on flue-cured tobacco in Fields 1, 2, and 3 (A) along with information on temperature (B) and rainfall (C) prevailed during that period in 1992. (Appendix 1)

*Observations were not made in Field 3 on 17 July.

After reaching a low on 21 July, the level of infection started to increase. Moderate rainfall during the second half of July might have helped the pathogen reestablish in aphid populations (Fig. 3.1C). Following a week of relatively cool and moderately wet weather, the epizootics peaked in all fields on 7 August, after which they started to decline. Specific observations on aphid population levels were not made during this period, but general observations indicated that populations decreased after topping tobacco during the second half of July. Although the level of infection was twice as much in Field 1 as in Field 2 on 7 August, the seasonal incidence of the pathogen followed a similar trend in both fields which were closely located. However, in the distant Field 3, there was a drop in infection level before it peaked, and a slight increase on the last two observation dates following a drastic fall after the peak was observed.

Laboratory observations: The level of infection in aphids sampled from the field and incubated in the laboratory was higher than that observed in the field throughout the observation period in Fields 1 and 2, and during most of the observation period in Field 3 (Figures 3.1A and 3.2A). This could be attributed to the conditions of moderate temperature and high humidity in the laboratory which were more conducive for disease development than field conditions. *P. neoaphidis* requires relative humidities near saturation for sporulation (Wilding 1971, Yu et al. 1995). The seasonal trends of field and laboratory observations did not correspond in Fields 1 and 2. The levels of infection were similar between 28 July and 4 August and were highest in aphids collected on 28 July from those two fields, while the highest level of infection in field observations was seen on 7 August. The seasonal trend was similar in field and laboratory observations for Field 3. However, the level of infection was slightly lower in the laboratory than in the field on 4 and 7 August.

1993:

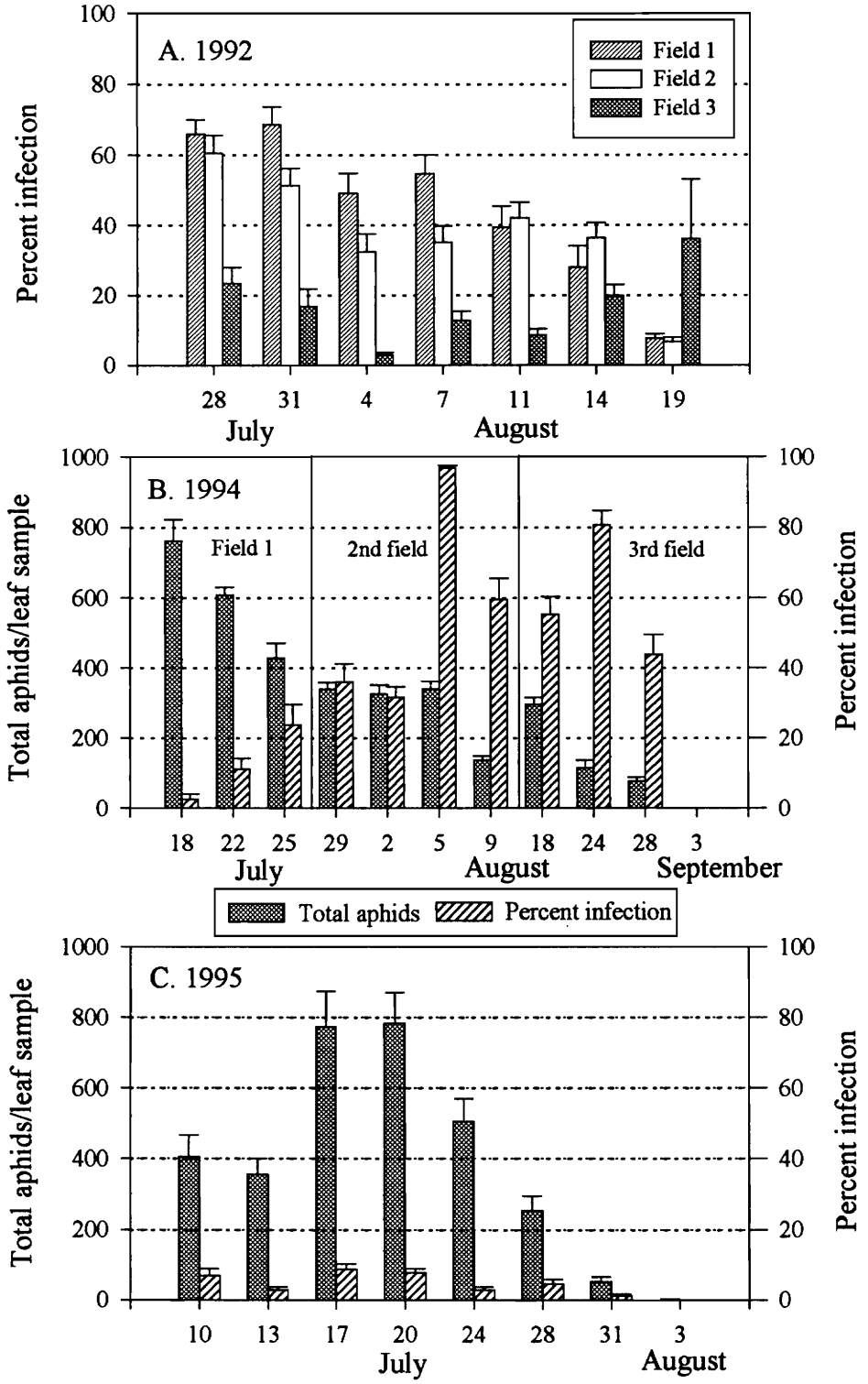

Fig. 3. 2. Development of *P. neoaphidis* in *M. nicotianae*, sampled from field tobacco and incubated in the laboratory, during tobacco growing seasons in 1992 (A), 1994 (B), and 1995 (C). (Appendix 2)

Field observations: Infections of *P. neoaphidis* in tobacco aphid populations would be expected in July based on the observations during 1992, 1994 and 1995. However, 1993 was relatively drier than the other three years during the tobacco growing season. Except for 76 mm of rainfall on 2 and 3 July, the rest of July received a total of 14 mm of rainfall. Daily average temperatures ranged between 25 and 31^{0}C, while maximum temperatures were between 35 and 39^{0}C during most of July. A very low level of infection by *P. neoaphidis* was seen in some populations of aphids in some fields at the SPAREC during early August, but the pathogen was not observed in the fields intended for this study.

Laboratory observations: The level of infection in tobacco aphids, on leaf samples collected from suckers of cut-back tobacco and held in the laboratory, fluctuated throughout the sampling period (between 30 August and 1 November) (Fig. 3.3). Higher levels of infection were recorded in October than in September, with a maximum of 30% aphids infected in the samples collected on 28 October. The monthly average temperatures for September and October were 22 and 14^{0}C, respectively. Although aphids were incubated under controlled conditions, cool weather during October might have promoted the spread of the pathogen in the field resulting in a corresponding increase in the infection in laboratory observations during that period.

1994:

Field observations: Occurrence of *P. neoaphidis* epizootics showed great variation among three fields. The pathogen was first observed on 7 July in Field 1, and infection peaked on 1 August (Fig. 3.4A). The epizootic remained at a very low level for about 3 wk (≤0.3% infection until 21 July) after it was first observed. Infection in tobacco aphids was seen on 1-2% of sample plants between 7 and 14 July and spread to about 32 and 40% of sample plants on 18 and 21 July, respectively. After a period of moderate rainfall with daily average temperatures

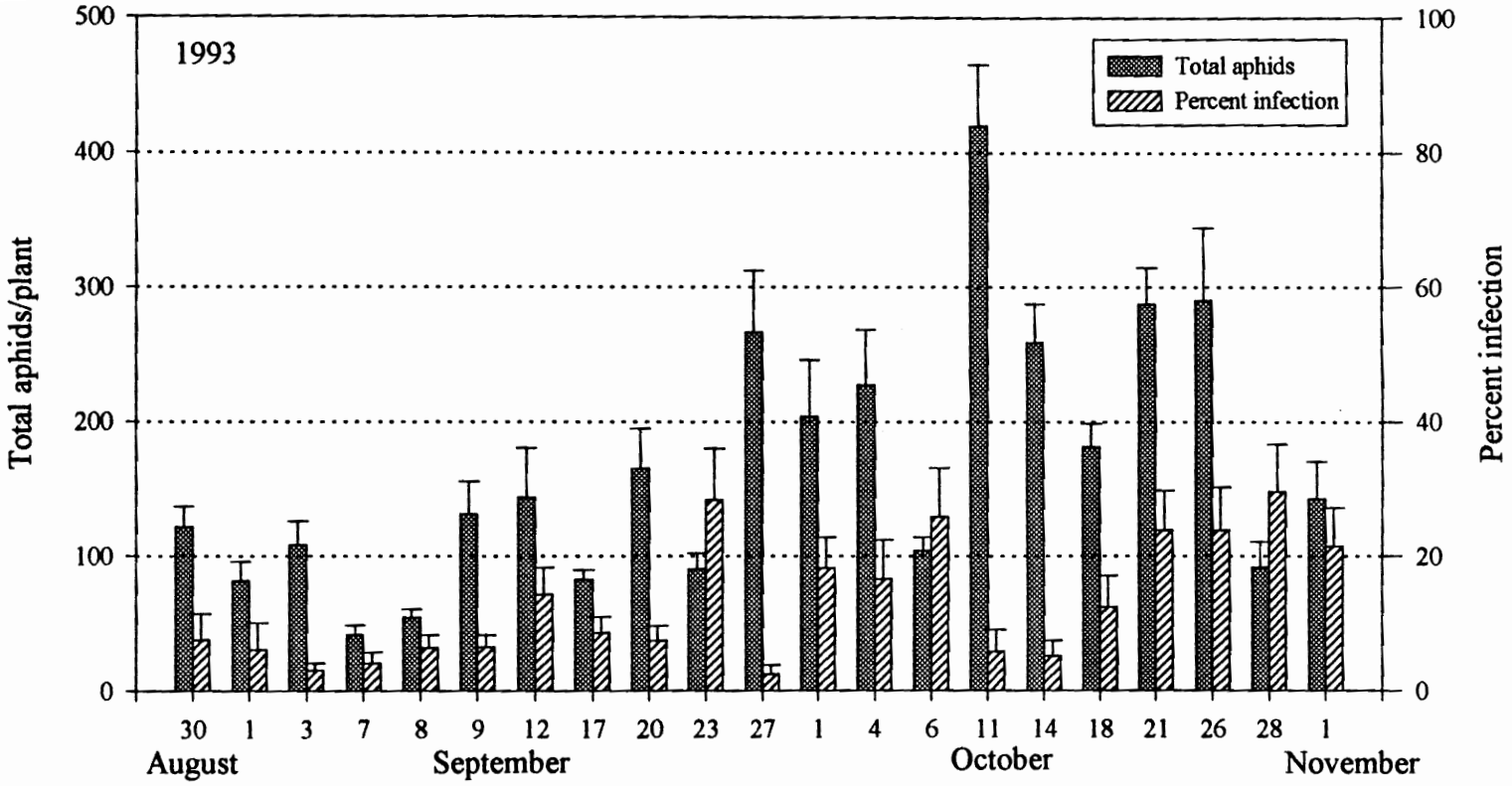

Fig. 3. 3. Development of *P. neoaphidis* in *M. nicotianae* sampled from suckers on cut tobacco in the field and incubated in the laboratory in 1993. (Appendix 3)

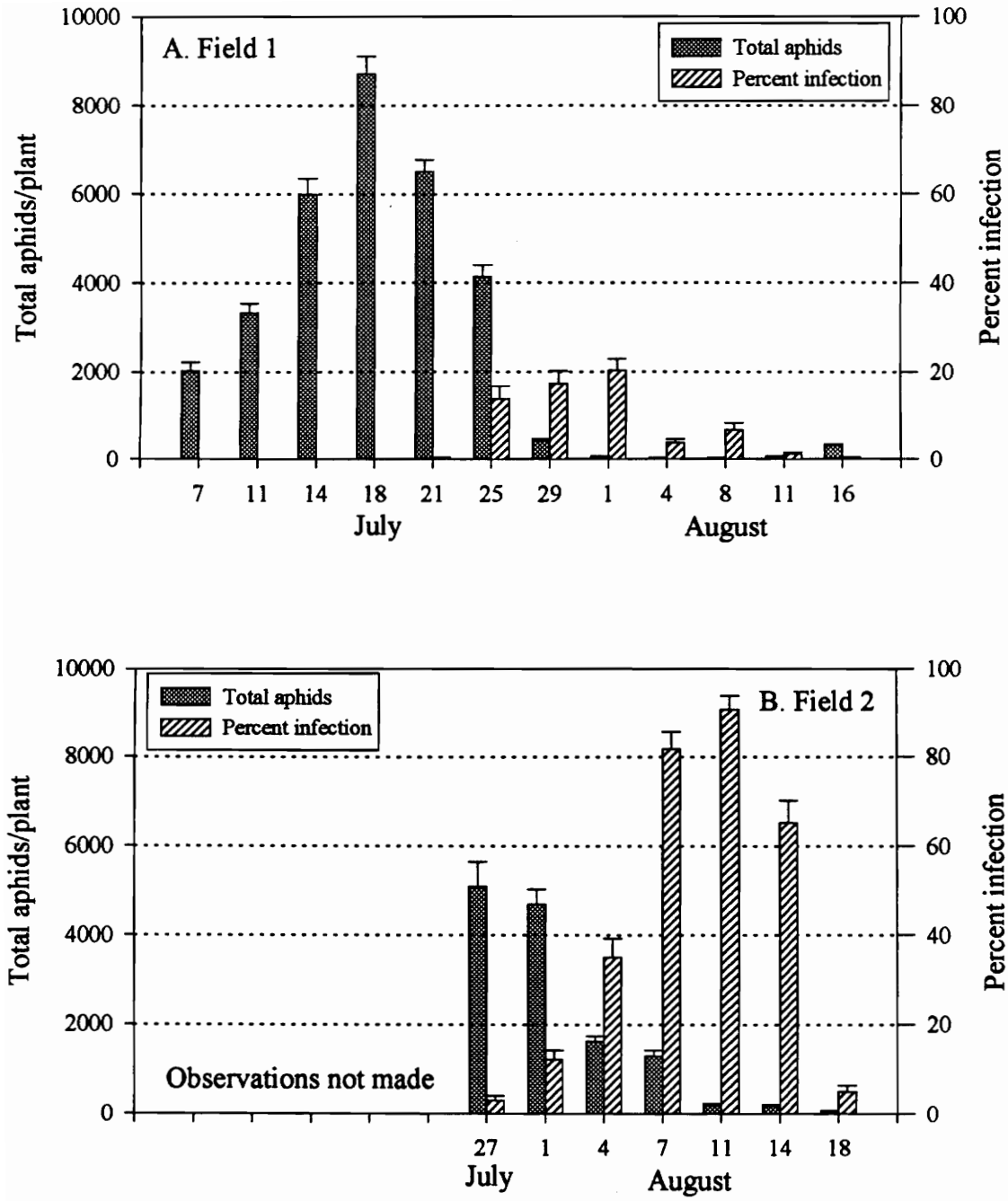

Fig. 3. 4. Seasonal incidence of *P. neoaphidis* in populations of *M. nicotianae* on flue-cured tobacco in Fields 1 and 2 in 1994.

below 26^0C, the epizootic increased to 6% infection on 25 July (Figures 3.4A, 3.5A and B) when aphids on nearly 90% of sample plants were infected. The weather was relatively cool and wet after 25 July (with average daily temperature at about 23^0C and total rainfall of 40 mm from 26 to 29 July) during which epizootic developed rapidly and peaked with 20% infection on 1 August. There was also an increase in the average daily relative humidity (from 85 to 96%) and leaf wetness (from 33 to 68%), and a decrease in the average daily solar radiation (from 883 to 385 $\mu molesec^{-1}m^{-2}$) during the last week of July. These conditions may have helped the establishment and further spread of the pathogen in tobacco aphid populations (Figures 3.5C and D). A sudden decrease in aphid populations after the tobacco was topped on 26 July prevented the epizootic from further development after 1 August. In the adjacent untopped tobacco in Field 2, the infection level increased from about one percent on 27 July to 91% on 11 August, and aphid populations declined from 1052 to 192 per plant on respective dates (Fig. 3.4B). There was a cool spell before the peak infection, when the minimum temperature was about 12^0C for 3 d which could have favored the incidence of *P. neoaphidis*. Surprisingly, in Field 3, which was also topped, the pathogen was not seen until 10 August when low level of infection (1.6%) was seen on only 12% of the sample plants. Wide variation occurred in the spatial and temporal distribution of the level of infection in aphid populations among the fields that were closely located within an area of about 2-3 ha.

Laboratory observations: Leaf samples were collected from three fields located adjacent to each other, and the number of tobacco aphids per leaf sample and the proportion of those infected by *P. neoaphidis* from the three fields were presented together (Fig. 3.2B). The level of infection increased from one percent in samples collected on 18 July to 16% in those collected on 25 July from Field 1. As topping of tobacco prevented further sampling from that field, an adjacent field was used

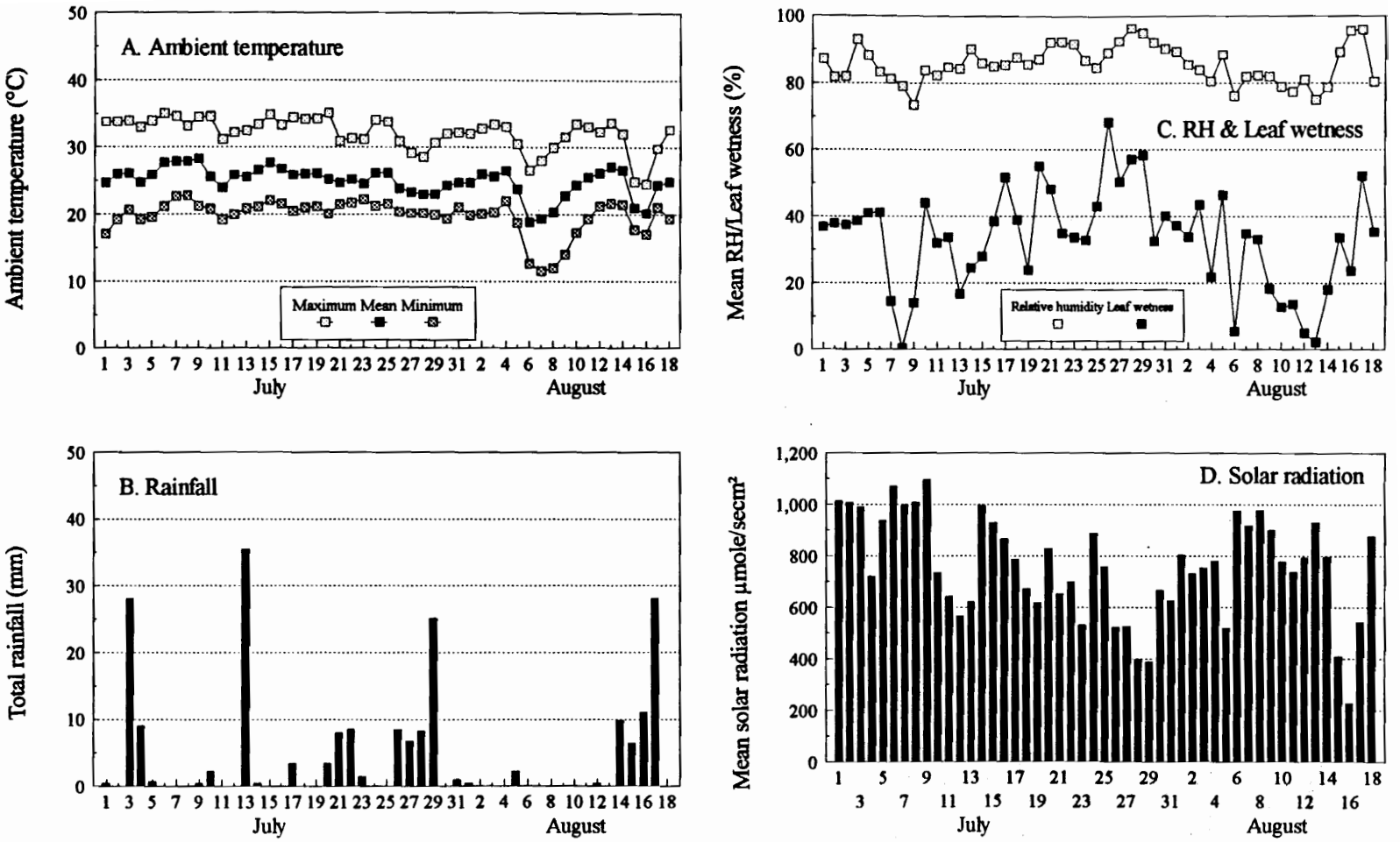

Fig. 3. 5. Weather conditions prevailed during the incidence of *P. neoaphidis* in populations of *M. nicotianae* on flue-cured tobacco in 1994. (Appendix 5)

for observations in which infection increased from 36 to 97% in samples collected on four dates between 29 July and 5 August. The number of aphids and percent infection were almost zero (data not presented in Fig. 3.2B) on 18 August, when the pathogen was still present in tobacco aphids on suckers of cut-back tobacco in another field adjacent to the second field. In the third field, the infection increased from 55 to 81% between 18 and 24 August, before it started to decline. No aphids or infection were found on leaf samples collected on 3 September. These results also indicate variation in the occurrence of epizootic among different fields within a small area.

1995:

Field observations: *P. neoaphidis* was first noticed at a very low level (<1%) on one sample plant in Field 1 on 1 July. Actual counts were started on 5 July when the level of infection was around one percent in different fields (Fig. 3.6). The maximum levels of infection found during the observation period ranged from 6 to 15% on 14 July in different sections of Fields 1 and 2. Total rainfall was 74 mm in the week before the infection was first seen on 1 July (data not presented in the graph), 45 mm between 1 and 5 July, and 63 mm between 5 and 13 July (Fig. 3.7B). This wet spell may have helped establishment of pathogen and progression of infection. The daily averages, maxima, and minima of temperature ranged between 23 and 26^{0}C, 31 and 34^{0}C, and 15 and 20^{0}C, respectively, between 5 and 13 July. However, high temperatures with daily averages $\geq$ 28^{0}C and maxima between 35 and 38^{0}C prevailed during most of the time after 14 July. There was a general decrease in the relative humidity in the second half of July when it ranged, most of the time, between 75 and 82%, compared to $\geq$84% during most of the first half of July (Fig. 3.7C). The leaf wetness averaged around 38% for the first 2 wk of July, but declined to an average of 19% between 14 and 18 July and was as low as 7% for 2 d during that time (Fig. 3.7C). The solar radiation was >850

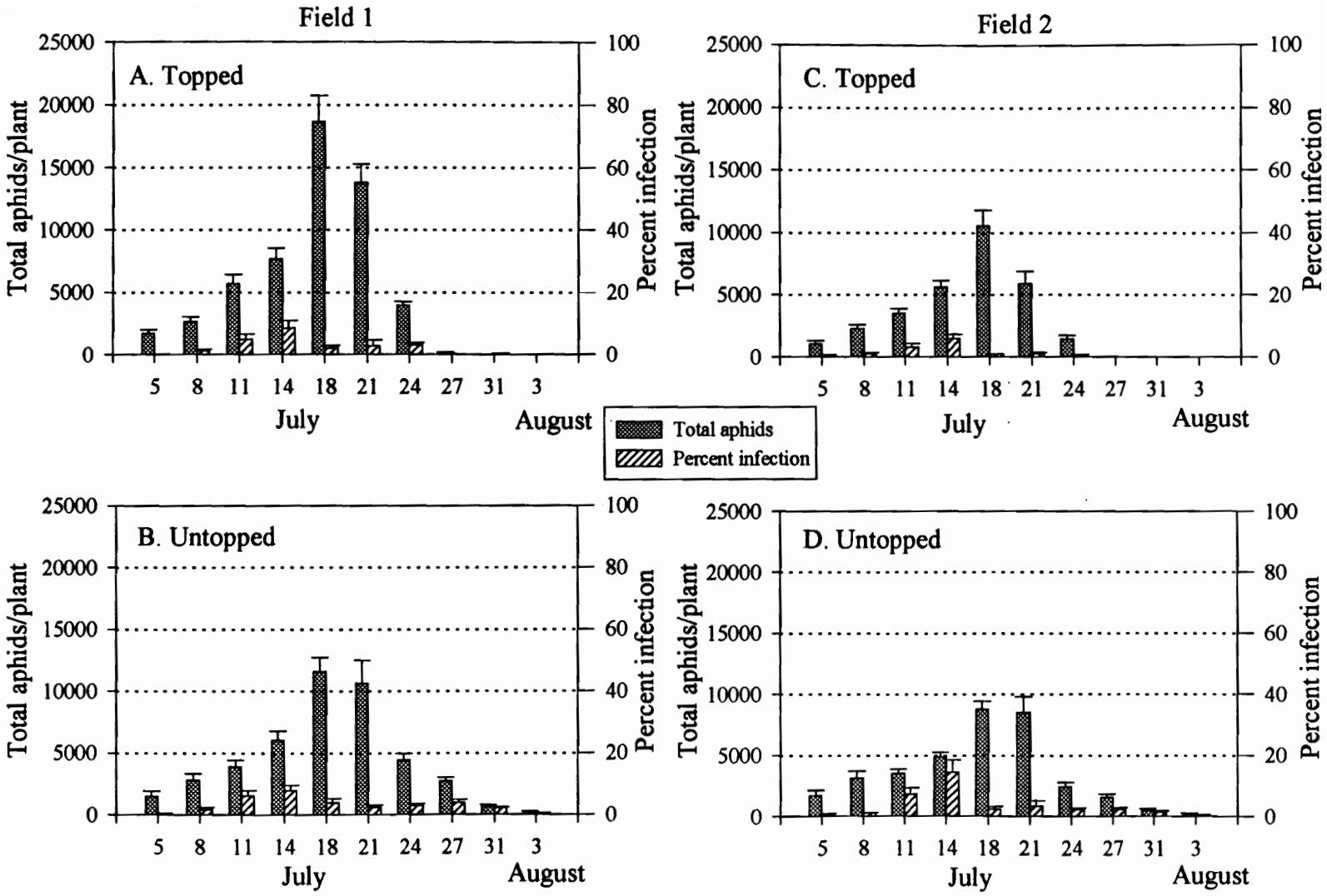

Fig. 3. 6. Seasonal incidence of *P. neoaphidis* in populations of *M. nicotianae* on topped and untopped flue-cured tobacco plants in Fields 1 and 2 in 1995. (Appendix 6)

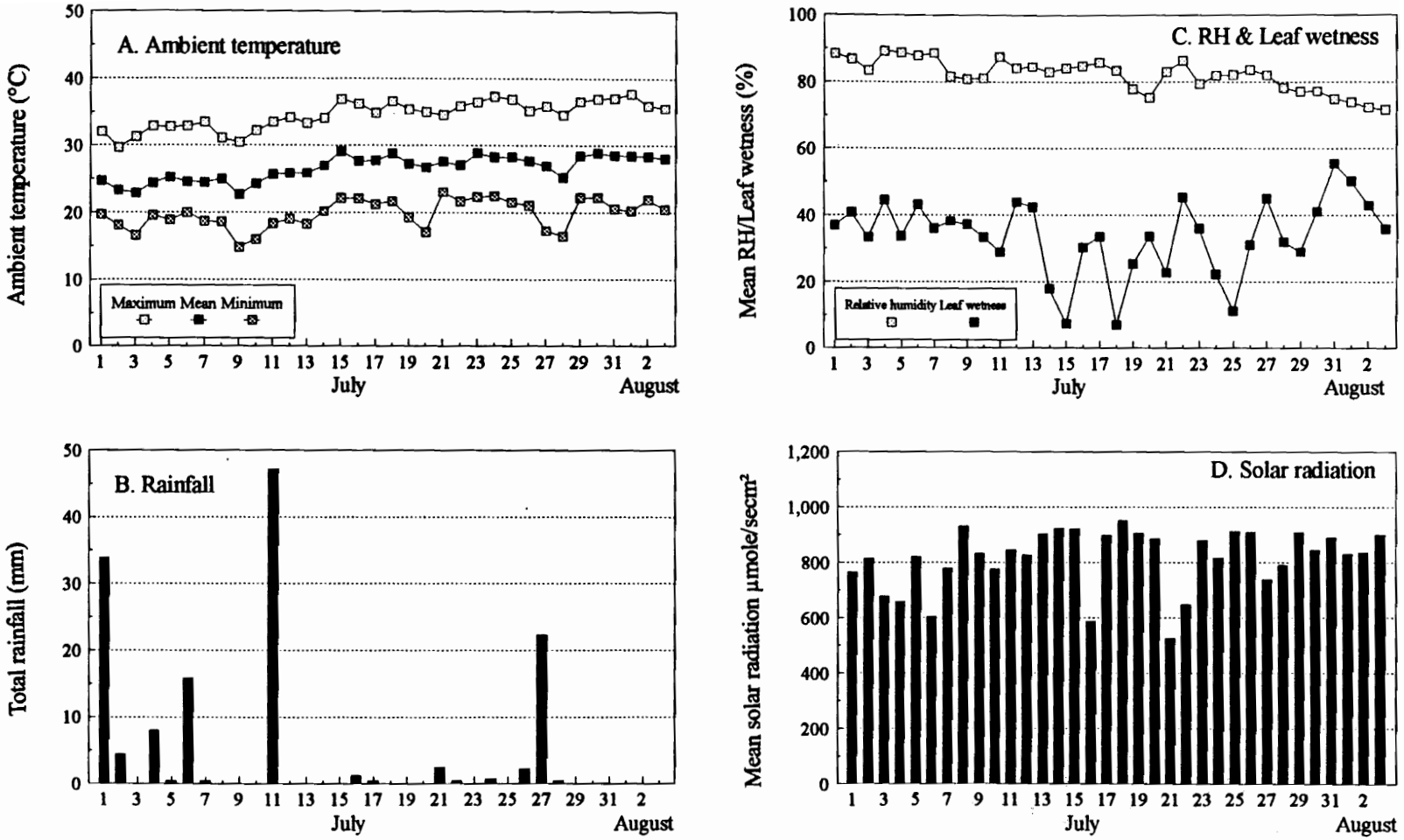

Fig. 3. 7. Weather conditions prevailed during the incidence of *P. neoaphidis* in populations of *M. nicotianae* on flue-cured tobacco in 1995. (Appendix 7)

$\mu molesec^{-1}m^{-2}$ during most of the second half of July (Fig. 3.7D). These conditions of hot and dry weather may have contributed to a rapid decline in aphid populations and prevented development of further infection towards the end of July. The seasonal progression of the epizootic followed a similar trend in all fields. Influence of topping tobacco plants on the level of infection could not be determined this year. Aphid populations and epizootic had already reached their peaks and were on the decline when tobacco plants were topped.

Laboratory observations: The level of infection in tobacco aphid populations observed in the laboratory was highest (about 9%) in samples collected on 17 July (Fig. 3.2C).

Discussion:

Entomophthorales require relative humidities near saturation for germination and host invasion (Carruthers and Hural 1990). Higher infections in aphid populations are associated with heavy rains or sprinkler irrigation in case of *P. neoaphidis* and other entomophthoraleans (Dean and Wilding 1973, Wilding 1975, Pickering et al. 1989). Dean and Wilding (1971) reported that infections of *Entomophthora* first appeared in cereal aphids after rainfall ended a long dry period. The occurrence of epizootics of entomophthorales in cereal aphid populations was preceded by periods of rainfall or heavy dew also in several investigations (Voronina 1971, Dean and Wilding 1973, Wilding 1975, Dedryver 1982). Similarly, in the present study, increase in the relative humidity and the leaf wetness, associated with the rainfall, preceded the establishment and peak incidence of *P. neoaphidis* in tobacco aphid populations. However, the influence of relative humidity and temperature was not clear on the infections of *P. neoaphidis* and *Conidiobolus* spp. in cereal aphid populations (Feng et al. 1991).

P. neoaphidis was the only pathogen infecting tobacco aphids during this study, but its influence in reducing aphid populations was unclear. As actual

counts of aphids were not taken in 1992, the influence of the pathogen on aphid populations could not be observed. The apparently increasing populations of tobacco aphids seemed to decrease as the infection increased in Field 2 in 1994 (Fig. 3.4B). However, in Field 1, aphid populations seemed to decrease before the pathogen became well established in the field (Fig. 3.4A). The number of aphids per plant decreased from 8700 on 18 July to 6500 on 21 July, when the infection increased from 0.003% to only 0.3% during the corresponding period. As this level of infection is too low to exert a major influence on aphid infestations, it would be appropriate to say that aphid populations were on decline at that time. Topping of tobacco resulted in the decrease of aphid numbers after 25 July. Further decrease in aphid numbers from 423/plant on 29 July to 57/plant on 1 August may have been due to the pathogen since the infection, during that period, increased from 17 to 20%. If the weather was not hot and dry during the second half of July, 1995, further increase in the level of infection may have occurred. Adverse effects of temperature were seen on the development of natural infections by *P. neoaphidis* and *Conidiobolus* spp. in populations of cereal aphids on irrigated spring wheat (Feng et al. 1991).

The incidence of *P. neoaphidis* was unpredictable as the timing and seasonal trends of the levels of infection in tobacco aphid populations varied widely among closely located fields. This variation could not be attributed to the differences in aphid populations among those fields. The number of aphids per plants was similar in 'untopped' and 'topped' sections of Field 2 between 11 and 14 July, in 1995, but the infection was nearly thrice and twice as high in 'untopped' as in 'topped' sections on 11 and 14 July, respectively (Figures 3.6C and D). In 1994, the number of aphids in Field 3 was close to that in Field 1, though it was lower in the former, but the pathogen did not occur until near the end of the observation period (data not presented). The difference in the seasonal

trend of infection in Field 3 from that in Fields 1 and 2, in 1992, could not have been due to the difference in cultivar (Fig. 3.1A). Tobacco aphids on 'NC 567' and 'K-326' of flue-cured tobacco had similar levels of infection by *P. neoaphidis* in another study in 1992 (see Chapter VI).

A maximum of 91% of aphids were infected on untopped plants compared with 20% on topped plants in 1994. However, influence of topping on the level of infection was not clear in 1995, as the epizootic was declining before the plants were topped (Fig. 3.6). This indicated that further increase in the infection levels could have been expected if the plants had not been topped in 1992. The proportion of infected aphids appeared to be higher in the upper most tobacco leaves (see Chapter IV) and topping probably reduces the major portion of inoculum from those leaves.

The development of *P. neoaphidis* requires high humidities (Rockwood 1950, Wilding 1971, Yu et al. 1995). In a greenhouse study, infections of *P. neoaphidis* as high as 100% were seen in tobacco aphids on the upper leaves of tobacco plants exposed to periodical water mist (personal observation). In the current study, provision of humid conditions, near saturation, might have resulted in higher infection levels in the laboratory observations. Another factor that could influence the infectivity of inocula of entomopathogenic fungi is solar radiation (Clerk and Madelin 1965, Ignoffo et al. 1977). Although the solar radiation information used in this study represented photosynthetically active radiation between the wavelengths of 400 and 700 nm, it gives a general idea about the intensity of radiation that could harm the pathogen.

The seasonal incidence of *P. neoaphidis* in tobacco aphids could be influenced by a combination of different factors including, weather conditions, reduction in aphid numbers as a result of topping tobacco, and spatial and temporal distributions of the pathogen in aphids within and between the fields. It

is also important to note that all these factors could act concurrently. The spread of epizootic may be slow if the pathogen occurs in aphids on limited number of plants. The level of infection increases as the pathogen spreads in aphids all over the field. Rainfall helps the establishment and development of the pathogen in aphid populations. Topping reduces the number of aphids including those infected by the pathogen and interferes with the further spread of the epizootic in the remaining aphid populations. Unfavorable weather conditions, in terms of high temperatures and solar radiation, and low rainfall, relative humidity, and leaf wetness, could interfere with epizootic at any level and would remain largely influential on the level of infection. However, the reason for wide variation in the extent and timing of infection between closely located fields is not known.

Summers and Newton (1989) reported 100% infection by *P. neoaphidis* of above ground populations of *Pemphigus populivenae* Fitch on sugarbeet. More than 90% *M. persicae* populations were killed by natural infections of *P. neoaphidis* on spinach (Elkassabany et al. 1992). As high as 74% of the *Acyrthosiphon pisum* collected from sprinkle-irrigated legume groundcover of an orchard were killed by *P. neoaphidis*, while no fungus-induced mortality was seen in aphids collected from drip-irrigated part of the legume groundcover (Pickering et al. 1989). In the current study, infections under field conditions up to 85% in 1994 indicate the potential of this pathogen in controlling the tobacco aphid. However, dependence of the pathogen on weather conditions and wide variation in its occurrence in different fields within a location and from year to year make it less reliable and largely unpredictable. But, the importance of this pathogen when the environmental conditions were favorable should not be overlooked.

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CHAPTER IV

Seasonal incidence of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) in red morph of *Myzus nicotianae* (Homoptera: Aphididae) on nonsolanaceous vegetable hosts.

Abstract:

Seasonal incidence of *Pandora neoaphidis* (Remaudière & Hennebert) Humber in populations of the tobacco aphid, *Myzus nicotianae* Blackman, was studied from fall to spring of 1993-94 and 1994-95 on 9 and 12 vegetable hosts (of Cruciferae, Compositae, and Chenopodiaceae families), respectively. The pathogen survived at moderate levels on these plants between October and December in 1993 and 1994. Aphid populations and plant stand were seriously affected after the first week of December in both years due to cold temperatures. No aphids and infection were seen in spring, 1994 on regrown plants, while a few aphids, some of them infected with the pathogen, were seen towards the end of winter, 1994 and early spring, 1995 on plants that overwintered. Plant stand was too low during 1995 to provide substantial counts. The overall level of infection was higher during the fall of 1993 than in 1994. In 1993, the pathogen occurred on tobacco late in the growing season (in August) and was maintained in aphid

populations on suckers growing from cut-back flue-cured tobacco. Infected aphids migrating from tobacco fields might have helped to promote high disease incidence in aphid populations on nonsolanaceous host plants in that year. Aphid populations on different host plants were significantly different almost the entire fall observation period, but the occurrence of the pathogen was not influenced by host plant on most of the observation dates in both 1993-94 and 1994-95 seasons. When the averages for the whole and, first and second halves of the fall observation period were considered, the level of infection in aphids was not significantly different ($P \geq 0.05$) among the host plants in 1993. But, in 1994, significant differences ($P < 0.05$) among host plants were noticed in averages for whole and second half of the fall, where turnip had the highest percent of infected aphids. Higher disease incidence was seen in the first than the second half of the fall in 1993, while in 1994, the incidence was higher in the latter half. Aphid populations and levels of infection were higher in 1993 than in 1994 on most of the host plants. Level of infection was higher in 1993 than in 1994. Most of the time, correlation between aphid numbers and percent infection was not significant ($P \geq 0.05$).

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, nonsolanaceous hosts, seasonal incidence, fall, winter, spring.

Introduction:

The tobacco aphid, *Myzus nicotianae* Blackman, is a major insect pest of tobacco. Blackman (1987) described the tobacco feeding form of *M. persicae* (Sulzer) as tobacco aphid, *M. nicotianae*. The tobacco aphid causes serious reductions in both yield and quality of tobacco leaf (Cheng and Court 1977, Mistric and Clark 1979, Cheng and Hanlon 1985, Lampert 1989, Reed and Semtner 1992). The tobacco aphid had been found to show resistance to chemical control (Koziol and Semtner 1984, Harlow and Lampert 1990, McPherson and Bass 1990, Chryssochoou and Bloukidis 1992, Abdel-Aal et al. 1992). This warrants the search for alternative methods of chemical control. A fungal pathogen, *Pandora neoaphidis* (Zygomycetes: Entomophthorales), has been found to be an important mortality factor for tobacco aphids on tobacco in Virginia (Dara and Semtner 1995). This pathogen infects several species of aphids on different hosts (Summers and Newton 1989, Elkassabany et al. 1992, Feng et al. 1990, Kish et al. 1994). Production of resting spores as a mechanism of overwintering is not known for *P. neoaphidis* (Rockwood 1950, Ben-Ze'ev and Kenneth 1982, Waterhouse and Brady 1982, Wilding and Brady 1984). Its conidia are known to survive winter temperatures in soil and on foliage (Latteur 1977, Schofield 1995), however, specific information on its overwintering mechanisms is unavailable. *P. neoaphidis* epizootics in aphids have been reported during spring, summer, fall, and parts of winter in different studies (Feng et al. 1990, Elkassabany et al. 1992,

Dara and Semtner 1995). There has been no report on year long occurrence of *P. neoaphidis* in a single study.

Since this pathogen appeared to be an important mortality factor in the natural control of tobacco aphid on tobacco (see Chapter III), investigation on its occurrence in tobacco aphids infesting other hosts after the tobacco season would provide information on the survival of the pathogen during nontobacco growing seasons of the year. This information could also enhance the knowledge on the year long cycle of the pathogen in tobacco aphid in a tobacco growing area. This paper presents the first detailed report on the occurrence of *P. neoaphidis* in Virginia during fall, winter, and spring. Part of the summer study was previously presented (Dara and Semtner 1995).

The objective of this study was to investigate the seasonal incidence of *P. neoaphidis* in populations of the red morph of the tobacco aphid on various nonsolanaceous vegetable host plants during fall, winter, and spring, when tobacco is not grown.

Materials and Methods:

This study was conducted at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Virginia between 1993 and 1995. The seasonal incidence of *P. neoaphidis* was studied on nine hosts during 1993-94 and on 12 hosts during 1994-95. The nine cruciferous host plants used in 1993 were,

broccoli (*Brassica oleracea* var. *botrytis* Mill. 'Green Comet'), cabbage (*B. oleracea* var. *capitata* L. 'Bonnie Hybrid), collards (*B. oleracia* var. *acephala* DC 'Georgia'), kale (*B. oleracea* var. *acephala* DC 'Premier'), mustard (*B. juncea* ssp. *juncea* L. 'Florida Broadleaf'), rape (*B. napus* L. 'Winter'), spinach mustard (*B. campestris* var. *perviridis* Bailey 'Tendergreen'), and two turnips (*B. campestris* var. *rapa* L. 'Purpletop White-Globe' and 'Seventop'). Three other hosts, lettuce, potato, and spinach, intended for study in 1993 were dropped due to poor plant stands. In 1994, only one turnip, 'Purpletop White-Globe', was used instead of two and four other hosts viz., lettuce (*Lactuca sativa* L. 'Black-seeded Simpson') (Family: Compositae), radish (*Raphanus sativus* L. 'White Tipped Scarlet') (Family: Cruciferae), rutabaga (*B. napus* var. *napobrassica* Mill.) (Family: Cruciferae), and spinach (*Spinacia oleracea* L. 'Long Standing Bloomsdale') (Family: Chinopodeaceae) were included. In both years, each host plant had one single-row (6 m long) plot replicated four times. These plots were established in a randomized complete block design. Broccoli, cabbage, and collards were transplanted at about 13 plants per row (46 cm within and 1.2 m between the rows) while others were sown using Precision Garden Seeder (Model 1001 B) (Earthway Products, Bristol, IN) with the spacing of 5 cm within the row (3.5 cm for radish) and 1.2 m between the rows. Plots were planted or sown on 15 and 8 September in 1993 and 1994, respectively.

Observations of *P. neoaphidis* incidence were made at weekly intervals from 22 October to 8 December 1993 and again on plant regrowth from 8 March to 18 May 1994 for 1993-94. On each observation date, the number of healthy and infected aphids on 5 randomly selected plants within each plot were counted from each leaf of the plant. Both living-infected (pale coloration) and fungus-killed aphids (golden brown cadavers) were considered as infected aphids. Loss of plants due to cold weather and occasional snow did not allow observations after 8 December, 1993. Counts were resumed in March 1994 on the plant regrowth. For 1994-95 season, observations were taken at weekly intervals from 13 October to 5 December 1994 (except on 10 November due to rain). Observations could not be taken until late January, 1995 due to cold temperatures. Plants were periodically checked for aphids and disease from late January to early April, 1995, but actual counts were not taken due to poor plant stand.

Weather data were recorded from a weather station (Omnidata International Inc., Logan, Utah) located about 0.5 km from the fields.

Identification of *P. neoaphidis*: On each sampling date, conidia collected on coverslips from 5-10 cadavers were examined in lactophenol under phase-contrast microscope. The identity of *P. neoaphidis* was confirmed based on the descriptions of Remaudière and Hennebert (1980), Wilding and Brady (1984), and Humber (1989).

Statistical analysis: Data were analyzed using PROC GLM of SAS software

(SAS Institute 1987). Means for total number of aphids and percent infection per five plants were obtained. Log transformed values of number of aphids and arcsine transformed values of percent infection were considered for means separation. Significant means were separated using Duncan's multiple range test ($P < 0.05$). The correlation between aphid populations and level of infection was obtained by analyzing the data using PROC CORR of SAS (SAS Institute, 1987).

Results:

1993: In general, lower numbers of aphids were observed on mustard and spinach mustard (Table 4.1, Figures 4.1A, B, and C). However, when the average for the entire fall observations (from 22 October to 8 December) was considered, the proportion of aphids infected by *P. neoaphidis* was lower on broccoli and cabbage, and higher on mustard and turnips, even though the difference was not significant ($P \geq 0.05$) (Fig. 4.2C). There was a decrease in the infection of aphids on all host plants 4 November, followed by an increase on most of the host plants during the next 2-3 observation dates before the infection began to decline finally (Table 4.2). Incidence of the pathogen was higher in the first (between 22 October and 10 November) than the second half (between 17 November and 8 December) of the fall (Figures 4.2A and B). Average level of incidence for these periods was not significantly different ($P \geq 0.05$) among host plants. The differences were also not significant on majority of dates when the infection level on individual observation

Table 4. 1. Seasonal abundance of tobacco aphids on different vegetable hosts during fall, 1993.

Host plant	Mean number of aphids per five plants ± SEM[1]							
	22 October	27 October	4 November	10 November	17 November	24 November	3 December	8 December
Broccoli	44.5 ± 16.1a	67.5 ± 23.2ab	41.8 ± 24.8ab	33.8 ± 10.8ab	37.8 ± 13.4abc	65.8 ± 22.6ab	23.3 ± 6.4a	22.8 ± 14.8b
Cabbage	34.5 ± 7.5a	118.3 ± 9.9a	51.3 ± 9.3ab	37.3 ± 8.4ab	62.3 ± 20.5a	102.5 ± 30.0ab	45.3 ± 13.1a	15.3 ± 3.8b
Collards	40.8 ± 6.5a	96.3 ± 35.5ab	70.5 ± 24.3a	51.5 ± 13.7a	67.5 ± 18.1a	178.5 ± 41.8a	23.8 ± 5.7a	21.0 ± 4.8ab
Kale	26.8 ± 10.1ab	36.0 ± 8.8bc	34.5 ± 16.5ab	41.0 ± 19.6ab	14.5 ± 8.4bc	60.5 ± 28.3ab	35.5 ± 13.5a	20.0 ± 6.7b
Mustard	5.3 ± 2.0c	3.8 ± 1.9c	1.3 ± 0.6d	5.5 ± 1.8c	8.5 ± 5.0c	32.5 ± 18.1b	25.3 ± 9.3a	20.3 ± 5.5ab
Rape	32.0 ± 11.8ab	94.0 ± 35.8ab	36.3 ± 7.7ab	47.3 ± 5.8a	39.0 ± 9.9ab	120.5 ± 46.4ab	67.8 ± 34.0a	72.3 ± 35.2ab
Spin. Mustard	4.0 ± 2.3c	6.0 ± 1.1de	4.3 ± 0.9cd	5.0 ± 1.8c	15.8 ± 5.9abc	31.8 ± 15.8b	21.8 ± 3.7a	26.8 ± 6.6ab
Turnip (ptop.)	15.3 ± 12.9bc	16.3 ± 5.9cd	20.3 ± 5.2b	18.0 ± 3.0ab	25.5 ± 8.6abc	82.0 ± 29.1ab	55.5 ± 26.2a	48.0 ± 7.6ab
Turnip (7top.)	3.5 ± 1.6c	13.3 ± 2.9d	8.5 ± 5.0c	16.0 ± 6.6bc	23.8 ± 10.1abc	51.0 ± 27.8b	80.5 ± 37.7a	71.3 ± 21.3a

[1]Means followed by the same letter within each column are not significantly different ($P \geq 0.05$; Duncan's multiple range test).

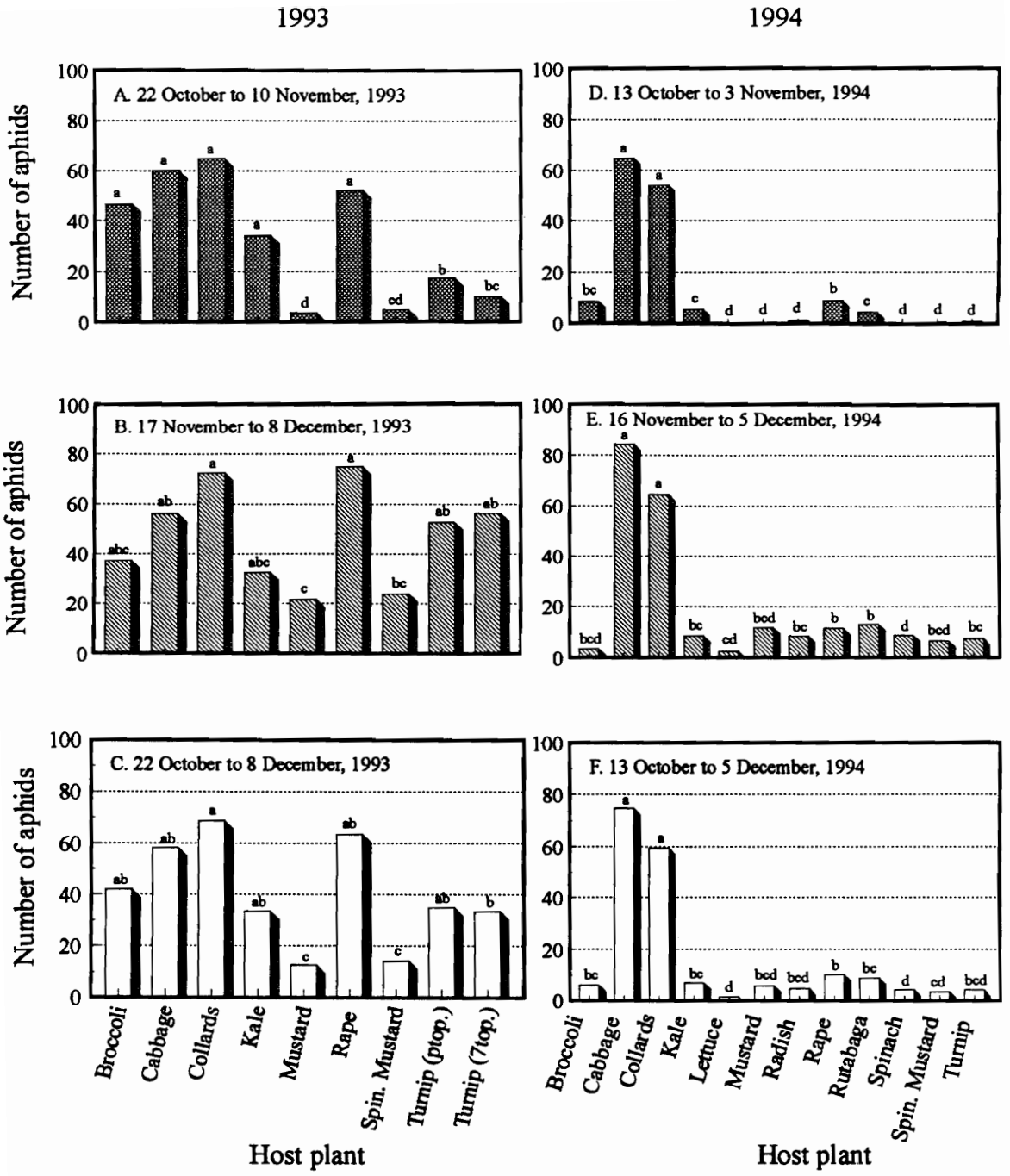

Fig. 4. 1. Average seasonal abundance of tobacco aphid populations (mean number/5 plants) on different host plants. Bars surmounted by same letters are not significantly different ($P \geq 0.05$; Duncan's multiple range test). (Appendix 8)

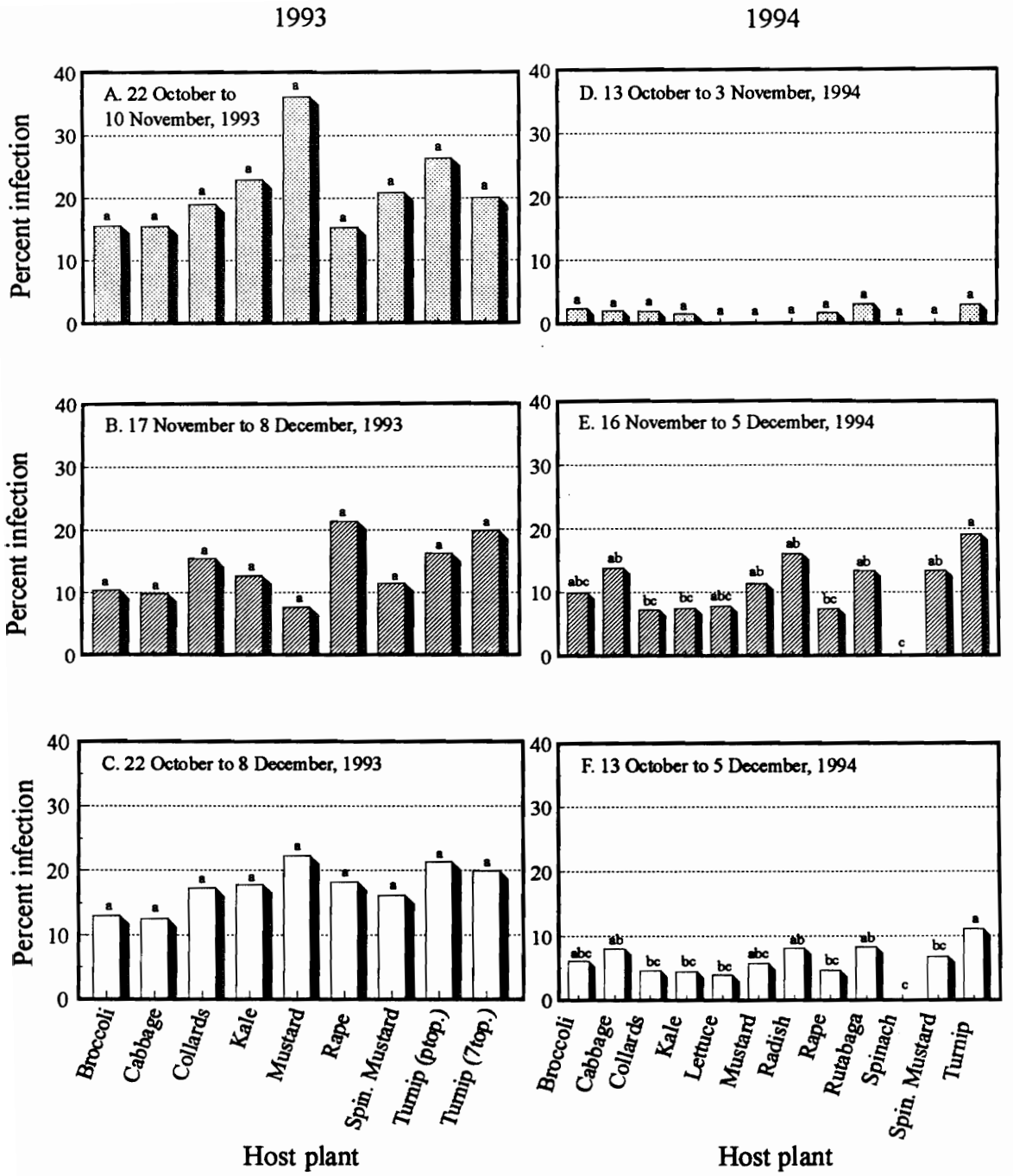

Fig. 4. 2. Average seasonal incidence of *P. neoaphidis* in tobacco aphid populations on different host plants. Bars surmounted by same letters are not significantly different ($P \geq 0.05$; Duncan's multiple range test). (Appendix 9)

Table 4. 2. Seasonal incidence of *P. neoaphidis* in tobacco aphid populations on vegetable hosts during fall, 1993.

Host plant	Mean percent of aphids infected on five plants ± SEM[1]							
	22 October	27 October	4 November	10 November	17 November	24 November	3 December	8 December
Broccoli	18.4 ± 8.6a	12.0 ± 4.9a	7.8 ± 3.4b	24.3 ± 7.9a	17.4 ± 9.5a	9.1 ± 4.7abc	9.0 ± 8.1ab	6.2 ± 6.2a
Cabbage	21.8 ± 8.5a	3.1 ± 1.3a	13.0 ± 2.6b	24.2 ± 4.4a	24.2 ± 6.1a	4.5 ± 3.0c	9.3 ± 3.8ab	1.1 ± 1.1a
Collards	28.7 ± 5.2a	6.3 ± 2.7a	13.4 ± 4.7b	28.1 ± 2.9a	24.4 ± 9.6a	7.7 ± 1.7bc	27.6 ± 27.6a	2.3 ± 2.3a
Kale	32.3 ± 7.8a	9.2 ± 5.5a	26.5 ± 3.1b	24.0 ± 9.2a	25.6 ± 12.9a	14.0 ± 5.3abc	6.8 ± 2.4ab	4.3 ± 4.3a
Mustard	38.1 ± 16.0a	17.4 ± 10.8a	58.3 ± 25.0a	30.8 ± 14.7a	18.2 ± 11.3a	11.3 ± 6.1abc	2.3 ± 1.5b	2.0 ± 1.2a
Rape	28.9 ± 12.4a	2.0 ± 1.2a	10.7 ± 5.6b	19.4 ± 2.5a	43.5 ± 16.1a	17.4 ± 4.3abc	22.3 ± 15.0ab	2.4 ± 0.5a
Spin. Mustard	9.9 ± 6.7a	7.1 ± 7.1a	35.4 ± 22.1ab	31.3 ± 23.1a	9.1 ± 3.4a	16.9 ± 6.5abc	14.9 ± 3.8ab	5.2 ± 2.8a
Turnip (ptop.)	29.6 ± 20.1a	10.6 ± 3.6a	42.1 ± 10.2ab	23.2 ± 4.5a	34.5 ± 11.5a	23.9 ± 10.3ab	4.4 ± 2.5ab	2.5 ± 0.9a
Turnip (7top.)	21.9 ± 12.9a	13.4 ± 7.7a	19.5 ± 7.7b	26.0 ± 12.3a	39.1 ± 20.1a	24.9 ± 10.8a	13.0 ± 2.6ab	2.6 ± 1.8a

[1]Means followed by the same letter within each column are not significantly different ($P \geq 0.05$; Duncan's multiple range test).

dates was considered ($P \geq 0.05$) (Table 4.2). The level of infection did not seem to be associated with aphid populations during the observation period. Significant positive correlation ($P < 0.05$) between aphid population and level of infection was seen only on 27 October and 3 December on mustard, on 17 November on spinach mustard, and on 'Purpletop' turnip on 22 October (Table 4.3).

The total amount of rainfall received during second half of the observation period was twice as much as that during first half, but the disease incidence was higher in first half (Fig. 4.3D). When the weekly averages for other weather parameters were considered, ambient temperatures decreased from the beginning to the end of both halves of the observation period (17.2 to 7.9 and 15.3 to 8.4^{0}C in first and second halves, respectively) (Fig. 4.3A). The weekly averages of relative humidity fluctuated between 60 and 80%, while that of leaf wetness between 7 and 34% (Figures 4.3B and C).

When observations were resumed on 8 March and continued until 18 May 1994, tobacco aphids were not seen until the first week of May. During the next two weeks in May, only one or two tobacco aphids per entire plot could be seen for some hosts, but no signs of infection were noticed.

1994: Cabbage and collards had higher numbers of aphids than the other hosts throughout the fall observation period (Table 4.4, Figures 4.1D, E, and F). However, both aphid populations and infection levels were relatively lower on all host plants in 1994 than in 1993 (Tables 4.4 and 4.5, Figures 4.1 and 4.2). The

Table 4. 3. The relationship between tobacco aphid populations and level of infection by *P. neoaphidis* on different vegetable hosts during fall, 1993.

Host plant	Pearson's correlation coefficient (r) [1]							
	22 October	27 October	4 November	10 November	17 November	24 November	3 December	8 December
Broccoli	-0.0254	-0.5837	0.8247	-0.4340	0.6016	0.8593	-0.3231	-0.6306
Cabbage	-0.3665	0.1833	-0.8600	0.7477	-0.7736	0.5045	-0.9424	0.5058
Collards	0.7976	-0.3575	0.3054	-0.3088	-0.2890	0.1039	0.7243	-0.7614
Kale	0.5532	-0.1773	0.2933	0.1845	0.3033	-0.9086	0.5896	0.3852
Mustard	0.1470	0.9998***	0.1743	0.8888	0.9023	-0.0300	0.9852*	0.5280
Rape	0.4769	0.5558	0.7849	-0.4211	-0.4561	0.2033	-0.8930	-0.9278
Spin. Mustard	0.8060	0.3292	0.4251	-0.8003	0.9957**	0.0374	0.6067	-0.8162
Turnip (ptop.)	0.9562*	-0.7507	-0.6325	-0.5271	-0.2204	-0.8664	-0.1176	0.2038
Turnip (7top.)	0.3904	0.7002	0.5953	0.9022	0.4121	-0.4827	-0.7620	0.0501

[1] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

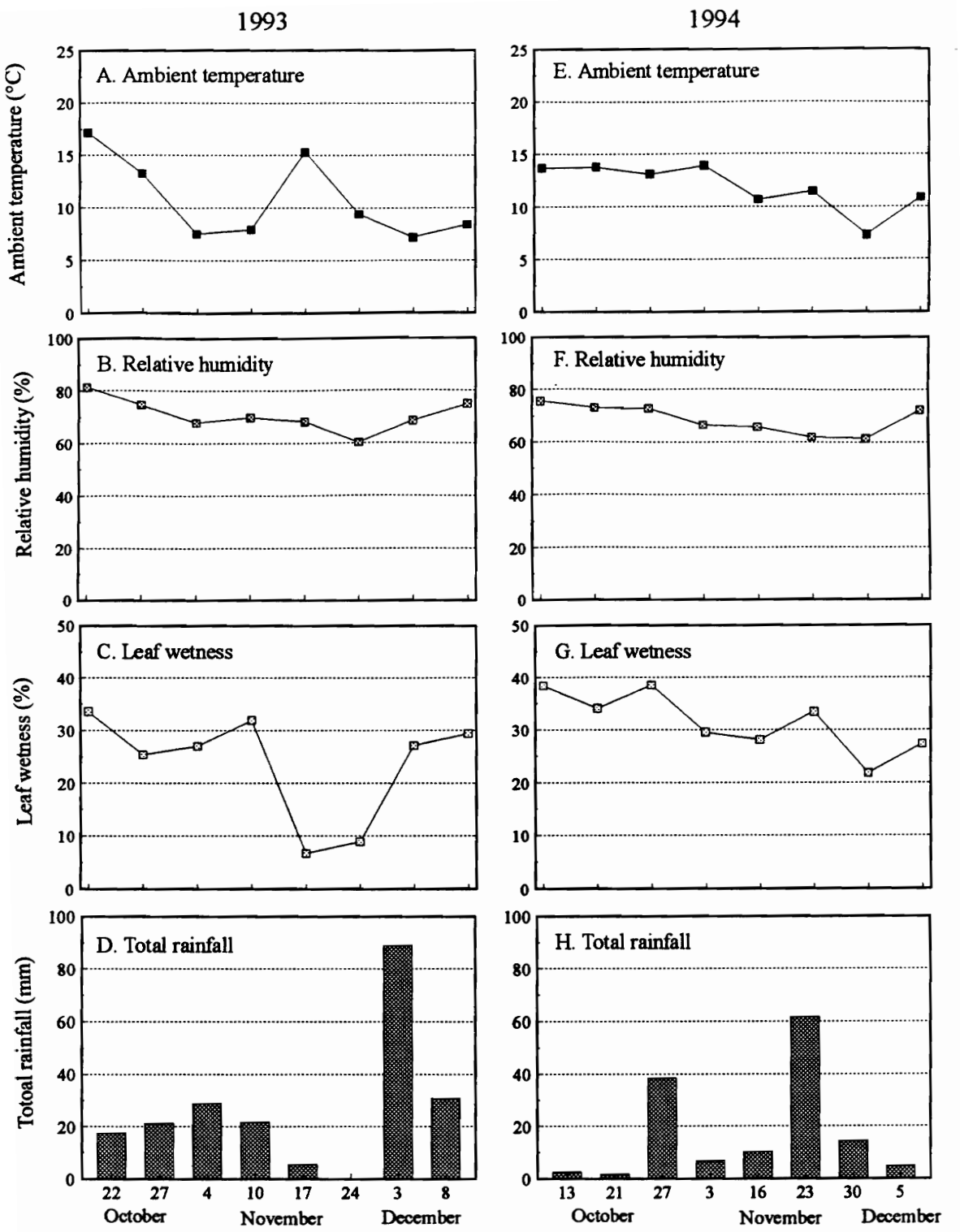

Fig.4. 3. Weekly averages of temperature, relative humidity, and leaf wetness and weekly total rainfall for the period when *P. neoaphidis* was observed during fall, 1993 and 1994. (Appendix 10)

Table 4. 4. Seasonal abundance of tobacco aphids on different vegetable hosts during fall, 1994.

Host plant	Mean number of aphids per five plants ± SEM[1]							
	13 October	21 October	27 October	3 November	16 November	23 November	30 November	5 December
Broccoli	10.5 ± 3.7b	12.8 ± 7.8bc	6.3 ± 3.8ab	5.3 ± 2.2bc	5.5 ± 2.3cde	4.3 ± 1.9cd	2.0 ± 1.1c	2.8 ± 1.3efg
Cabbage	56.0 ± 27.2a	106.3 ± 29.8a	20.5 ± 10.4a	76.3 ± 23.1a	80.3 ± 22.7a	123.0 ± 18.3a	54.0 ± 22.0ab	80.5 ± 21.9a
Collards	27.3 ± 5.5a	95.0 ± 41.3a	26.0 ± 15.0a	68.3 ± 15.3a	64.0 ± 28.3ab	84.0 ± 48.8ab	67.5 ± 32.8a	43.0 ± 25.7b
Kale	1.8 ± 0.6cd	4.8 ± 4.4cd	8.0 ± 5.7ab	7.8 ± 2.6b	8.5 ± 3.8cd	15.5 ± 7.4bc	7.5 ± 2.5abc	2.5 ± 1.3fg
Lettuce	0.3 ± 0.3d	0.0 ± 0.0d	1.0 ± 1.0b	0.3 ± 0.3e	0.8 ± 0.5ef	4.8 ± 2.6cd	1.3 ± 0.7c	3.8 ± 2.1defg
Mustard	0.0 ± 0.0d	0.0 ± 0.0d	0.3 ± 0.3b	0.8 ± 0.3de	2.5 ± 0.8def	28.3 ± 26.6cd	4.5 ± 2.2c	11.8 ± 5.0bcdef
Radish	1.3 ± 0.8cd	1.3 ± 1.3cd	0.3 ± 0.3b	2.5 ± 1.2cd	2.3 ± 0.6def	8.8 ± 3.2bc	10.3 ± 5.6abc	13.0 ± 4.8bcd
Rape	7.8 ± 2.0b	17.5 ± 8.2b	4.3 ± 1.6ab	7.3 ± 1.7b	13.8 ± 5.0bc	16.8 ± 2.2bc	5.3 ± 2.5bc	10.3 ± 2.8bcde
Rutabaga	3.5 ± 1.2bc	4.0 ± 1.4bcd	5.3 ± 3.6ab	5.8 ± 2.4bc	8.0 ± 2.4cd	14.5 ± 4.3bc	11.5 ± 2.3abc	18.5 ± 7.0bc
Spinach	0.0 ± 0.0d	1.3 ± 1.3cd	0.0 ± 0.0b	0.0 ± 0.0e	0.3 ± 0.3f	0.0 ± 0.0d	34.5 ± 34.5c	0.0 ± 0.0g
Spin. Mustard	0.5 ± 0.3cd	0.3 ± 0.3d	0.3 ± 0.3b	1.8 ± 0.6cde	3.8 ± 2.7def	10.5 ± 1.6bc	5.8 ± 2.5bc	6.3 ± 2.8cdef
Turnip	0.3 ± 0.3d	1.0 ± 0.4cd	0.5 ± 0.5b	1.8 ± 0.8cde	2.8 ± 1.3def	7.5 ± 3.2bc	8.5 ± 2.9abc	11.8 ± 2.0bcd

[1]Means followed by the same letter within each column are not significantly different ($P \geq 0.05$; Duncan's multiple range test).

Table 4. 5. Seasonal incidence of *P. neoaphidis* in tobacco aphid populations on different vegetable hosts during fall, 1994.

Host plant	Mean percent of aphids infected on five plants ± SEM[1]							
	13 October	21 October	27 October	3 November	16 November	23 November	30 November	5 December
Broccoli	2.1 ± 2.1a	0.0 ± 0.0a	7.5 ± 7.5a	0.0 ± 0.0b	11.1 ± 11.1a	3.6 ± 3.6a	0.0 ± 0.0b	25.0 ± 25.0a
Cabbage	0.0 ± 0.0a	0.8 ± 0.8a	7.0 ± 4.2a	0.0 ± 0.0b	7.9 ± 7.9a	12.3 ± 3.0a	7.1 ± 4.2ab	28.5 ± 12.2a
Collards	0.7 ± 0.7a	0.8 ± 0.8a	4.8 ± 2.9a	1.4 ± 1.4a	5.6 ± 5.6a	4.6 ± 2.4a	3.5 ± 2.3b	15.0 ± 13.5a
Kale	0.0 ± 0.0a	0.0 ± 0.0a	6.0 ± 6.0a	0.0 ± 0.0b	15.4 ± 11.8a	0.0 ± 0.0a	6.2 ± 6.2ab	8.3 ± 8.3a
Lettuce	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a	6.2 ± 6.2a	12.5 ± 12.5ab	12.5 ± 12.5a
Mustard	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a	13.0 ± 9.4a	12.5 ± 12.5ab	20.0 ± 8.6a
Radish	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a	11.4 ± 6.6a	25.0 ± 14.4a	28.4 ± 11.4a
Rape	1.9 ± 1.9a	1.9 ± 1.9a	3.1 ± 3.1a	0.0 ± 0.0b	12.5 ± 12.5a	6.5 ± 5.0a	0.0 ± 0.0b	10.7 ± 10.7a
Rutabaga	0.0 ± 0.0a	0.0 ± 0.0a	12.5 ± 12.5a	0.0 ± 0.0b	5.6 ± 5.6a	15.6 ± 11.8a	1.9 ± 1.9b	30.7 ± 9.1a
Spinach	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0ab	0.0 ± 0.0a
Spin. Mustard	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0b	12.5 ± 12.5a	3.6 ± 3.6a	10.6 ± 7.8ab	27.5 ± 11.9a
Turnip	0.0 ± 0.0a	12.5 ± 12.5a	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a	16.7 ± 11.8a	19.9 ± 12.2ab	40.1 ± 6.0a

[1]Means followed by the same letter within each column are not significantly different ($P \geq 0.05$; Duncan's multiple range test).

average incidence of *P. neoaphidis* over the entire fall was significantly higher ($P < 0.05$) in aphids infesting turnip than those on collards, kale, lettuce, rape, and spinach mustard (Fig. 4.2F). Either no or very low aphid populations were seen on spinach throughout the observation period, except on 30 November, and none of the aphids were infected on this host (Tables 4.4 and 4.5). In contrast to 1993 observations, average level of pathogen incidence was much lower in the first than the second half of the fall observation period (Figures 4.2A, B, D, and E). There were no significant differences ($P \geq 0.05$) among the host plants during first half of fall (between 13 October and 3 November) and there was no infection on lettuce, mustard, radish, spinach and spinach mustard. During the second half of fall (between 16 November and 5 December), aphids on turnip had significantly ($P < 0.05$) higher level of infection than those on collards, kale and rape. When the counts on individual observation dates were considered, influence of host plants on disease incidence was seen only on 30 November (Table 4.5). The level of infection was also significant ($P \geq 0.05$) on 3 November, but collards was the only host that had infected aphids (Table 4.5). Variation in infection levels did not show any relation to variation in aphid numbers on different hosts (Figures 4.1D, E, and F, 4.2D, E, and F). No correlation was seen between aphid populations and infection levels on different host plants, except for 27 October on cabbage and collards ($P < 0.05$) (Table 4.6).

Table 4. 6. The relationship between tobacco aphid populations and level of infection by *P. neoaphidis* on different vegetable hosts during fall, 1994.

Host plant	Pearson's correlation coefficient (r)							
	13 October	21 October	27 October	3 November	16 November	23 November	30 November	5 December
Broccoli	0.2649	-	0.4911	-	0.5288	0.4797	-	0.5468
Cabbage	-	-0.5188	0.9564*	-	0.7944	0.1741	0.7577	-0.8298
Collards	0.5277	0.6856	0.9842*	0.7596	0.5856	0.4996	0.8972	0.4026
Kale	-	-	0.8412	-	-0.4481	-	0.2081	0.7421
Lettuce	-	-	-	-	-	0.1381	0.4378	-0.0826
Mustard	-	-	-	-	-	0.3417	0.3699	0.5976
Radish	-	-	-	-	-	0.7200	-0.1683	0.4102
Rape	0.7827	0.6526	0.5512	-	-0.7852	-0.2502	-	-0.2683
Rutabaga	-	-	0.1770	-	0.2490	-0.4980	0.2647	-0.4223
Spinach	-	-	-	-	-	-	-	-
Spin. Mustard	-	-	-	-	0.0057	0.6212	0.7048	0.1816
Turnip	-	0.6978	-	-	-	-0.3760	0.6194	-0.6720

[1]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The amount of rainfall was similar during both halves of the observation period (Fig. 4.3E), but lower levels of infection were observed during the first half. The weekly averages of ambient temperature, relative humidity and leaf wetness were slightly lower in second half of the observation period (Fig. 4.3F, G, and H).

When the field was checked between January and April 1995, only a few of the host plants were surviving with very few aphids on them. Some of these aphids were infected with *P. neoaphidis*, but counts were not taken because of poor plant stand. Infected aphids were seen throughout the observation period between January and April, 1995.

Discussion:

Unlike other entomophthoraleans, *P. neoaphidis* does not produce overwintering resting spores (Rockwood 1950, Ben-Ze'ev and Kenneth 1982, Waterhouse and Brady 1982, Wilding and Brady 1984) and it is not known how it overwinters. In vitro formation of spores, thought to be resting spores, was known for *P. neoaphidis*, under special conditions (Uziel and Kenneth 1986). However, it has not been reported elsewhere without any ambiguity and the resting spore status given by Uziel and Kenneth (1986) is debatable (R.A. Humber personal communication). Earlier reports stated survival of *P. neoaphidis* (syn. *Empusa/ Entomophthora aphidis*) for 3-32 wk in aphid hosts (Rockwood 1950, Remaudière and Michel 1971, Wilding 1973, Feng et al. 1992). Rockwood (1950) reported

that in vitro grown cultures were viable for four months at about 2^0C. Infectivity of the pathogen lasted for 3.5 mo at temperatures ranged between 2 and -196^0C (Remaudière and Michel 1971), and for at least 32 wk at 0^0C and 20 or 50% relative humidity (Wilding 1973). When the cadavers of fungus-killed aphids were moistened after incubation at these conditions, the pathogen produced infective conidia. Feng et al. (1992) also reported that *P. neoaphidis* conidia from cadavers of pea aphid, *Acyrthosiphon pisum*, kept at 4^0C in refrigerator or at outdoor conditions in cold winter with continuous snow, were infective for about six months. Inside the cadavers of these pea aphids, Feng et al. (1992) found spherical hyphal bodies of *P. neoaphidis*, which were thought to play a role in overwintering. Latteur (1977) reported that conidia of *P. neoaphidis* in the soil were infective to aphids after several months of storage at 5^0C. Morgan et al. (1992) found that primary conidia of *P. neoaphidis* survived in the soil at -3 and 3^0C for at least 128 d. They were capable of producing secondary conidia when the temperature was raised to 18^0C. In another study, conidia of *P. neoaphidis* remained infective up to 32 d at 5^0C and 85% relative humidity and for 16 d in winter field conditions on oilseed rape leaves in the UK (Schofield et al. 1995). Feng et al. (1990) and Elkassabany et al. (1992) reported occurrence of *P. neoaphidis* epizootics in different species of aphids on winter wheat and spinach during parts of winter. All these reports indicate that the pathogen can remain viable at cold temperatures in the aphid hosts, in soil, or on foliage, but there is no

complete information available to describe the continuation of *P. neoaphidis* epizootics to spring through winter in a single study, except for Rockwood's (1950) findings on pea aphids. Rockwood found that infections of *P. neoaphidis* in populations of pea aphid infesting vetches and Austrian winter field peas occurred in the fall and continued through winter to next spring. My observations also suggest that *P. neoaphidis* overwintered in tobacco aphids infesting nonsolanaceous vegetable hosts as it was seen until early December, 1994 and again from late January, 1995. Based on these findings, it could be proposed that survival in the host insects is one of the mechanisms of overwintering for *P. neoaphidis*.

Higher infection levels in 1993 than in 1994 could be attributed to the source of inoculum in a nearby tobacco fields. An epizootic was observed until early November, 1993 in tobacco aphid populations on regrowth of cut tobacco in other fields at the research station (see Chapter III). Alate aphids are more susceptible than apterae to entomophthorans and infected alatae are thought to be responsible for long-distance transmission of infection (Rockwood 1950, Soper 1981, Steinkraus and Hollingsworth 1994). Infected alatae migrating from tobacco to winter hosts might have served as the source of inoculum to result in higher infection in aphids on nonsolanaceous hosts in 1993. This could also have influenced higher levels of infection during the first half of the fall in that year. Aphid populations were also higher in 1993.

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CHAPTER V

Seasonal incidence and within-plant distribution of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) in populations of red morph of *Myzus nicotianae* (Homoptera: Aphididae) on flue-cured tobacco.

Abstract:

The within-plant distribution of the entomophthoralean *Pandora neoaphidis* (Remaudière & Hennebert) Humber was investigated in populations of the tobacco aphid, *Myzus nicotianae* Blackman, on flue-cured tobacco at two growth stages viz., pre-flower and full-flower. Leaves on plants were divided into three and six leaf positions and the aphid population and infection levels were compared among leaf positions. Total number of aphids, number of infected aphids, and percentage of infected aphids tended to increase from the bottom to the top of the plant and the upper 1/3 of the plant usually had significantly higher values (Waller-Duncan K-ratio T test; K-ratio = 100) than the other two leaf positions. When six leaf positions per plant were considered, this trend was slightly inconsistent with some lower leaf positions having higher values than the leaf positions immediately above them.

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, within-plant distribution, tobacco.

Introduction:

The tobacco aphid, *Myzus nicotianae* Blackman, previously known as green peach aphid, *M. persicae* (Sulzer), is the most important insect pest of tobacco in North America (Blackman 1987, Mistric and Clark 1979, Cheng and Hanlon 1985, Lampert 1989, Reed and Semtner 1992). In recent years, the common green morph of the tobacco aphid has been replaced by a red morph (McPherson 1989, Reed and Semtner 1991). Distribution of the aphids varies within the tobacco plant (Webster et al. 1983, Lampert 1989). Lampert (1989) reported that the density of tobacco aphids was higher in the upper 1/3 of the tobacco plant and the numbers were higher on the middle 1/3.

The enotomophthoralean, *Pandora (Erynia) neoaphidis* (Remaudière & Hennebert) Humber is the most common (Wilding and Brady 1984, Sivcev 1991, Elkassabany et al. 1992, Kish et al. 1994, Dara and Semtner 1995) and dominant (Pickering et al. 1989, Feng et al. 1990, Feng et al. 1991, Sivcev 1992, Feng and Nowierski 1992) pathogen of various species of aphids on different hosts plants. Fungal pathogens (unidentified) were reported as important biocontrol agents of *M. nicotianae* on tobacco in Greece (Lykouressis and Mentzos 1995). Nordin et al. (1993) and Yu et al. (1995) reported *P. neoaphidis* infecting *M. nicotianae* in the United States. Dara and Semtner (1995) found that *P. neoaphidis* is an important mortality factor for the tobacco aphid on tobacco in Virginia.

Brobyn et al. (1985) found differences in the persistence of infectivity of *E. neoaphidis* conidia to pea aphid, *Acyrthosiphon pisum* (Harris), on the upper and lower leaves of field beans (*Vicia faba* L.). This indicates that the level of infection in the host might vary in different regions within a plant. Feng et al. (1991) hypothesized that the location of an aphid species on a wheat plant would influence the disease development. Aphids that occupy the upper portions of a plant would be more influenced by rainfall than those that occupy lower portions. Feng et al. (1991) also reported that infections are correlated to aphid densities. Lykouressis and Mentzos (1995) reported the differences in the occurrence of the tobacco aphid, infected by unidentified fungi, in upper and lower halves of tobacco plants. These findings suggest the possibility of variation in the levels of *P. neoaphidis* infection in aphids within the tobacco plant. Information on the within-plant distribution of *P. neoaphidis* would provide a better understanding of the ecology of the pathogen in the crop ecosystem. This information could be used to modify sampling techniques for better assessment of the infection level in the field. The objective of this research was to determine the within-plant distribution of *P. neoaphidis* in populations of the red morph of the tobacco aphid on flue-cured tobacco in the field.

Materials and Methods:

I conducted this research at the Southern Piedmont Agricultural Research

and Extension Center (SPAREC), Blackstone, Virginia in 1994 and 1995. The flue-cured tobacco 'K-326' was transplanted on 17 May in 1994 (0.08 ha) and on 16 May in 1995 (0.06 ha). Standard agronomic practices recommended by the SPAREC for flue-cured tobacco production in Virginia were followed, except that plants were not treated with aphicides or topped (removal of terminal floral portion) (Reed et al. 1995). Aphid populations usually decrease after topping (Semtner 1984, Lampert 1989). Topping was, therefore, avoided to promote high aphid populations.

Within-plant distribution of *P. neoaphidis* in the tobacco aphid was determined by counting the number of healthy and *Pandora*-infected aphids on each leaf of randomly selected sample plants. Both fungus-killed (golden brown cadavers) and living fungus-infected (with pale coloration) aphids were counted as infected aphids. Aphid populations were monitored on tobacco at two growth stages viz., pre-flower (no flowering, only flower buds) and full-flower (at least 50% of buds flowering). Observations were made at weekly intervals on four dates each year, from 27 July to 15 August in 1994 and from 12 July to 2 August in 1995. Tobacco plants at the two growth stages occurred simultaneously in the same field throughout the observation period. Ten plants of each growth stage, with *P. neoaphidis* infections in aphids, were sampled on all dates in 1994, except for the last date when 12 pre-flower and 10 full-flower plants were sampled. In 1995, seven plants of each growth stage were sampled on all observation dates.

Sample plants were randomly selected, cut at the base, taken to a shaded area in the field or into the laboratory one at a time, and healthy and infected aphids were counted on each leaf. The green morph of the tobacco aphid appeared on some plants in negligible numbers and they were excluded from counts. Variation in the number of leaves per plant did not allow generalization of disease distribution in aphid populations, on a per leaf basis, within each plant. Hence, each plant was divided into six leaf positions (LP), each representing 1/6 of the plant. LP1 to LP6 represented leaves in the bottom 1/6 to the top 1/6 of the plant, respectively. When a fraction of a leaf came under a leaf position, the proportion of aphids corresponding to that fraction was included in that particular leaf position. Inflorescences in full-flower plants and flower buds in pre-flower plants were included in the LP6 category of respective plants. Results were also compared by dividing each plant into three leaf positions (bottom, middle, and top).

Weather data were obtained from a weather station (Omnidata International Inc., Logan, Utah) located about 0.5 km from the test fields.

Identification of *P. neoaphidis*: On each sampling date, conidia collected on coverslips from 5-10 cadavers were examined in lactophenol under phase contrast. The identity of *P. neoaphidis* was confirmed based on the descriptions of Remaudière and Hennebert (1980), Wilding and Brady (1984), and Humber (1989).

Statistical Analysis: Data were analyzed by PROC ANOVA or PROC GLM (for

unbalanced data) of SAS software (SAS Institute 1987) and mean number of aphids and percent infection per leaf position were determined. Means of logarithmically-transformed aphid numbers and arcsine-transformed percent infection were considered for means separation. Significant means were separated by Waller-Duncan K-ratio T test (K-ratio = 100). Regression of number of aphids and percent infection on leaf position (6 leaf positions/plant) was performed by PROC REG of SAS.

Results and Discussion:

In 1994, infections of *P. neoaphidis* increased from 27 July onwards and peaked on 8 August (Tables 5.1 and 5.2). In 1995, infections were maximum on 12 July, when they were first observed, and declined thereafter, except for some increase on 2 August (Tables 5.3 and 5.4). In general, the aphid populations were 3-5 times higher in 1995 than in 1994, throughout the sampling period, however, the level of infection in 1995 was much less than that observed in 1994. Low rainfall and high temperatures after 12 July in 1995 may have prevented further development of the infection in aphid populations (Fig. 5.1).

Distribution among three leaf positions:

The distribution of aphids in this study was similar to that reported by Reed (1987), but differed somewhat from that reported by Lampert (1989). Most of the time, the total number of aphids was significantly higher (Waller-Duncan K-ratio

Table 5. 1. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among six leaf positions in pre-flower plants. 1994.

Leaf position	n[1]	Means ± SEM[2]			
		27 July	1 August	8 August	15 August
			Aphids		
LP6	10	1,545.2 ± 190.7a	1,301.1 ± 181.3a	467.6 ± 45.1a	385.0 ± 62.9a
LP5	10	1,302.9 ± 131.6a	1,015.1 ± 126.3a	433.7 ± 75.9a	219.7 ± 52.8b
LP4	10	506.3 ± 80.8b	549.9 ± 74.9b	176.4 ± 45.6b	32.3 ± 7.6cd
LP3	10	435.9 ± 90.6bc	447.4 ± 71.8bc	105.1 ± 26.6c	14.3 ± 3.0d
LP2	10	327.9 ± 63.8c	403.4 ± 92.1c	77.5 ± 18.9c	33.5 ± 6.6c
LP1	10	96.1 ± 24.8d	78.1 ± 20.7d	54.1 ± 13.1d	12.8 ± 4.6e
			Percent infection		
LP6	10	1.6 ± 0.3a	23.7 ± 7.2a	85.2 ± 6.4a	15.1 ± 1.5bc
LP5	10	1.4 ± 0.2a	13.6 ± 4.5b	82.4 ± 7.7a	39.2 ± 5.0a
LP4	10	2.0 ± 0.7a	9.6 ± 2.4b	66.6 ± 8.9b	47.0 ± 8.7a
LP3	10	1.6 ± 0.5a	7.2 ± 1.0b	46.0 ± 11.2c	21.4 ± 6.4b
LP2	10	1.0 ± 0.3a	7.7 ± 1.5b	42.8 ± 11.1c	5.9 ± 2.6c
LP1	10	1.6 ± 0.9a	14.5 ± 3.1ab	42.3 ± 11.5c	3.2 ± 1.8c

[1] *n* is 12 for pre-flower plants on 15 August.

[2] Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

Table 5. 2. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among six leaf positions in full-flower plants. 1994.

Leaf position	n	Means ± SEM[1]			
		27 July	1 August	8 August	15 August
			Aphids		
LP6	10	2,092.6 ± 386.5a	1,183.9 ± 172.1a	502.0 ± 98.5a	198.0 ± 28.8a
LP5	10	1,397.7 ± 333.4b	1,037.1 ± 167.2a	170.8 ± 36.1b	85.9 ± 17.8c
LP4	10	794.0 ± 206.0c	872.1 ± 88.4ab	124.5 ± 27.9bc	50.2 ± 16.7d
LP3	10	735.5 ± 149.7c	645.1 ± 93.4bc	109.8 ± 36.9cd	112.4 ± 33.3bc
LP2	10	504.8 ± 109.1c	465.8 ± 49.6cd	180.0 ± 54.6bcd	169.9 ± 40.3ab
LP1	10	164.0 ± 36.0d	184.8 ± 32.3d	67.9 ± 19.0d	83.5 ± 25.1c
			Percent infection		
LP6	10	12.4 ± 7.8a	38.6 ± 10.7a	89.7 ± 3.4a	38.9 ± 7.0ab
LP5	10	6.6 ± 3.1ab	16.4 ± 8.2b	60.3 ± 8.5b	46.0 ± 11.7a
LP4	10	3.7 ± 1.6ab	5.5 ± 1.2b	43.1 ± 10.1c	19.6 ± 10.0bc
LP3	10	1.6 ± 0.5sb	5.6 ± 1.2b	26.4 ± 7.5d	5.9 ± 4.9c
LP2	10	0.6 ± 0.3b	4.2 ± 0.8b	9.8 ± 4.0e	0.6 ± 0.3c
LP1	10	1.7 ± 0.9ab	6.9 ± 1.1b	4.5 ± 1.9e	1.2 ± 0.8c

[1]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

Table 5. 3. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among six leaf positions in pre-flower plants. 1995.

Leaf position	n	Means ± SEM[1]			
		12 July	19 July	26 July	2 August
			Aphids		
LP6	7	2,121.7 ± 456.2c	3,713.1 ± 590.9ab	1,392.6 ± 216.3b	385.1 ± 69.9a
LP5	7	5,139.1 ± 1,035.8a	5,507.4 ± 988.4a	3,607.9 ± 603.6a	578.5 ± 197.2a
LP4	7	3,786.0 ± 888.1ab	2,834.0 ± 410.0b	1,218.6 ± 206.9b	52.0 ± 18.3b
LP3	7	3,383.1 ± 667.2b	1,294.0 ± 224.5c	263.0 ± 78.5c	9.3 ± 2.6b
LP2	7	1,373.9 ± 360.4c	780.5 ± 166.2d	59.9 ± 11.5d	1.7 ± 1.2b
LP1	7	284.7 ± 72.1d	205.7 ± 54.1e	10.5 ± 2.7e	0.0 ± 0.0b
			Percent infection		
LP6	7	22.5 ± 10.9a	3.6 ± 0.9a	2.4 ± 0.4ab	18.2 ± 13.7a
LP5	7	24.6 ± 12.5a	4.5 ± 1.6a	0.9 ± 0.2bc	15.6 ± 14.1a
LP4	7	19.7 ± 10.4ab	3.2 ± 0.6a	0.9 ± 0.3bc	20.2 ± 13.7a
LP3	7	9.2 ± 2.7ab	9.8 ± 6.0a	1.4 ± 0.3abc	10.7 ± 5.1a
LP2	7	3.8 ± 1.2b	8.4 ± 4.1a	2.7 ± 1.0a	0.0 ± 0.0a
LP1	7	12.7 ± 9.4ab	9.1 ± 4.1a	0.0 ± 0.0c	0.0 ± 0.0a

[1]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

Table 5. 4. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among six leaf positions in full-flower plants. 1995.

Leaf position	n	Means ± SEM[1]			
		12 July	19 July	26 July	2 August
			Aphids		
LP6	7	1,640.6 ± 300.8b	5,309.2 ± 744.8a	2,535.5 ± 382.7a	172.0 ± 30.7a
LP5	7	3,653.5 ± 1,055.1a	4,823.7 ± 845.9a	1,247.4 ± 213.6a	6.2 ± 1.7b
LP4	7	3,772.0 ± 1,110.3a	4,144.2 ± 871.7ab	349.5 ± 94.1b	1.5 ± 0.5c
LP3	7	2,525.2 ± 457.4ab	2,469.0 ± 458.0b	170.2 ± 43.0b	2.3 ± 1.4c
LP2	7	982.7 ± 229.3c	1,278.2 ± 462.7c	24.5 ± 9.8c	0.5 ± 0.2d
LP1	7	496.9 ± 188.4d	376.8 ± 89.1d	3.4 ± 1.1d	0.04 ± 0.04e
			Percent infection		
LP6	7	29.4 ± 12.2a	5.8 ± 1.6ab	3.0 ± 0.6ab	4.8 ± 1.1a
LP5	7	27.6 ± 11.7a	6.2 ± 1.6ab	1.6 ± 0.5b	0.0 ± 0.0b
LP4	7	14.4 ± 5.7ab	3.7 ± 0.7b	2.6 ± 0.6ab	0.0 ± 0.0b
LP3	7	6.3 ± 2.3b	2.8 ± 0.5b	5.2 ± 2.5a	0.0 ± 0.0b
LP2	7	2.7 ± 0.8b	4.7 ± 1.1b	0.2 ± 0.2b	0.0 ± 0.0b
LP1	7	4.0 ± 1.1b	9.9 ± 3.1a	0.0 ± 0.0b	0.0 ± 0.0b

[1]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

T test; K-ratio = 100) in the 'top' than the other two leaf positions of both pre- and full-flower plants in both 1994 and 1995 (Tables 5.5 and 5.6). The number of infected aphids was always significantly higher (K-ratio = 100) in the 'top' leaf position (data not presented). In 1994, percent infection in aphids followed a similar trend except that the 'middle' leaf position had significantly higher level of infection on pre-flower plants on 15 August (Table 5.5). In 1995, distribution of the pathogen was not as distinct as it was in 1994. Significant differences in the level of infection among the leaf positions were not seen most of the time in 1995 (Table 5.6). The proportion of infected aphids in pre-flower plants was higher in upper leaf positions on 12 July and 2 August, but differences were not significant (K-ratio = 100) during the entire observation period. On full-flower plants, the proportion of infected aphids was significantly higher in the 'top' than in the 'bottom' leaf position and the rest of the plant on 12 July and 2 August, respectively.

Distribution among six leaf positions:

The number of aphids tended to increase toward the top of the plant in both pre- and full-flower plants in both 1994 and 1995 (Tables 5.1 to 5.4). The percent infection also followed similar trend throughout the season on full-flower plants (Table 5.2) and in the middle of the season on pre-flower plants (Table 5.1) in 1994, and on first observation date on both stages of plants in 1995 (Tables 5.3 and 5.4). High temperatures and dry weather after 8 August, and a decline in

Table 5. 5. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among three leaf positions. 1994.

Leaf position	n[1]	Means ± SEM[2]			
		27 July	1 August	8 August	15 August
Pre-flower plants:			Aphids		
Top	10	2,848.2 ± 286.4a	2,316.2 ± 271.0a	901.3 ± 99.2a	604.6 ± 80.1a
Middle	10	942.2 ± 162.3b	997.3 ± 139.2b	281.4 ± 68.4b	46.5 ± 9.5b
Bottom	10	424.0 ± 83.4c	481.5 ± 100.3c	131.5 ± 27.7c	46.2 ± 10.4b
			Percent infection		
Top	10	1.5 ± 0.3a	19.2 ± 6.3a	84.0 ± 6.9a	22.0 ± 2.5b
Middle	10	1.7 ± 0.6a	8.2 ± 1.5a	60.2 ± 9.2b	39.6 ± 6.5a
Bottom	10	1.0 ± 0.3a	8.9 ± 1.8a	41.2 ± 9.3c	5.1 ± 1.8c
Full-flower plants:			Aphids		
Top	10	3,490.3 ± 662.2a	2,220.9 ± 274.8a	672.8 ± 99.6a	283.8 ± 33.7a
Middle	10	1,529.5 ± 332.0b	1,517.2 ± 159.5b	234.3 ± 63.0b	162.7 ± 48.7b
Bottom	10	668.8 ± 137.8c	650.6 ± 61.3c	247.9 ± 68.5b	253.4 ± 63.9ab
			Percent infection		
Top	10	10.1 ± 5.9a	27.6 ± 9.1a	81.3 ± 3.5a	39.2 ± 7.4a
Middle	10	2.6 ± 1.0a	5.4 ± 1.2b	38.1 ± 9.1b	7.6 ± 3.8b
Bottom	10	1.0 ± 0.5a	4.8 ± 0.8b	7.8 ± 3.1c	0.9 ± 0.5b

[1]*n* is 12 for pre-flower plants on 15 August.
[2]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

Table 5. 6. Within-plant distribution of the tobacco aphid and *P. neoaphidis* among three leaf positions. 1995.

Leaf position	n	Means ± SEM[1]			
		12 July	19 July	26 July	2 August
Pre-flower plants:			Aphids		
Top	7	7,260.7 ± 1,370.9a	8,752.1 ± 908.8a	5,000.4 ± 762.7a	963.7 ± 250.0a
Middle	7	7,169.1 ± 1,536.6a	4,128.1 ± 597.4b	1,481.6 ± 262.5b	61.3 ± 20.0b
Bottom	7	1,658.6 ± 411.6b	986.3 ± 206.0c	70.5 ± 13.8c	1.7 ± 1.2b
			Percent infection		
Top	7	24.2 ± 12.2a	3.9 ± 1.0a	1.3 ± 0.2a	16.5 ± 13.9a
Middle	7	14.4 ± 6.3a	5.1 ± 2.1a	1.0 ± 0.3a	19.2 ± 13.5a
Bottom	7	4.0 ± 1.3a	8.5 ± 4.1a	2.4 ± 0.9a	0.0 ± 0.0a
Full-flower plants:			Aphids		
Top	7	5,294.1 ± 1,317.3a	10,133.0 ± 1,137.7a	3,783.0 ± 449.1a	178.2 ± 31.5a
Middle	7	6,297.1 ± 1,556.4a	6,613.3 ± 1,304.7b	519.7 ± 133.7b	3.8 ± 1.4b
Bottom	7	1,479.6 ± 322.9b	1,655.0 ± 520.9c	27.9 ± 9.9c	0.6 ± 0.2c
			Percent infection		
Top	7	28.1 ± 11.9a	5.8 ± 1.6a	2.5 ± 0.5a	4.6 ± 1.0a
Middle	7	10.9 ± 4.0ab	3.3 ± 0.5a	2.9 ± 0.6a	0.0 ± 0.0b
Bottom	7	3.2 ± 0.7b	5.5 ± 0.9a	0.2 ± 0.2a	0.0 ± 0.0b

[1]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

aphid populations could have altered the distribution of the pathogen on the last observation date (15 August) in 1994. Proportion of infected aphids in LP6 was significantly lower (Waller-Duncan K-ratio T test: K-ratio = 100) than that in the lower leaf positions (LP4 and LP5) on pre-flower plants on 15 August, 1994. In 1995, distribution of the pathogen in aphids was inconsistent after the first observation date when infection was declining during hot and dry weather conditions.

In 1994, the daily average temperatures were $<27^0$C for nearly 3 wk before the first observation date, 27 July (Fig. 5.1). Rainfall was moderate during the week before 27 July. A brief period of moderately cool (daily averages around 23^0C from 27 to 29 July) and wet weather, after 27 July and of cool weather (daily average about 19^0C on 6 and 7 August) before 8 August may have promoted the rapid spread of the pathogen with peak infection occurring on 8 August. In 1995, the occurrence of *P. neoaphidis* was first noticed on 1 July before the actual counts were made (data not presented). There was a wet and moderately cool period where average daily temperatures were about 23^0C for about one week with fair amount of rainfall before the pathogen first appeared (Fig. 5.1). The daily temperature minima were between 15 and 18^0C for 3 d and the daily average temperatures were $\leq 26^0$C before 12 July when the infection was at its maximum. There was also 47 mm of rainfall on 11 July. These conditions of cool and moist weather could have helped the establishment and development of the pathogen in

the aphids after it first appeared. However, high temperatures (daily maximum 33-38^{0}C) coupled with dry weather after 12 July contributed to a rapid decline in aphid populations and infection levels towards the end of July, 1995. A decrease in temperature and moderate rainfall after 25 July may have favored the pathogen and helped the increase in infection on 2 August.

The relationship of aphid populations and percent infection to the leaf position varied among the observation dates (Table 5.7) and appeared to be influenced by the environmental factors and the extent of epizootic in the field. In 1994, the number of aphids significantly increased towards the upper leaf positions on all observation dates in pre-flower plants (Table 5.1) and on first two dates in full-flower plants (Table 5.2). Similar increase in infection towards upper leaf positions was seen only on 8 August in pre-flower plants and on all, except the second, observation dates in full-flower plants. In 1995, increase in aphid populations with increasing leaf positions was seen on 19 July in pre-flower plants (Table 5.3) and on 19 and 26 July in full-flower plants (Table 5.4). In case of the infection, this increase was significant on 12 July in full-flower plants. Higher proportion of infected aphids in LP1 on 12 July altered that relationship in pre-flower plants.

Distribution of the pathogen in aphids within a tobacco plant seemed to be influenced by the overall level of incidence in the field, as evinced by the level of infection on 27 July in pre-flower plants in 1994, and by weather conditions as

Table 5. 7. Relationship of number of tobacco aphids and proportion infected by *P. neoaphidis* to the leaf position (x[1]).

Date	Pre-flower plants		Full-flower plants	
	Regression equation (y = a + bx)	r^2	Regression equation (y = a + bx)	r^2
1994:		Aphids		
27 July	y = -321.71 + 292.6**[2]x	0.888	y = -289.92 + 353.72**x	0.915
01 August	y = -172.76 + 230.1**x	0.932	y = 37.83 + 198.18***x	0.989
08 August	y = -101.67 + 91.64**x	0.865	y = -23.26 + 61.65x	0.539
15 August	y = -127.49 + 69.65x	0.720	y = 90.82 + 7.38x	0.060
		Percent infection		
27 July	y = 1.37* + 0.05x	0.068	y = -2.93 + 2.10*x	0.783
01 August	y = 6.11 + 1.89x	0.329	y = -6.63 + 5.57x	0.609
08 August	y = 25.49* + 10.11**x	0.903	y = -20.45* + 16.98***x	0.962
15 August	y = 3.47 + 5.29x	0.309	y = -15.14 + 9.67*x	0.838
1995:		Aphids		
12 July	y = 594.47 + 596.41x	0.401	y = 680.71 + 427.93x	0.342
19 July	y = -936.65 + 950.22*x	0.781	y = -630.52 + 1056.39***x	0.973
26 July	y = -758.93 + 528.86x	0.523	y = -929.10 + 471.67*x	0.777
02 August	y = -198.76 + 105.67x	0.630	y = -57.19 + 25.03x	0.455
		Percent infection		
12 July	y = 3.23 + 3.48x	0.636	y = -6.91 + 5.99**x	0.887
19 July	y = 11.01** - 1.31*x	0.673	y = 7.03* - 0.43x	0.105
26 July	y = 0.77 + 0.17x	0.103	y = 0.44 + 0.47x	0.208
02 August	y = -3.95 + 4.21*x	0.776	y = -1.60 + 0.69x	0.429

[1]x = six leaf positions per plant.

[2]*, $P < 0.05$; ** $P < 0.01$; ***$P < 0.001$; H_0, coefficient, 0.

seen in inconsistent trends on 15 August in 1994 and after 12 July in 1995. Very low disease incidence on 27 July, higher proportion of infected aphids in lower leaf positions on 1 August, and declined aphid populations and disease incidence on 15 August may have altered the distribution trend in pre-flower plants on those days. Higher infection level on lower leaf positions had similar influence on pathogen distribution on 1 August in full-flower plants. Somewhat similar to my observations, infection levels of unidentified fungal pathogens in tobacco aphids in Greece differed in upper and lower halves of tobacco plants when the disease incidence was low (Lykouressis and Mentzos 1995). Tobacco aphids on upper than on lower half of the plants had significantly more infection when the disease was at its maximum.

In general, infections in aphids were similar on both pre- and full-flower plants when the epizootic was at low levels. However, pre-flower plants had higher levels of infection than full-flower plants during peak of the epizootic.

Brobyn et al. (1985) found that *P. neoaphidis* conidia were viable for longer periods on lower leaves than on upper leaves of field beans (*Vicia faba* L.). This was attributed to the protection of lower leaves from harmful radiation that would inactivate the conidia and from rainfall that would wash conidia off the leaves. The lower leaf positions of tobacco hold dew for longer periods than the upper leaf positions (Norse 1971). One would, therefore, expect higher infection levels on lower leaf positions since microclimatic conditions are more favorable

for infection process on the sheltered parts of the plant. But, my observations showed lower levels of infection on the lower leaf positions. Factors other than microclimatic conditions may also contribute for higher infection levels in upper leaf positions. More exposure of upper leaf positions to wind currents would suggest that aphids in this part are more exposed to airborne inoculum than those in the lower parts. Thurston (1961) reported that aphid populations were higher in the upper than the middle or bottom leaves of some tobacco cultivars and the opposite was seen in the case of highly resistant species of tobacco (*Nicotiana gossei* and *N. repanda*). These differences were attributed to the variation in leaf chemistry. The possibility of variation in the leaf surface chemistry among leaf positions and its influence on the pathogen should also be considered since leaf surface chemicals of tobacco are known to be toxic to some phytopathogenic fungi (Cruickshank et al. 1977, Severson et al. 1985, Menetrez et al. 1987, Menetrez et al. 1990, Kennedy et al. 1992). Diterpene compounds isolated from *Nicotiana* spp. inhibit germination and development of several phytopathogenic fungi, including *Erysiphe cichoracearum* (tobacco powdery mildew pathogen) and *Peronospora tabacina* (tobacco blue mold pathogen) (Sheperd and Mandryk 1963, Bailey et al. 1974, Cruickshank et al. 1977, Cohen et al. 1983, Menetrez et al. 1987, Menetrez et al. 1990). Such influence on entomopathogen fungi, however, is not known. The overall difference in the leaf surface chemistry of pre- and full-flower plants might have influenced the infection levels in aphids on plants at

these two stages of development. Severson et al. (1985) reported that older tobacco plants with higher levels of duvatrienediols on the leaf surfaces were less susceptible to *P. tabacina* than the younger plants. However, Court (1982) found that duvatrienediol content increased towards the upper leaf positions in flue-cured tobacco before topping. Since infections in aphid populations were higher in upper leaf positions in the current study, it is not clear if duvatrienediols influence inoculum of *P. neoaphidis*. Higher numbers of aphids in upper leaf positions also seems to be an important factor which could have promoted the spread of the inoculum. Feng et al. (1991) stated that the development of entomophthoralean infections in cereal aphids on wheat appeared to be associated more with host density than with temperature and rainfall. Similarly infections of *P. neoaphidis* and another fungal pathogen, *Schizolachnus piniradiatae* (Davidson) were associated with levels of inoculum and densities of their aphid hosts (Wilding 1975, Soper and MacLeod 1981). My results also suggest that, within each tobacco plant, higher infection levels, in general, were associated with higher numbers of aphid populations, which were seen in the upper parts of the plant. Harper and Carner (1995) found that levels of airborne conidia of *P. gammae* infecting the soybean looper, *Pseudoplusia includens* (Walker), increased as the epizootic progressed in the host populations. A study based on the measurement of inoculum levels and microenvironmental conditions at different leaf positions could provide further insight on the interrelation among levels of infection and of

inoculum, and microenvironmental differences among leaf positions.

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CHAPTER VI

Occurrence of *Pandora neoaphidis* (Zygomycotina: Entomophthorales) in populations of *Myzus nicotianae* (Homoptera: Aphididae) on different cultivars of tobacco and influence of cultural practices.

Abstract:

The natural incidence of the entomophthoralean, *Pandora neoaphidis* (Remaudière & Hennebert) Humber in populations of the tobacco aphid, *Myzus nicotianae* Blackman, on different cultivars of flue-cured, burley, dark fire-cured, and sun-cured tobacco, tobacco introductions, and a breeding line was compared in the field during 1992, 1994, and 1995. The incidence of artificially introduced *P. neoaphidis* was compared on six cultivars representing three tobacco types in the greenhouse during 1995. The influence of planting date, topping (removal of terminal inflorescence of tobacco), and stage of topping on the occurrence of *P. neoaphidis* was also investigated during 1994 and 1995. In general, aphid populations and level of infection were higher on flue-cured and burley tobaccos ($P < 0.05$) in the field. Infection was higher ($P < 0.05$) in aphids on Tobacco Introduction 1462 than on burley or flue-cured tobaccos in the greenhouse, where aphid populations were more uniform. Planting date, topping, and stage of topping

did not have significant influence on the level of infection.

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, tobacco cultivar, tobacco type, cultural practices.

Introduction:

Blackman (1987) described the tobacco feeding form of the green peach aphid, *Myzus persicae* (Sulzer), as the tobacco aphid, *M. nicotianae* Blackman. The tobacco aphid is a major pest of tobacco in North America and is capable of causing serious reductions in the yield and quality of tobacco under heavy infestations (Cheng and Court 1977, Mistric and Clark 1979, Cheng and Hanlon 1985, Reed and Semtner 1992). The number of effective insecticides against the tobacco aphid has decreased considerably in the last 15 years due to its increased resistance to some insecticides (Koziol and Semtner 1984, Harlow and Lampert 1990, McPherson and Bass 1990, Abdel-Aal et al. 1992, Chryssochoou and Bloukidis 1992) and the removal of other insecticides from the market. This necessitates development of alternative strategies to chemical control.

There have been several studies of entomopathogenic fungi infecting various species of aphids (Milner et al. 1980, Glare et al. 1986, Feng et al. 1990, Poprawski et al. 1992). The entomophthoralean, *Pandora (Erynia) neoaphidis* (Remaudière & Hennebert) Humber is the most common (Wilding and Brady 1984, Sivcev 1991, Kish et al. 1994) and dominant (Pickering et al. 1989, Feng et al. 1990, Feng et al. 1991, Sivcev 1992) pathogen of various species of aphids on different host plants. Yu et al. (1995) reported their laboratory studies on *P. neoaphidis* infecting *M. nicotianae* in the United States. However, field studies on the occurrence of *P. neoaphidis* in tobacco aphids in the United States have not

been published.

The occurrence of *P. neoaphidis* epizootics in tobacco aphid populations showed considerable variation in the time and distribution among different fields (see Chapter III). It also appeared that topping (removal of terminal inflorescence) of tobacco may have interfered with the incidence of the pathogen. Research on influence of cultural practices and different cultivars of tobacco on the occurrence of *P. neoaphidis* would enhance our knowledge of this pathogen in the tobacco ecosystem.

The objective of this study was to compare the occurrence of *P. neoaphidis* in tobacco aphids on various cultivars of tobacco and to determine how infection levels were influenced by planting date and stage at which flue-cured tobacco plants were topped.

Materials and Methods:

This study was conducted at the Southern Piedmont Agricultural Research and Extension Center (SPAREC) of Virginia Polytechnic Institute and State University, Blackstone, Virginia between 1992 and 1995. The occurrence of *P. neoaphidis* in tobacco aphid populations on different cultivars of four types of tobacco was monitored in the field during 1992, 1994, and 1995 and in the greenhouse during 1995. The influence of planting date, topping, and stage of topping was studied during 1994 and 1995 on flue-cured tobacco. Standard

agronomic practices recommended by the SPAREC for tobacco cultivation were followed for this study, except that tobacco was not treated with aphicides (Reed et al. 1995). Fenamiphos (Nemacur 3), pebulate (Tillam 6E), chlorpyrifos (Lorsban 4E), and metalaxyl (Ridomil 2E) were applied as preplant treatments for the control of nematodes, weeds, wireworms, and blackshank and blue mold, respectively.

Influence of cultivars:

Field, 1992: Twelve cultivars of flue-cured tobacco (Coker 139, Coker 319, Coker 371 Gold, Hicks, K-326, K-399, NC 27 NF, NC 37 NF, NC 567, Reams 134, Reams 158, and Reams 713), five cultivars of burley tobacco (Burley 21 X Kentucky 10, Kentucky 14, Kentucky 14-L8, Tennessee 86, and Tennessee 90), two cultivars of dark fire-cured tobacco (Kentucky Black and Virginia 309), and one cultivar of sun-cured tobacco (Virginia 409) were used in this study along with two tobacco introductions (TI 1068 and TI 1462) and a breeding line (NC 745). All entries were planted on 12 May in 0.26 ha.

Field, 1994: Fifteen cultivars of flue-cured tobacco (Coker 139, Coker 319, Coker 371 Gold, Hicks, K-326, K-346, K-394, K-730, NC 27 NF, NC 37 NF, Oxford 940, Reams M1, RG-8, RG-89, Speight G-126, and Virginia 116), three cultivars of burley tobacco (Burley 21 X Kentucky 10, Kentucky 14, and Tennessee 90), two cultivars of dark fire-cured tobacco (Kentucky Black and Virginia 309), and one cultivar of sun-cured tobacco (Virginia 409) were used in this study along

with a tobacco introduction (TI 1462). All entries were planted on 11 May in 0.23 ha.

Field, 1995: Thirteen cultivars of flue-cured tobacco (Coker 139, Coker 319, Coker 371 Gold, Hicks, K-326, K-346, K-394, K-730, NC 27 NF, NC 37 NF, Oxford 940, RG-89, and Speight G-126), five cultivars of burley tobacco (Burley 21, Burley 21 X Kentucky 10, Kentucky 14, NC BH-129, and Tennessee 90), two cultivars of dark fire-cured tobacco (Kentucky Black and Virginia 309), and one cultivar of sun-cured tobacco (Virginia 409) were used in this study along with a tobacco introduction (TI 1462). All entries were planted on 24 May in 0.21 ha.

Each entry had a single 12.2 m long row and was replicated six times during each year of the study. Entries were arranged in a randomized complete block design. Observations of aphids and the *P. neoaphidis* infection were made on 28 July, 5 August, and 21 July in 1992, 1994, and 1995, respectively. Plants were topped by the time of observations in all years, but the observations in 1994 were made on suckers growing from topped plants.

The number of aphids, on a 0-8 scale (0=0, 1=1-10, 2=11-50, 3=51-100, 4=101-500, 5=501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids), and percentage of infected aphids (0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7=76-90, 8=91-95, and 9=>95%), on a 0-9 scale, were rated on top four leaves of each of the five randomly selected plants in each plot on the observation date.

Greenhouse, 1995: *P. neoaphidis* was artificially introduced and its incidence in tobacco aphid populations was monitored under water mist conditions. Approximately 4-wk old plants of Coker 371 Gold, K-326, and NC 37 NF of flue-cured tobacco, Kentucky 14 and Tennessee 90 of burley tobacco, and the tobacco introduction TI 1462 were the treatments used for this study. Each entry had a single potted plant growing in sterile vermiculite (Palmetta Vermiculite Co. Inc.) and was replicated six times. Pots were placed in shallow pans with 150 ppm Fisons Technigro® 17-5-24 (N-P-K) (Fisons Horticulture Inc., Warwick, NY) fertilizer solution. The plants were arranged in four rows (about 68 cm spacing within the rows and 60 cm between the rows), with nine plants per row. Entries were arranged in a randomized complete block design . A mist system, with three nozzles aligned 1.8 m apart on a pipe, was set up with the pipe running in the middle with two rows of tobacco on either side of the pipe. Mist from each nozzle covered the area within a radius of about 90 cm and ensured coverage of all plants from three nozzles. A Nelson Rainmatic® 3000 electronic water timer (L.R. Nelson Corporation, Peoria, IL) was used to regulate the mist schedule. The timer was set up to mist water for 1 min at 30 min intervals, except between 200 and 800 h. The temperature in the greenhouse was maintained at a minimum of 21^0C.

Plants were artificially infested with laboratory colonies of tobacco aphids. Aphids were allowed to build up their populations. Natural infestations of tobacco aphids were also seen at this time. After about 2-wk, *P. neoaphidis* was

introduced into aphid populations in the form of living-infected and fungus-killed aphids collected from field tobacco. Ten living-infected and fungus-killed aphids were placed on each plant using a camel's hair brush. The controlled conditions of high humidity and moderate temperature provided a favorable environment for the establishment and development of infection in aphid populations.

Estimations of aphid populations and percent infection from all leaves of each plant were made on 30 October and 2 November, about 2-wk after inoculation, using the ratings considered for field observations. Plants were not topped in this study.

Influence of cultural practices:

In 1994, the incidence of *P. neoaphidis* in tobacco aphids was observed on flue-cured tobacco cultivars K-394 and K-326 that were planted on two dates, 12 and 26 May, and NC 27 NF that was planted on 12 May. Plants were topped at three stages of development viz., button, late button, and flowering. In 1995, three flue-cured tobacco cultivars, K-394, K-326, and NC 37 NF, were used with two planting dates, 8 and 22 May, and two stages of topping, button and flowering stages. In both years, each treatment had a single 12.2 m long row replicated six times (in 0.3 ha in 1994 and 0.24 ha in 1995). Treatments were arranged in randomized complete block design. Ratings (on the scale described above) of aphid numbers and percent infection were taken on 29 July and 2 August in 1994, and on 14 and 21 July in 1995 on top four leaves of each of the five randomly

selected plants per treatment.

In 1994, all early planted (12 May) tobacco, and part of late planted K-326 (26 May) had been topped by the time of observations. Comparison of planting date was made for all topped plants of K-326 from both planting dates. Comparison was not possible for K-394 as late planted part was not topped at the time of observations. Influence of stage of topping was compared for early planted tobacco. Incidence of pathogen on topped and untopped plants was also compared for K-326.

In 1995, none of the late planted (22 May) tobacco was topped at the time of observation. Early planted (8 May) tobacco had been topped in the button stage by the time of first observation on 14 July, and also at flowering stage by the time of second observation on 21 July. Influence of planting date was evaluated for untopped plants after excluding topped part of early planted tobacco. Comparisons for stage of topping were possible for early planted tobacco, only on 21 July. Influence of topping was compared for early planted tobacco on 14 July.

Statistical analysis: The data were analyzed using PROC GLM or PROC ANOVA of SAS software (SAS Institute 1987). Significant means were separated by Waller-Duncan K-ratio T test (K-ratio = 100). The data from test for cultivars were analyzed using PROC UNIVARIATE NORMAL PLOT statement to confirm normal distribution of residual error. Comparisons between different types of tobacco were made by orthogonal contrasts.

Identification of *P. neoaphidis*: On each sampling date, conidia collected on coverslips from 5-10 fungus-killed aphids sampled from the field were examined in lactophenol under phase contrast microscope. The identity of *P. neoaphidis* was confirmed based on the descriptions of Remaudière and Hennebert (1980), Wilding and Brady (1984), and Humber (1989).

Results and Discussion:

Influence of cultivar:

The occurrence of *P. neoaphidis* in tobacco aphid populations varied widely on various cultivars of different types of tobacco, and tobacco introductions, and the breeding line during this study (Tables 6.1, 6.2, 6.3, and 6.4). In general, the level of infection and aphid numbers were higher on burley and flue-cured tobaccos than on other tobacco types (Waller-Duncan K-ratio T test; K-ratio = 100), in the field over 3 years (Table 6.5). However, in the greenhouse, higher levels of infected aphids were seen on the tobacco introduction than on flue-cured or burley tobaccos, when aphid populations were similar.

In the field, variation in aphid numbers among the cultivars may have contributed to the variation in infection levels among different cultivars. This variation in aphid numbers might have interfered with and altered the influence of cultivars. Greenhouse results also showed higher infections in aphids on the tobacco introduction, where aphid populations were similar to or higher than those

Table 6. 1. Occurrence of *P. neoaphidis* in tobacco aphid populations on various tobacco cultivars, types, introductions, and a breeding line in the field, 28 July, 1992.

Cultivar	Type	Ratings for	
		Aphid numbers[1]	Percent infection[2]
Coker 139	Flue-cured	3.93ab[3]	2.80bcdef
Coker 319	Flue-cured	3.80abc	1.47def
Coker 371-Gold	Flue-cured	4.07a	2.23cdef
Hicks	Flue-cured	3.93ab	1.37def
K-326	Flue-cured	3.68abcd	3.47abc
K-399	Flue-cured	3.33abcde	2.17cdef
NC 27 NF	Flue-cured	3.73abcd	3.57abc
NC 37NF	Flue-cured	4.00ab	4.27ab
NC567	Flue-cured	3.67abcd	3.10abc
Reams 134	Flue-cured	4.13a	2.67bcdef
Reams 158	Flue-cured	4.22a	2.40bcdef
Reams 713	Flue-cured	3.32abcde	2.63bcdef
Virginia 309	Dark fire-cured	2.90cde	1.87cdef
Kentucky Black	Dark fire-cured	1.40fg	0.95f
Virginia 409	Sun-cured	3.00bcde	1.23def
Burley 21 X KY 10	Burley	2.80cde	2.67bcdef
Kentucky 14	Burley	2.42ef	4.73a
Kentucky 14-L8	Burley	2.83cde	2.88abcdef
Tennessee 86	Burley	3.20abcde	2.67bcdef
Tennessee 90	Burley	2.72de	2.97abcde
NC 745	Breeding Line	0.50g	2.40bcdef
TI 1068	Tobacco Introduction	3.75abc	2.57bcdef
TI 1462	Tobacco Introduction	0.60g	1.00ef

[1]Ratings for aphid numbers: 0 rating for 0 aphids, 1 for 1-10, 2 for 11-50, 3 for 51-100, 4 for 101-500, 5 for 501-1,000, 6 for 1,001-5,000, 7 for 5,001-10,000, and 8 for >10,000 aphids.

[2]Ratings for percent infection: 0 rating for 0% infection, 1 <1, 2 for 1-5, 3 for 6-10, 4 for 11-25, 5 for 26-50, 6 for 51-75, 7 for 76-90, 8 for 91-95, and 9 for >95% infection.

[3]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio=100).

Table 6. 2. Occurrence of *P. neoaphidis* in tobacco aphid populations on various tobacco cultivars, types, and an introduction in the field, 5 August, 1994.

Cultivar	Type	Ratings for	
		Aphid numbers[1]	Percent infection[2]
Coker 139	Flue-cured	3.70a[3]	4.07a
Coker 319	Flue-cured	3.57ab	2.90abc
Coker 371-Gold	Flue-cured	2.13cde	2.27bcd
K-326	Flue-cured	2.63abcde	2.63abc
K-346	Flue-cured	2.50bcde	1.57cde
K-394	Flue-cured	2.93abcd	2.63abc
K-730	Flue-cured	3.27abc	2.23bcd
NC 27 NF	Flue-cured	1.73efg	1.27cde
NC 37 NF	Flue-cured	2.93abcd	2.67abc
Oxford 940	Flue-cured	2.80abcde	2.63abc
Reams M1	Flue-cured	3.43ab	3.7ab
RG-8	Flue-cured	3.20abc	3.77ab
RG-89	Flue-cured	2.97abcd	2.77abc
Speight G-126	Flue-cured	3.30ab	2.70abc
Virginia 116	Flue-cured	2.80abcde	2.80abc
Virginia 409	Sun-cured	0.90fgh	0.73de
Kentucky Black	Dark fire-cured	0.57h	0.30de
Virginia 309	Dark fire-cured	0.63gh	0.37e
Burley 21 X KY 10	Burley	2.73abcde	3.27ab
Kentucky 14	Burley	0.83gh	1.57cde
Tennessee 90	Burley	2.03def	2.10bcd
TI 1462	Tobacco Introduction	0.07h	0.23e

[1]Ratings for aphid numbers: 0 rating for 0 aphids, 1 for 1-10, 2 for 11-50, 3 for 51-100, 4 for 101-500, 5 for 501-1,000, 6 for 1,001-5,000, 7 for 5,001-10,000, and 8 for >10,000 aphids.

[2]Ratings for percent infection: 0 rating for 0% infection, 1 <1, 2 for 1-5, 3 for 6-10, 4 for 11-25, 5 for 26-50, 6 for 51-75, 7 for 76-90, 8 for 91-95, and 9 for >95% infection.

[3]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio=100).

Table 6. 3. Occurrence of *P. neoaphidis* in tobacco aphid populations on various tobacco cultivars, types, and an introduction in the field, 21 July, 1995.

Cultivar	Type	Ratings for	
		Aphid numbers[1]	Percent infection[2]
Coker 139	Flue-cured	6.16ab[3]	1.04bc
Coker 319	Flue-cured	6.90a	0.53cdef
Coker 371-Gold	Flue-cured	6.60a	0.53cdef
Hicks	Flue-cured	3.63e	0.03f
K-326	Flue-cured	6.30ab	0.37cdef
K-346	Flue-cured	6.26ab	0.37cdef
K-394	Flue-cured	6.30ab	0.97bcd
K-730	Flue-cured	6.53ab	0.40cdef
NC 27 NF	Flue-cured	6.23ab	0.37cdef
NC 37 NF	Flue-cured	6.57a	0.23ef
Oxford 940	Flue-cured	5.24bcd	0.36cdef
RG-89	Flue-cured	6.43ab	0.80bcde
Speight G-126	Flue-cured	6.30ab	0.67cdef
Virginia 409	Sun-cured	4.07de	0.10f
Kentucky Black	Dark fire-cured	2.00f	0.04f
Virginia 309	Dark fire-cured	4.76cde	0.32def
Burely 21	Burley	5.23bcd	0.53cdef
Burley 21 X KY 10	Burley	5.63abc	0.97bcd
Kentucky 14	Burley	5.84abc	1.44ab
NC BH-129	Burley	5.67abc	1.73a
Tennessee 90	Burley	4.56cde	0.84bcde
TI 1462	Tobacco Introduction	1.03f	0.33def

[1]Ratings for aphid numbers: 0 rating for 0 aphids, 1 for 1-10, 2 for 11-50, 3 for 51-100, 4 for 101-500, 5 for 501-1,000, 6 for 1,001-5,000, 7 for 5,001-10,000, and 8 for >10,000 aphids.

[2]Ratings for percent infection: 0 rating for 0% infection, 1 <1, 2 for 1-5, 3 for 6-10, 4 for 11-25, 5 for 26-50, 6 for 51-75, 7 for 76-90, 8 for 91-95, and 9 for >95% infection.

[3]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio=100).

Table 6. 4. Occurrence of *P. neoaphidis* in tobacco aphid populations on various flue-cured and burley cultivars, and an introduction in the greenhouse, 1995.

Cultivar	Type	Ratings for			
		Aphid numbers[1]		Percent infection[2]	
		30 Oct.	2 Nov.	30 Oct.	2 Nov.
Coker 371-Gold	Flue-cured	6.67ab	7.00a	3.00c	3.33c
K-326	Flue-cured	7.12a	7.12a	4.00b	4.00bc
NC 37NF	Flue-cured	6.40b	6.40b	3.60bc	4.00bc
Kentucky 14	Burley	6.33b	6.12b	4.00b	4.00bc
Tennessee 90	Burley	6.50b	6.12b	4.00b	4.50ab
TI 1462	Tobacco Introduction	6.67ab	6.50b	4.67a	5.00a

[1]Ratings for aphid numbers: 0 rating for 0 aphids, 1 for 1-10, 2 for 11-50, 3 for 51-100, 4 for 101- 500, 5 for 501-1000, 6 for 1,001-5,000, 7 for 5,001-10,000, and 8 for >10,000 aphids.
[2]Ratings for percent infection: 0 rating for 0% infection, 1 <1, 2 for 1-5, 3 for 6-10, 4 for 11-25, 5 for 26-50, 6 for 51-75, 7 for 76-90, 8 for 91-95, and 9 for >95% infection.
[3]Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio=100).

Table 6. 5. Occurrence of *P. neoaphidis* in the tobacco aphid populations on various types of tobacco in the field (1992, 1994, and 1995) and the greenhouse (1995).

Type of tobacco	Field			Greenhouse	
	28 July 1992	5 August 1994	21 July 1995	30 October 1995	2 November 1995
			Aphids[1]		
Breeding Line	0.50d[2]	-	-	-	-
Burley	2.79b	1.87b	5.40b	6.33b	6.17c
Dark fire-cured	2.15bc	0.60cd	3.38c	-	-
Flue-cured	3.82a	2.93a	6.12a	6.77a	6.88a
Sun-cured	3.00b	0.90bcd	4.07c	-	-
Tobacco Introduction	2.18bc	0.07d	1.03d	6.58ab	6.33bc
			Percent infection[3]		
Breeding Line	2.40abc	-	-	-	-
Burley	3.19a	2.31a	1.10a	4.00b	4.00b
Dark fire-cured	1.50c	0.33b	0.18b	-	-
Flue-cured	2.68ab	2.71a	0.51b	3.53c	3.77b
Sun-cured	1.23c	0.73b	0.10b	-	-
Tobacco Introduction	1.94bc	0.23b	0.33b	4.33a	4.75a

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.

[2]Means separation based on orthogonal contrasts. Means followed by the same letter within each column are not significantly different ($P \geq 0.05$).

[3]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7=76-90, 8=91-95, and 9=>95% infection.

on other tobaccos, compared to the lower infections in the field where aphid populations were relatively low (Table 6.5). Leaf surface chemistry varies among different tobacco cultivars (Girardeau et al. 1973, Severson et al. 1984, Severson et al. 1985, Johnson et al. 1985) and this could have been another factor responsible for the variation in infection levels. Influence of leaf surface chemicals on entomopathogenic fungi is not known, however, such an influence is known for phytopathogenic fungi (Cruickshank et al. 1977, Severson et al. 1985, Menetrez et al. 1987, Menetrez et al. 1990).

Influence of cultural practices:

There was no influence of planting date on the occurrence of *P. neoaphidis* in tobacco aphids on K-326 in 1994 (Table 6.6) and both K-394 and NC 37 NF in 1995 (Table 6.7). However, aphids on early planted K-326 had higher infection ratings than those on late planted K-326 ($F = 14.00$, df = 1,17, $P = 0.003$) in 1995. Aphid populations were also similar on tobacco from both planting dates in both years, except on K-394 where the early planted plots had significantly higher aphid ratings ($F = 14.33$, df = 1,17, $P = 0.005$) (Tables 6.6 and 6.7). Although the differences were not generally significant ($P \geq 0.05$), aphid populations and infection levels were higher on early planted tobacco in 1995.

The stage at which tobacco was topped did not appear to influence the incidence of *P. neoaphidis*. Topping stage influenced the level of infection only on K-394 in 1995 (Tables 6.6 and 6.8). Aphids on K-394 topped at the flowering

Table 6. 6. Influence of planting date and stage of topping on the occurrence of *P. neoaphidis* in the tobacco aphid populations on flue-cured tobacco after topping, 1994.

Cultivar	Planting date	Stage of topping	Ratings for			
			Aphid numbers[1]		Percent infection[2]	
			July 29	August 2	July 29	August 2
Planting date[3]:						
K-326	12 May		4.80NS	3.80NS	0.68NS	1.98NS
	26 May		4.53	3.80	1.60	2.67
Topping stage[4]:						
K-394	12 May	B[5]	4.63a	3.47a	0.60a	2.40a
		L	4.67a	3.17a	1.33a	1.73a
		F	4.87a	4.23a	0.73a	2.23a
K-326	12 May	B	4.93a	4.04a	0.67a	1.96a
		L	5.13a	4.50a	1.10a	2.23a
		F	4.24b	2.72b	0.20a	1.68a
NC 27 NF	12 May	B	5.37a	4.10a	1.40a	2.40a
		L	5.00a	3.92a	0.40a	2.80a
		F	5.23a	4.60a	0.83a	2.00a

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.

[2]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7= 76-90, 8=91-95, and 9=>95% infection.

[3]Means separation for planting dates. NS, not significantly different ($P \geq 0.05$).

[4]Means separation for stage of topping within cultivar. Means followed by the same letter within each column are not significantly different (Waller-Duncan K-ratio T test; K-ratio = 100).

[5]B, L, and F represent button, late button, and flowering stages, respectively, at which plants were topped.

Table 6. 7. Influence of planting date on the occurrence of *P. neoaphidis* in the tobacco aphid populations on various flue-cured tobacco cultivars before topping. Observations on 14 July, 1995.

Cultivar	Planting date	Ratings for	
		Aphid numbers[1]	Percent infection[2]
K-394	8 May	5.97**[3]	3.07NS
	22 May	4.65	2.50
K-326	8 May	5.53NS	4.10**
	22 May	5.07	2.03
NC 37 NF	8 May	5.77NS	2.90NS
	22 May	5.12	2.22

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.
[2]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7=76-90, 8=91-95, and 9=>95% infection.
[3]Means separation for planting dates within cultivar. NS, not significantly different, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; $P < 0.001$; H_0, coefficient, 0.

Table 6. 8. Influence of stage of topping on the occurrence of *P. neoaphidis* in the tobacco aphid populations on various flue-cured tobacco cultivars after topping. Observations on 21 July, 1995.

Cultivar	Planting date	Topping stage	Ratings for	
			Aphid numbers[1]	Percent infection[2]
K-394	8 May	B[3]	4.30NS[4]	1.70*
		F	5.03	2.93
K-326	8 May	B	4.80NS	2.40NS
		F	4.30	1.77
NC 37 NF	8 May	B	4.06NS	2.20NS
		F	4.43	2.47

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.
[2]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7= 76-90, 8=91-95, and 9=>95% infection.
[3]B, and F represent button and flowering stages, respectively, at which plants were topped.
[4]Means separation for stage of topping within cultivar. NS, not significantly different, $P \geq 0.05$; *, $P < 0.05$; H_0, coefficient, 0.

stage had significantly higher infection levels than those on that cultivar topped at button stage ($F = 12.56$, df = 1,11, $P = 0.0165$), in 1995. Higher numbers of aphids on tobacco topped at flowering stage might have been responsible for higher infections in aphids on those plants. Although significant differences in aphid populations on K-326 in 1994 did not significantly influence the infection level, higher infections were observed on K-326 topped at button and late button stages where aphid populations were also higher (Table 6.6). Similarly aphid populations and level of infection were higher on K-326 and NC 37 NF in 1995, even though the differences were not significant ($P \geq 0.05$) (Table 6.8).

Topping did not have a significant influence on the incidence of *P. neoaphidis* in tobacco aphid populations (Tables 6.9 and 6.10), but the level of infection was generally higher in aphids on untopped than topped plants in both 1994 (on K-326) and 1995 (on K-326 and K-394). On NC 37 NF, a non-flowering cultivar aphids were slightly higher on topped plants. Incidence of *P. neoaphidis* appeared to be higher in aphids on the upper most leaves in untopped plants (see Chapter V) and hence, lower infections would be expected in the topped plants, which lost major portion of the inoculum.

Table 6. 9. Influence of topping on the occurrence of *P. neoaphidis* in the tobacco aphid populations on flue-cured tobacco 'K-326' planted on 26 May, 1994.

Topping	Ratings for			
	Aphid numbers[1]		Percent infection[2]	
	July 29	August 2	July 29	August 2
Topped	4.53NS[3]	3.80NS	1.60NS	2.67NS
Not topped	5.87	4.53	3.20	5.40

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.
[2]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7= 76-90, 8=91-95, and 9=>95% infection.
[3]Means separation for topped and untopped plants. NS, not significantly different ($P \geq 0.05$).

Table 6. 10. Influence of topping on the occurrence of *P. neoaphidis* in the tobacco aphid populations on various flue-cured tobacco cultivars on 1 May, 1995. Observations on 14 July, 1995.

Cultivar	Topping	Ratings for	
		Aphid numbers[1]	Percent infection[2]
K-394	Topped	4.53*[3]	2.00NS
	Not topped	5.97	3.07
K-326	Topped	5.57NS	3.27NS
	Not topped	5.53	4.10
NC 37 NF	Topped	4.83NS	3.07NS
	Not topped	5.77	2.90

[1]Ratings for aphid numbers: 0=0, 1=1-10, 2=11-50, 3=51-100, 4=101- 500, 5 for 501-1,000, 6=1,001-5,000, 7=5,001-10,000, and 8=>10,000 aphids.
[2]Ratings for percent infection: 0=0, 1=<1, 2=1-5, 3=6-10, 4=11-25, 5=26-50, 6=51-75, 7= 76-90, 8=91-95, and 9=>95% infection.
[3]Means separation for topped and untopped plants within cultivar. NS, not significantly different, $P \geq 0.05$; *, $P < 0.05$; H_0, coefficient, 0.

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CHAPTER VII

Introducing *Pandora neoaphidis* (Remaudière & Hennebert) Humber (Zygomycotina: Entomophthorales) into populations of *Myzus nicotianae* Blackman (Homoptera: Aphididae) on flue-cured tobacco.

Abstract:

The entomophthoralean, *Pandora neoaphidis* (Remaudière & Hennebert) Humber was successfully introduced into populations of the tobacco aphid, *Myzus nicotianae* Blackman, on flue-cured tobacco in the field and greenhouse. In 1993, the success of introducing pathogen using different forms of inocula was evaluated in the field. In a greenhouse test in 1994, the potential of artificially introduced pathogen to control tobacco aphids was compared with insecticidal control. The pathogen, introduced as living-infected aphids and fungus-killed aphids, living-infected aphids alone, and triturated fungus-killed aphids, was able to initiate infection in tobacco aphids in the field. Infections of 33-37% were seen, 2 wk after introducing the pathogen, when living-infected aphids were distributed alone and along with fungus-killed aphids, compared to 2% infection in untreated control. Application of triturated fungus-killed aphids resulted in low infections (maximum of 6%) similar to those in untreated control. In the greenhouse

(maximum of 6%) similar to those in untreated control. In the greenhouse experiment, both fungus-killed and living-infected aphids were equally effective in introducing the pathogen in tobacco aphid populations. The treatments of two forms of pathogen and insecticide were initiated when there were about 3000 aphids/plant. There was a 100% control in aphids with insecticide treatment by 4 days after treatment (DAT). Though there was an increase in infection levels, the pathogen failed to prevent development of aphid populations. There was nearly a six-fold increase in aphid populations from 4 to 19 DAT. Almost no aphids were seen on insecticide treated plant except for a few on 19 DAT.

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, introducing pathogen, insecticidal control, tobacco.

Introduction:

The tobacco aphid, *Myzus nicotianae* Blackman, is a major insect pest of tobacco resulting in serious damage to the yield and quality of tobacco leaf (Cheng and Court 1977, Cheng and Hanlon 1985, Lampert 1989, Mistric and Clark 1979, Reed and Semtner 1992). The entomophthoralean, *Pandora neoaphidis* (Remaudière & Hennebert) Humber has been found to cause great reductions in tobacco aphid populations (Dara and Semtner 1995).

Studies have been conducted on artificially introducing *P. neoaphidis* into populations of cereal aphids and black bean aphids (Latteur and Godefroid 1982, Wilding 1982, Wilding et al. 1986a, Wilding et al. 1986b, Wilding et al. 1990). While the pathogen applied in vivo was successfully established in the aphid hosts, in vitro formulations of the fungus failed in some of these studies supposedly due to lack of virulence or lower resistance to weather factors compared to in vivo formulations. The yield of *Vicia* beans was twice as high in insecticide treated plots as in *P. neoaphidis* treated plots (Wilding 1982). Wilding et al. (1986b) also reported that pirimicarb resulted in significantly greater reductions in *A. fabae* populations compared to *P. neoaphidis* on *Vicia* beans. The absence of resting spores and failure of in vitro formulations to introduce infection limit this pathogen's commercial potential. Slow development in the host populations and high dependence on weather factors are major limitations to the use of this pathogen as an alternative to chemical control. However, its potential in

controlling aphids should not be ignored as it is an important mortality factor of various species of aphids in naturally occurring epizootics (Pickering et al. 1989, Summers and Newton 1989, Dara and Semtner 1995). Artificial introduction of the pathogen might help early inducement of the epizootic and could be a part of integrated pest management programs, even though it does not seem to be as effective as the use of insecticides.

Introducing *P. neoaphidis* by artificial means has not been reported in North America. This study was conducted to evaluate the possibility of introducing *P. neoaphidis* into tobacco aphid populations on tobacco. The relative effectiveness of the forms of inoculum were compared as well as the influence of the pathogen on tobacco aphid control against that with insecticidal treatment.

Materials and Methods:

This study was conducted at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, Virginia.

1993, Field trial to introduce *P. neoaphidis* in tobacco aphid populations: A field of flue-cured tobacco 'K-326' with suckers, about 3-4 wk old, grown from cut tobacco plants was used for this study. Aphids in this field were free from *P. neoaphidis* infection and was distant from the other fields containing infected aphid populations. Three methods for introducing *P. neoaphidis* were evaluated for their effectiveness in initiating infection in tobacco aphid populations on flue-

cured tobacco. Treatments included were, application of triturated fungus-killed aphids (about 10 mg/plant), distribution of living-infected aphids (10/plant), distribution of both living-infected and fungus-killed aphids (10-15/plant) and an untreated control, each replicated four times. Treatments were arranged in a randomized complete block design. Each plot consisted of a single 6 m long row that was separated from the adjacent plots by two buffer rows on either side. Treatments were applied to five randomly selected plants within each plot.

Inoculum of fungus-killed and living-infected aphids was obtained from a tobacco field located about 0.5 km from the test field where natural infection by *P. neoaphidis* had occurred. Fungus-killed aphids were air-dried and ground by sieving through a No. 45 strainer (355 μm opening) to obtain a fine powder.

Treatments were initiated between 1700 and 1830 h on 13 August. Plants were sprayed with water just before initiation of treatments to promote adhesion to leaf surfaces of the powder of fungus-killed aphids and to induce favorable conditions for conidial germination and sporulation of the pathogen. Powder was applied by hand to the plants by sprinkling above them, living-infected aphids were distributed using a camel's hair brush, and fungus-killed aphids attached to tobacco leaf, cut into small pieces of about 5 cm^2, were placed on plants along with living-infected aphids, in respective treatments.

Numbers of healthy and infected (both fungus-killed and living-infected) aphids were recorded at 1-3 d intervals from 14 August to 2 September on the

sample plants in the treated and untreated plots. The number of total aphids and those infected by *P. neoaphidis* from five plants were used to calculate mean number of aphids and percent infection per plant.

Weather data were obtained from a weather station (Omnidata International Inc., Logan, Utah) located about 1 km from the test site.

1994, Greenhouse trial to compare the use of *P. neoaphidis* with insecticidal use for aphid control: The experiment was initiated on 11 November with four treatments viz., i. distribution of fungus-killed aphids (40/plant), ii. release of living-infected aphids (40/plant), iii. acephate spray (Orthene® 75S 1.1 kg/ha), and iv. untreated control. Each treatment had a single potted plant replicated four times. The temperature in the greenhouse was variable and maintained at a minimum of 15^0C. Flue-cured 'K-326' tobacco plants, approximately 4-wk old, growing in sterile vermiculite (Palmetta Vermiculite Co. Inc.) were used. These pots were placed in shallow pans with 50 ppm Peters 20-10-10 (N-P-K) (Scotts-Sierra Horticultural Products Co., Marysville, OH) fertilizer solution. The plants were arranged in two rows (about 90 cm spacing within the rows and 1.2 m between the rows), with two replications (eight plants) per row. Treatments were arranged in randomized complete block design. Plants were artificially infested with laboratory colonies of tobacco aphids during the last week of October, and aphid populations were allowed to build up for about 2 wk. On 11 November, infected aphids were placed on the plants with the help of a camel's hair brush.

Plants to be treated with insecticide were set aside, and acephate was sprayed using a CO_2-pressurized backpack sprayer with a delivery rate of 234 l/ha through three TX-10 (Spraying Systems Co., Wheaton, IL) hollow-cone nozzles per row at 414 kPa pressure. Acephate-treated plants were put back in their original places the next day. A mist system with three nozzles aligned 1.8 m apart on a pipe was set up with the pipe running in between the two rows of tobacco plants. Mist from each nozzle covered the area within a radius of about 90 cm and ensured coverage of all plants. A Nelson Rainmatic® 3000 electronic water timer (L.R. Nelson Corporation, Peoria, IL) was used to regulate the timing of mist schedule. The timer was set up to mist water for 15 sec at 20 min intervals throughout the day to encourage establishment and development of infection in aphid populations. The number of healthy and/or infected (both fungus-killed and living-infected) aphids on each plant was counted from 11 to 30 November at 3-5 d intervals.

Laboratory colonies of aphids were infected by subjecting them to conidial showers from *P. neoaphidis* cultures to obtain inocula used in the test. *P. neoaphidis* was isolated from field collected tobacco aphids using the method described by G.L. Nordin (personal communication; see Appendix 12). The fungus was grown on modified SEMA (Sabouraud maltose-egg-milk-agar) (Yu et al. 1995; see Appendix 12). Ingredients of SEMA included 74.6% fresh yolk from unwashed eggs, 14.4% vitamin D milk, 11% Sabouraud maltose agar (Difco Laboratories, Detroit, MI), 0.1% gentamicin (Sigma Chemical Co., St. Louis, MO)

and 0.05% penicillin (Agri Laboratories Ltd., St. Joseph, MO). These cultures were incubated at 20^{0}C and 14L:10D photophase. Two to three week old cultures were used for inoculating the aphids. Sporulating cultures were selected for the experiment based on the conidial deposition on the lids of Petri plates.

Aphids to be inoculated were placed on a tobacco leaf disc (about 55 mm dia) in a small Petri plate (60 mm dia by 15 mm). Tobacco leaves from greenhouse plants were rinsed with deionized water before the discs were cut. A filter paper (Whatman No. 1, 55 mm circle) was placed on the bottom and a similar one was attached to the lid of each Petri plate with non-toxic Permanent Glue Stic (Avery Dennison, Framingham, MA). These filter papers were moistened to maintain high humidity ($\geq$ 98%) in the plate to facilitate conidial germination (Wilding 1971, Yu et al. 1995).

Fungus cultures in the large Petri plates (100 mm dia by 15 mm) were inverted over the small plates containing aphids in order to expose the aphids to conidial showers. These plates were covered with moist paper towels, placed in a sealed plastic bag, and incubated in the dark at 22^{0}C in a growth chamber. Relative humidity inside the plastic bags was measured using a hygrometer (127 mm dia by 32 mm) (Abbeon Cal. Inc., Santa Barbara, CA). Aphids were exposed to conidial showers for 4-5 h. Culture plates were rotated 90^0 every quarter of exposure time to promote uniform conidial showers over the exposed area. At the end of the inoculation, the culture plates were removed and small plates were

covered with their lids after moistening the filter papers attached to them. After about 16 h, the paper towels and plastic bags were removed and the chamber was changed from 24D to 16L:8D photophase. Aphids were provided with new leaf discs every other day. After about 3-5 d, inocula for the test (living-infected aphids and fungus-killed aphids) were collected from these infected aphid colonies.

Statistical analysis: The data were analyzed using PROC ANOVA of SAS software (SAS Institute 1987) and means per plant total number of aphids and the proportion of those infected by the pathogen were obtained. Means of logarithmically-transformed aphid numbers and arcsine-transformed percent infection from the analysis were used for means separation. Significant means were separated using Waller-Duncan K-ratio T test (K-ratio = 100).

Results:

1993, Field trial: Infection by *P. neoaphidis* was first noticed on 17 August, 4 d after inoculation (Fig. 7.1B). Coincident natural occurrence of the pathogen resulted in infections in untreated plots after the pathogen was introduced into treatment plots. However, infection in control plots remained lower than in the treated plots during most of the observation period. In general, higher infections in aphids were resulted from distribution of living-infected aphids alone and along with fungus-killed aphids. Infections resulted from the application of triturated

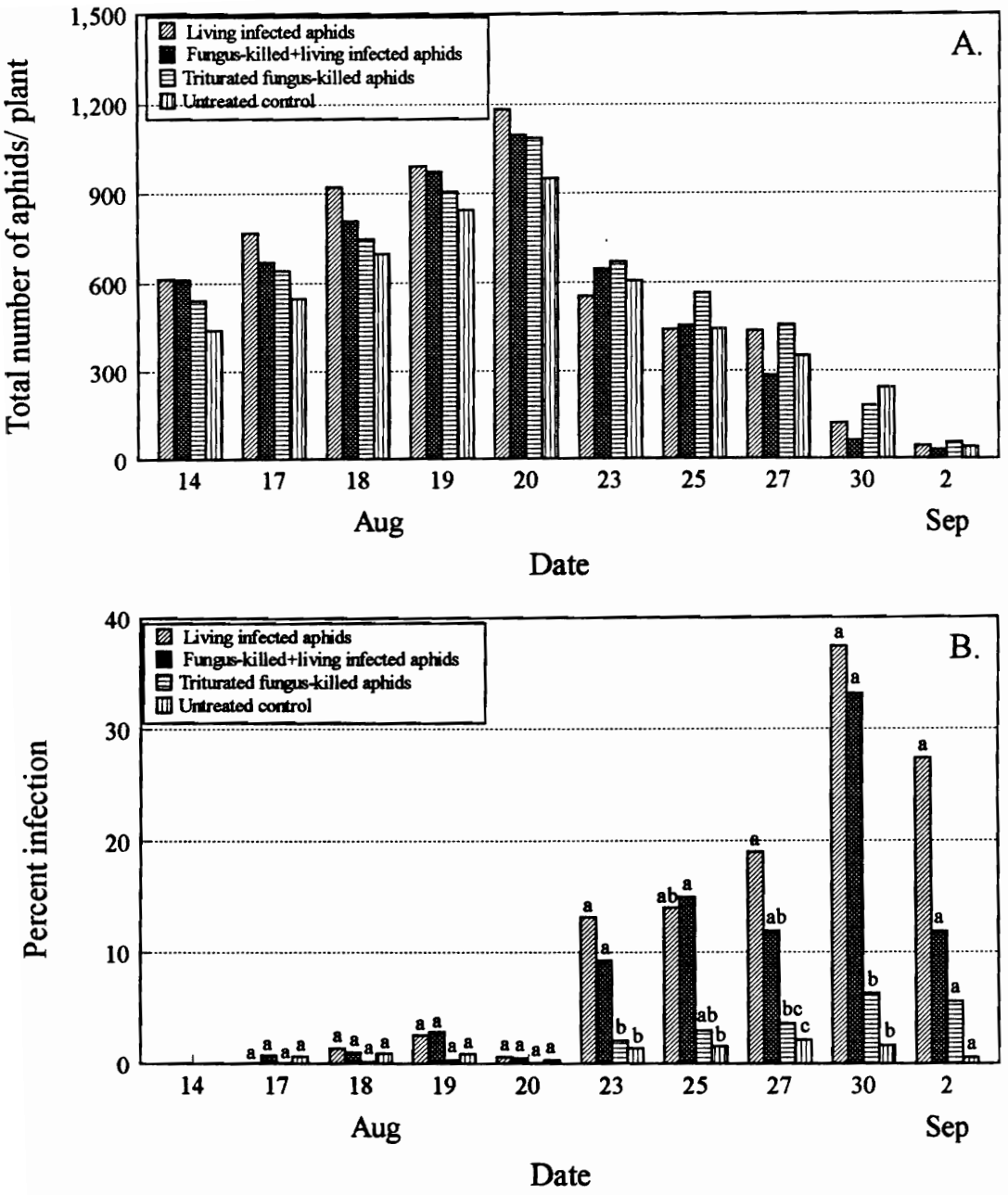

Fig. 7. 1. Development of *P. neoaphidis* artificially introduced into *M. nicotianae* populations on flue-cured tobacco in the field, 1993. Aphid populations (A) and their proportion infected by the pathogen (B), introduced by artificial means. A. Aphid populations among treatments were not significantly different on all dates (Waller-Duncan K-ratio T test; K ratio=100); B. Bars on each date surmounted by the same letter are not significantly different (Waller-Duncan K-ratio T test; K ratio=100). (Appendix 13)

fungus-killed aphids were similar to those in untreated controls. Maximum levels of infection caused by different forms of inoculum during this test were 37.2, 33.2 and 6.4% for living-infected aphids alone, living-infected and fungus-killed aphids, and for triturated fungus-killed aphids, respectively (on 30 August), compared to the maximum of 2.2% in the control (on 27 August). Percent infection increased gradually until it peaked on 30 August. Aphid populations increased from about 550/plant on 14 August to about 1100/plant on 20 August and then steadily declined to about 40/plant on 2 September (Fig. 7.1A). However, aphid populations on individual observation dates were uniform in both treated and control plots.

Influence of environmental factors on disease development was unclear. The average daily temperatures during the observation period ranged between 23 and 29^{0}C (Fig. 7.2A). The total amount of rainfall between 14 and 31 August was only 3 mm and the daily averages of leaf wetness were below 35% during most of that period (Fig. 7.2B). The average daily relative humidity ranged between 69 and 86% during the observation period (Fig. 7.2B). The average number of hours in each day with >90% relative humidity and >70% leaf wetness were about 6 and 5.5, respectively, during the period of observation.

1994, Greenhouse trial: There was an immediate control of tobacco aphids on plants treated with acephate, where the number of aphids was reduced from about 3000 to almost zero per plant by 4 DAT, providing 100% control (Fig. 7.3A).

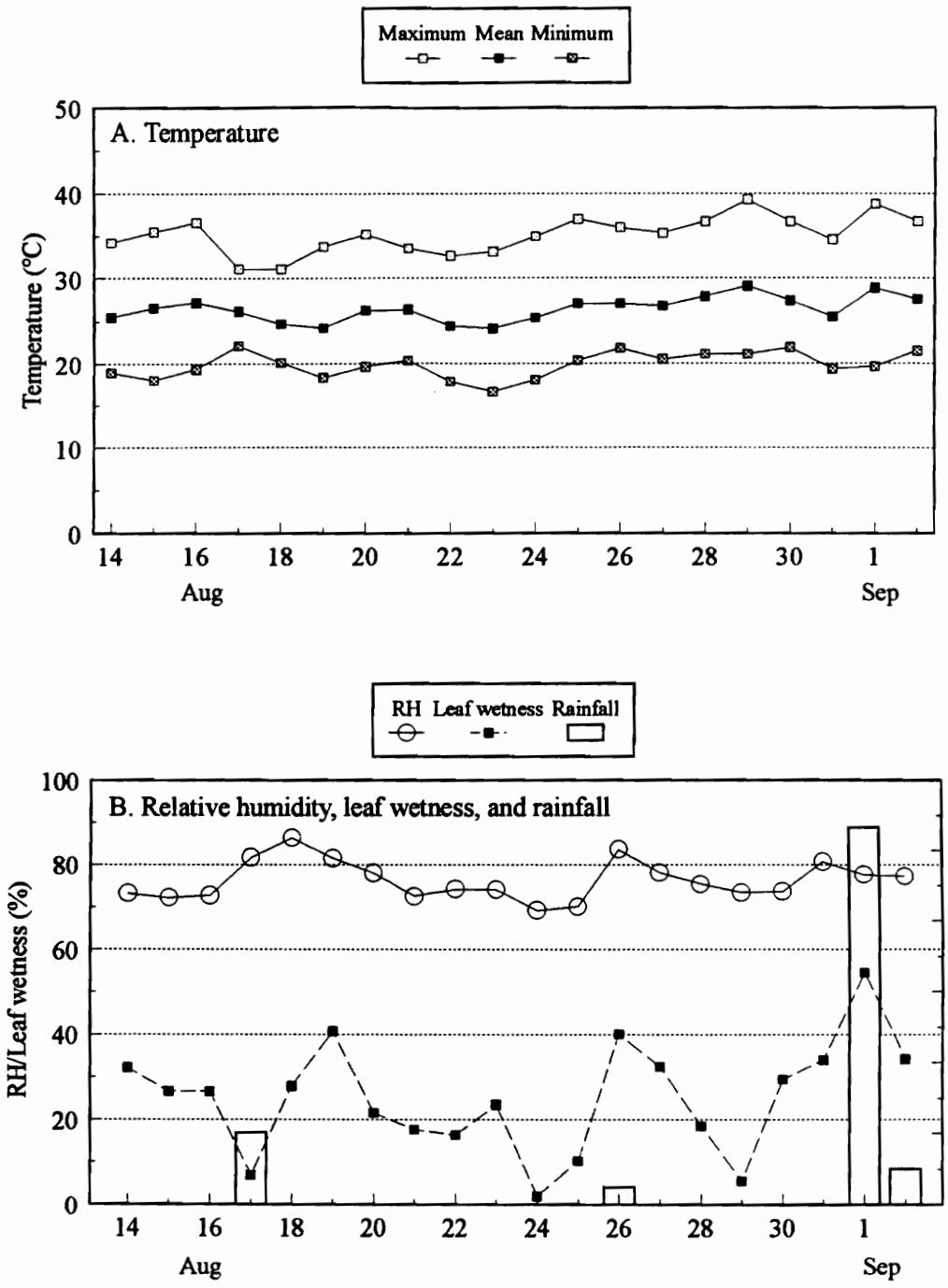

Fig. 7. 2. Environmental conditions during development of introduced *P. neoaphidis* in tobacco aphid populations on flue-cured tobacco in the field, 1993. Daily averages of temperature (A), relative humidity and leaf wetness, and daily total of rainfall (B). (Appendix14)

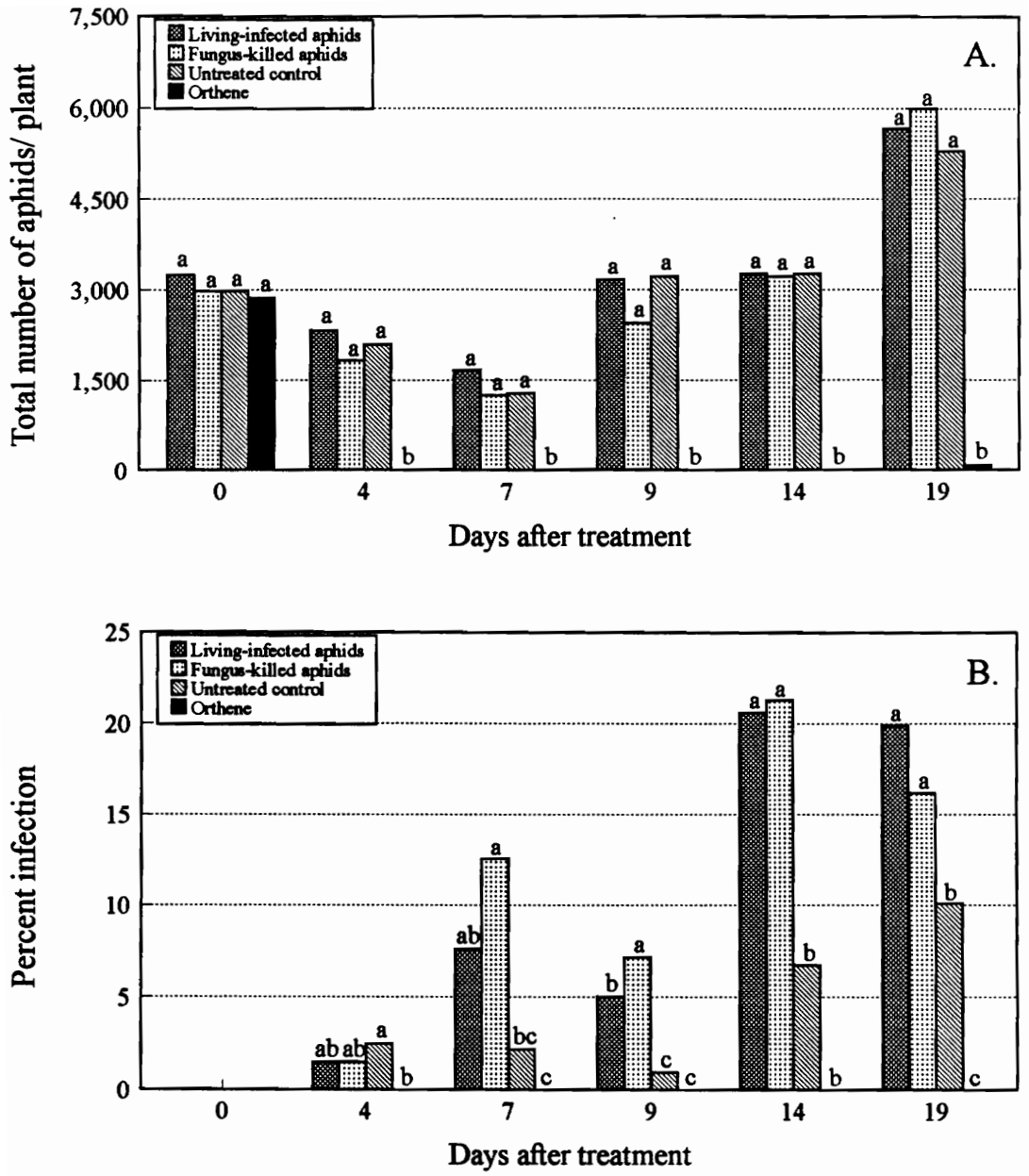

Fig. 7. 3. Efficacy of *P. neoaphidis* and Orthene in controlling *M. nicotianae* in the greenhouse, 1994. Tobacco aphid populations (A) and their proportion infected by the pathogen (B). Bars on each date surmounted by the same letter are not significantly different (Waller-Duncan K-ratio T test; K ratio=100). (Appendix 15)

Aphids populations never rebounded on acephate treated plants, although few aphids were seen by 19 DAT. Introduction of *P. neoaphidis* did not reduce aphid populations, even though infection levels progressed in aphid populations (Fig. 7.3). Infection levels were similarly low on both pathogen-treated and control plants on 4 DAT. However, infection in aphid populations was significantly lower ($P < 0.05$) in controls than in plants treated with fungus-killed aphids, but not those treated with living-infected aphids, on 7 DAT. Except for a slight decrease on 9 DAT, the level of infection gradually increased in both treated and control plants towards the end of the observation period and was significantly higher ($P < 0.001$) in treated than control plants between 9 and 19 DAT.

Discussion:

Weather conditions during the summer of 1993 were relatively hot and dry. Natural occurrence of *P. neoaphidis* was not detected until the first week of August in fields about 0.5 km from the test site. The pathogen had become established in the experimental plots, despite extremely low rainfall during the experiment (total of 3 mm between 14 and 31 August and about 6 mm between 1 and 2 September) and caused higher infections in treated than in untreated plots. Development of infection was influenced neither by the narrow range of fluctuations in temperature (daily averages from 23 to 29^{0}C) and relative humidity (daily averages from 69 to 86%) nor by wide range of fluctuations in leaf wetness

(daily averages from 2 to 55%). Despite the dry weather in terms of rainfall, the relative humidity was >90% for 5-13 h, and leaf wetness was >70% for 5-9 h at nights during most of the observation period. The average number of hours per day with relative humidity >90% was 6 between 14 August and 2 September. While the average number of hours per day with >70% leaf wetness were 5.5 during the same period. High humidities ($\geq$ 90%) are required for the spread of infection by *P. neoaphidis* in the field (Rockwood 1950). The favorable range of leaf wetness for the development of infection is not known. However, it appears reasonable to consider that 70% leaf wetness could fall in a favorable range for the pathogen. Periods of high humidity and leaf wetness prevailed during this experiment might have helped development of *P. neoaphidis* in tobacco aphid populations.

Although natural incidence of the pathogen caused infection in untreated plots, it was lower than that in treated plots and did not seem to be responsible for reduction in aphid populations. Aphid populations decreased in all plots towards the end of the observation period. Decline in aphid populations is common after topping tobacco (Semtner 1984, Lampert 1989), but the tobacco in test plots was not topped. Migration of aphids, as the end of the season was approaching, might have been responsible for reduction in their populations.

Application of in vitro grown *P. neoaphidis* mycelium failed to establish infection in aphid populations in earlier studies (Latteur and Godefroid 1982,

Wilding 1982, Wilding et al. 1986a). Spraying in vitro grown *P. neoaphidis* as a mycelial suspension in water also failed to cause infection in tobacco aphids on tobacco, in my preliminary laboratory and greenhouse trials. In other studies, application of fungus in vivo (as living-infected or fungus-killed aphids or triturated fungus-killed aphids) was successful in establishing infections and application of triturated fungus-killed aphids was equally effective as distribution of living-infected aphids (Wilding 1982, Wilding et al. 1986a, Wilding et al. 1986b, Wilding et al. 1990). However, triturated fungus-killed aphids were not as effective in the present study as fungus-killed and living-infected aphids in causing infections in aphid populations. Due to the insufficiency of inoculum, the rate of triturated fungus-killed aphids was limited to only 10 mg/plant (65 g/ha). The rate of triturated fungus-killed aphids used by Wilding (1982) and Wilding et al. (1986b) was 0.5-2.8 mg/plant on *Vicia* beans and 1.15 kg/ha, respectively. Irrigation increased infection levels in black bean aphid, *Aphis fabae* Scopoli, on *Vicia* beans in artificial introductions of *P. neoaphidis* (Wilding et al. 1986b). In a greenhouse study, where *P. neoaphidis* was introduced as fungus-killed and living-infected aphids, virtually 100% of tobacco aphids were infected in the upper 1/4 of some tobacco plants while those on the lower parts had lower levels of infection (personal observation). A mister system was established to mist water for 1 min every half hour for 18 h/day in that experiment and the vertically oriented upper leaves were directly exposed to the mist and thus had more favorable conditions

for disease development than more horizontally oriented lower leaves. Influence of overhead irrigation on natural *P. neoaphidis* epizootic in pea aphid, *Acyrthosiphon pisum* (Harris), on legume ground cover of a pecan orchard was previously reported, where the pathogen infected 70% of the aphids in overhead irrigated portion against no infection in drip irrigated portion of the orchard (Pickering et al. 1989). However, tobacco was not irrigated in the present study.

The pathogen was unable to prevent the build up of aphid populations after the initial decrease and the number of aphids in pathogen treated plants and controls was similar and significantly higher ($P = 0.0001$) than in insecticide treated plants throughout the observation period. Wilding et al. (1986b) observed the influence of treatments on *A. fabae* populations from the use of an insecticide and *P. neoaphidis* on *Vicia* beans, which was similar to the findings in the current study. Wilding (1982) reported higher yields in insecticide treated plots than in *P. neoaphidis* treated plots of *Vicia* beans.

Use of *P. neoaphidis* does not appear to provide a practical alternative to chemical control, but its potential as an important mortality factor of aphid should be considered in designing pest management programs. Introducing the pathogen early in the season in some parts of the field might help in initial establishment and further development in aphid populations. Further research needs to be done in this area to determine if the pathogen introduced in some parts of the field could spread in aphid populations all over the field and reduce or delay insecticide

treatments. However, its success in controlling aphids, being largely dependent upon environmental conditions, remains unpredictable.

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CHAPTER VIII

Virulence of Virginia isolate of *Pandora neoaphidis* (Zygomycotina: Entomophthorales) to apterae of *Myzus nicotianae* and *M. persicae* (Homoptera: Aphididae) from different geographic locations in the eastern United States.

Abstract:

The virulence of an isolate of *Pandora neoaphidis* (Remaudière & Hennebert) Humber from the red morph of the tobacco aphid, *Myzus nicotianae* Blackman, was evaluated in laboratory bioassays to apterae of three biotypes of the red morph and two biotypes of the green morph of tobacco aphid and one biotype of the green peach aphid, *M. persicae* (Sulzer). The biotypes evaluated in this study were of red tobacco aphid collected from tobacco (*Nicotiana tabacum* L.) near Windsor, CT, Tifton, GA and Blackstone, VA; green tobacco aphid obtained from tobacco near Duplin, NC and Blackstone, VA; and green peach aphid collected from climbing nightshade (*Solanum dulcamara* L.) near Glade Spring, VA. Three-day old aphids were inoculated with conidia of *P. neoaphidis* and their mortality was monitored daily, beginning on the third day after inoculation (DAI) until infected aphids were no longer observed. The

susceptibilities of different biotypes of aphids were significantly different (Waller-Duncan K-ratio T test; K-ratio = 100) only on 4 and 5 DAI in Tests 1 and 2, respectively. When mortality of different biotypes within red and green tobacco aphids was combined, significant differences (K-ratio = 100) were seen only on 6, 7, and 8 DAI in Test 3 when red tobacco aphid was more susceptible than green peach aphid. *P. neoaphidis* is equally virulent to the red and green morphs of tobacco aphid, and green peach aphid and also to their biotypes.

Key words: *Myzus nicotianae*, *M. persicae*, *Pandora neoaphidis*, red morph, green morph, virulence, susceptibility.

Introduction:

Pandora neoaphidis (Remaudière & Hennebert) Humber is an entomophthoralean infectious to several species of aphids on different host plants (Humber 1992). The tobacco aphid, *Myzus nicotianae* Blackman, and the green peach aphid, *M. persicae* (Sulzer), are two of the hosts of *P. neoaphidis*. The tobacco aphid, a major insect pest of tobacco (*Nicotiana tabacum* L.), was formerly considered as the tobacco feeding form of the green peach aphid (Blackman 1987, Lampert 1989, Reed and Semtner 1992). First reports on the occurrence of *P. neoaphidis* in Virginia demonstrated the importance of the pathogen in reducing populations of tobacco aphid (Dara and Semtner 1995). The red morph of the tobacco aphid is more tolerant of high temperatures and has higher rates of development, survival and fecundity than the green morph (Lampert and Dennis 1987, Reed and Semtner 1991). In addition, it has greater resistance to many aphicides (McPherson and Bass 1990, Harlow and Lampert 1990).

Variation in the susceptibility of biotypes to *P. neoaphidis* has been studied for pea aphid, *Acyrthosiphon pisum* (Harris) in Australia, where resistance in field populations of aphids to a fungal pathogen was found for the first time (Milner 1982 and 1985). An isolate of *P. neoaphidis* was found to be specific to one of the two species of aphids on alfalfa in New Zealand (Cameron and Milner 1981). It is not known if the isolate of *P. neoaphidis* infecting the tobacco aphid in Virginia would be virulent to the tobacco aphid from different geographic locations.

Information is also not available on possible differences in susceptibility of different biotypes of the tobacco aphid to *P. neoaphidis*.

This study was conducted to evaluate the virulence of *P. neoaphidis* to different biotypes of apterous red and green morphs of tobacco aphid, and green peach aphid obtained from different geographic locations in the eastern United States.

Materials and Methods:

The virulence of *P. neoaphidis* to 3-d old apterous aphids of three biotypes of red tobacco aphid, two biotypes of green tobacco aphid and a biotype of green peach aphid were evaluated in a laboratory study conducted at the Southern Piedmont Agricultural Research and Extension Center (SPAREC), Blackstone, Virginia. The red tobacco aphids were collected from Windsor, Connecticut (1989), Tifton, Georgia (1990) and Blackstone, Virginia (1994). The green tobacco aphids were obtained from Duplin, North Carolina (1985) and Blackstone, Virginia, while the green peach aphid was obtained from Glade Spring, Virginia (1994). All these aphids were collected from tobacco, except for the green peach aphid which was obtained from climbing nightshade (*Solanum dulcamara* L.). All their colonies were maintained on the leaves of flue-cured tobacco 'K-326' in the laboratory at the SPAREC. Aphids were inoculated with *P. neoaphidis* by exposing them to conidial showers from fungal cultures. The experiment was

repeated three times. In Tests 1 and 2, each biotype had six replications each of an untreated control of 25 aphids and a treatment of 25 aphids inoculated with *P. neoaphidis*. Test 3 was similar except that the fungal treatments was replicated 12 times, but insufficiency of aphids limited the number of replications in controls to six. The treatments were arranged in randomized complete block design.

Rearing aphids: Aphid colonies were reared on tobacco leaves in Styrofoam® cups at 22^0C, 18L:6D photophase (18 h light helped to keep aphids from developing into alatae). Petioles of tobacco leaves were inserted in 1% water agar (Fisher Scientific, New Jersey, NJ) in Styrofoam® cups. Lids were placed on cups to keep aphids inside. Aphids were transferred weekly to new leaves in new cups using a camel's hair brush. Leaves were obtained from greenhouse tobacco plants and rinsed with deionized water before placing aphids on them. Newly born aphids were collected from viviparae using a camel's hair brush and used to start a new colony on a tobacco leaf in a new cup. Leaves were replaced 5 or 6 d after the colony was started. When the aphids were 9-d old, they were transferred to new leaves in new cups and allowed to larviposit for 6-8 h. After larviposition, viviparae were gently removed using moist camel's hair brush and the neonate nymphs were retained in the cups. These nymphs were used for the test when they became 3-d old.

Culturing *P. neoaphidis*: *P. neoaphidis* was isolated from the red morph of tobacco aphid collected from tobacco fields during the summer of 1994 in

Blackstone, VA. The fungus was grown on modified SEMA (Sabouraud maltose-egg-milk-agar) described by Yu et al. (1995; see Appendix 12). Ingredients of SEMA included 74.5% fresh yolk from unwashed eggs, 14.4% vitamin D milk, 11% Sabouraud maltose agar (Difco Laboratories, Detroit, MI), 0.1% gentamicin (Sigma Chemical Co., St. Louis, MO) and 0.05% penicillin (Agri Laboratories Ltd., St. Joseph, MO). These cultures were incubated at 20^0C and 14L:10D photophase. The cultures used for the experiment had been subcultured five times in vitro after they had been isolated from an aphid host. Two to 3 wk old cultures were used for inoculating the aphids. Sporulating cultures were selected for the experiment based on the conidial deposition on the lids of Petri plates.

Inoculation of aphids: On the day of the experiment, 25 aphids of each biotype were placed on a separate tobacco leaf disc (about 55 mm dia) in a small Petri plate (60 by 15 mm). Before the discs were cut, tobacco leaves from greenhouse plants were rinsed with deionized water. A filter paper (Whatman No. 1, 55 mm circle) was placed on the bottom and another one was attached to the lid of each Petri plate with non-toxic Permanent Glue Stic (Avery Dennison, Framingham, MA). Filter papers were moistened to maintain high humidity ($\geq$ 98%) in the Petri plate which is required for conidial germination (Wilding 1971, Carruthers and Hural 1990, Yu et al. 1995). A small piece of coverslip (about 40 mm^2) was also placed in each Petri plate to sample the conidia showered from the fungal culture.

Test aphids were inoculated with conidial showers of *P. neoaphidis* by inverting fungus cultures in the large Petri plates (100 by 15 mm) over the small plates containing the aphids. The plates were then covered with moist paper towels, placed in a sealed plastic bag, and incubated in the dark at 22^0C in a growth chamber. Relative humidity inside the plastic bags was measured using a circular Certified Hygrometer & Temperature Indicator (Model HTAB-176, Abbeon Cal. Inc., Santa Barbara, CA). Aphids were exposed to conidial showers for 4-5 h. Culture plates were rotated 90^0 every quarter of exposure time to promote uniform conidial showers over the exposed area. At the end of the conidial showers, the culture plates were removed, pieces of coverslips were collected and lids were placed over the small plates after the filter papers had been moistened. After about 16 h, the paper towels and plastic bags were removed and the chamber was changed from 24D to 18L:6D photophase. Aphids in the controls were subjected to similar conditions, except that they were not exposed to conidial showers. Leaf discs in the Petri plates were renewed every other day. Aphids treated with the fungus started to show symptoms of infection (light coloration of living-infected aphids and golden brown cadavers, with or without sporulation) 3 d after inoculation (DAI). Aphids in both treatments and controls were checked daily for mortality, starting at 3 DAI. Counts of healthy aphids and those killed by fungus and/or other causes were made until no further infection was seen or all aphids had died in treatments. The number of conidia collected on the pieces of

coverslip were counted under light microscope at six random locations per coverslip and the conidial density per mm^2 was estimated.

Mortality of aphids inoculated with fungus represented death due to *P. neoaphidis* and other or natural causes. Mortality of aphids in untreated controls represented death due to causes other than the pathogen. Several aphids in treatments died before showing the symptoms of infection. This implied that the fungus may cause mortality without showing the symptoms of infection. Hence, the mean mortality in controls was deducted from that of treated aphids and percent mortality due to pathogen was calculated based on the survival in controls using Abbott's formula (Abbott 1925).

Statistical analysis: The percent mortality on each day during the observation period was calculated. The means of cumulative mortality of untreated aphids in controls was subtracted from the cumulative mortality of treated aphids. Percent mortality due to the pathogen was calculated based on the numbers surviving in controls on respective days using the formula: (Number dead in treatments-Mean number dead in controls)/Number alive in controls. The data were analyzed by PROC GLM of SAS software (SAS Institute 1987). Means for untransformed and arcsine-transformed cumulative mortality due to the fungus were obtained. Means of arcsine-transformed data were considered for comparing treatments using Waller-Duncan K-ratio T test (K-ratio = 100) of SAS. Data were combined across biotypes within morphs for analyses comparing the two morphs of the tobacco

aphid with the green peach aphid.

Results:

The mean conidial density to which aphids were exposed was similar in each test ($F = 0.62$, df = 5,30 $P = 0.69$ for Tests 1 and 2; $F = 0.34$, df = 5,31 $P = 0.88$ for Test 3). The dosage ranged from 30-37 conidia/mm^2. Cumulative percent mortality of different biotypes of red and green tobacco aphids, and green peach aphid for Tests 1, 2, and 3 are shown in Table 8.1. Disease symptoms were observed in aphids in 3-4 DAI. The susceptibility of different biotypes to *P. neoaphidis* was similar in all tests throughout the observation period, except for 4 and 5 DAI in Tests 1 and 2, respectively (Table 8.1). However, the range of differences (Waller-Duncan K-ratio T test; K-ratio = 100) was very narrow on those two days. There were no significant interactions ($P \geq 0.05$) between tests and biotypes during the entire observation period. Red and green tobacco aphids and the green peach aphid were similarly susceptible to *P. neoaphidis* during the entire observation period in Tests 1 and 2 and half of the time in Test 3 (Table 8.2). The red morph of tobacco aphid was significantly more susceptible (Waller-Duncan K-ratio T test; K-ratio = 100) to the pathogen than green peach aphid on 6, 7, and 8 DAI in Test 3. None of the interactions between tests and aphid species or morphs were significant ($P \geq 0.05$) during the entire observation period.

Table 8. 1. Cumulative daily percent mortality of different morphs of apterous *M. nicotianae* and *M. persicae* due to *P. neoaphidis*, Tests 1 to 3.

Aphid	Cumulative percent mortality (Mean ± SEM)[1]						
	3 DAI[2]	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI
Test 1							
CTRED[3]	41.3 ± 6.7[4]	52.9 ± 5.6b	71.1 ± 4.1	74.4 ± 4.6	74.5 ± 5.2	76.3 ± 6.0	73.7 ± 6.9
GARED	64.6 ± 9.8	79.4 ± 9.8a	85.2 ± 1.6	89.3 ± 3.6	89.4 ± 3.0	89.9 ± 4.3	87.3 ± 5.4
VARED	57.4 ± 3.0	66.4 ± 4.3ab	77.8 ± 4.4	74.1 ± 6.6	75.0 ± 6.9	72.0 ± 7.8	75.6 ± 10.7
VAGRN	51.1 ± 6.0	70.6 ± 7.7ab	84.5 ± 6.0	84.6 ± 6.1	81.1 ± 7.5	80.5 ± 6.0	77.3 ± 6.0
NCGRN	57.1 ± 10.6	75.6 ± 8.7a	85.9 ± 5.9	88.9 ± 5.9	89.1 ± 5.5	89.1 ± 5.1	87.7 ± 5.8
VAGPA	58.0 ± 5.0	77.8 ± 5.0a	85.4 ± 3.7	84.4 ± 4.3	84.6 ± 4.1	83.3 ± 4.4	84.3 ± 5.1
Test 2							
CTRED	78.7 ± 4.8	90.2 ± 3.5	97.9 ± 2.1a	99.3 ± 0.7	99.3 ± 0.7	99.2 ± 0.8	-
GARED	53.2 ± 20.1	78.1 ± 8.1	91.5 ± 3.0ab	96.0 ± 2.2	95.7 ± 2.4	96.3 ± 2.7	-
VARED	60.5 ± 6.2	73.3 ± 8.4	91.9 ± 5.9ab	95.8 ± 3.0	95.6 ± 3.2	95.3 ± 3.4	-
VAGRN	60.1 ± 9.0	67.0 ± 16.1	73.1 ± 18.0b	75.6 ± 19.6	72.4 ± 23.1	70.3 ± 26.0	-
NCGRN	59.9 ± 8.7	82.1 ± 5.2	95.5 ± 2.8ab	96.9 ± 2.2	98.4 ± 1.6	98.4 ± 1.6	-
VAGPA	76.4 ± 3.6	86.7 ± 3.8	95.4 ± 1.1ab	96.9 ± 1.5	96.8 ± 1.6	96.7 ± 1.6	-
Test 3							
CTRED	53.8 ± 9.6	86.2 ± 5.8	-	90.3 ± 4.2	89.2 ± 4.5	90.0 ± 4.1	79.0 ± 12.0
GARED	55.3 ± 7.4	92.1 ± 2.6	-	94.6 ± 2.3	94.2 ± 2.6	92.7 ± 3.7	84.3 ± 6.9
VARED	56.6 ± 7.5	88.5 ± 4.9	-	94.6 ± 1.6	93.6 ± 2.2	93.1 ± 2.6	91.4 ± 4.0
VAGRN	50.6 ± 9.0	82.6 ± 5.7	-	88.6 ± 3.6	91.6 ± 2.6	91.7 ± 2.5	89.6 ± 3.4
NCGRN	51.1 ± 8.2	82.1 ± 4.7	-	87.6 ± 2.7	88.5 ± 2.7	87.9 ± 2.8	81.7 ± 4.6
VAGPA	45.5 ± 7.2	83.5 ± 3.1	-	86.7 ± 2.3	86.0 ± 2.4	86.2 ± 2.5	80.7 ± 3.6

[1]Obtained by subtracting the mean mortality in untreated aphids from mortality in aphids inoculated with *P. neoaphidis*.

[2]Days after inoculation.

[3]CTRED, GARED and VARED are red morphs of the tobacco aphid, *M. nicotianae*, from Windsor, CT, Tifton, GA and Blackstone, VA, respectively.
VAGRN and NCGRN are green morphs of the tobacco aphid, *M. nicotianae*, from Blackstone, VA and Duplin, NC, respectively.
VAGPA is the green peach aphid, *M. persicae* from Glade Spring, VA.

[4]Means followed by the same or no letter within each column are not significantly different (Waller-Duncan K-ratio T test; K ratio = 100).

Table 8. 2. Cumulative daily percent mortality of apterae of red and green morphs of *M. nicotianae* and *M. persicae* due to *P. neoaphidis*, Tests 1 to 3.

Aphid	Cumulative percent mortality (Mean ± SEM)[1]						
Aphid	3 DAI[2]	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI
Test 1							
REDTA[3]	52.9 ± 4.4[4]	64.3 ± 4.0	77.1 ± 2.7	78.3 ± 3.3	78.6 ± 3.4	78.5 ± 3.9	77.9 ± 4.7
GRNTA	54.1 ± 5.8	73.1 ± 5.6	85.2 ± 4.0	86.8 ± 4.1	85.1 ± 4.6	84.8 ± 4.0	82.5 ± 4.3
GPA	58.0 ± 5.0	77.8 ± 5.0	85.4 ± 3.8	84.4 ± 4.3	84.6 ± 4.1	83.3 ± 4.4	84.3 ± 5.1
Test 2							
REDTA	65.0 ± 6.9	81.0 ± 4.1	93.9 ± 2.1	97.1 ± 1.2	96.9 ± 1.3	97.0 ± 1.4	-
GRNTA	60.0 ± 6.0	74.5 ± 8.4	84.3 ± 9.3	86.3 ± 9.9	85.4 ± 11.7	84.3 ± 13.1	-
GPA	76.4 ± 3.6	86.7 ± 3.8	95.4 ± 1.2	96.9 ± 1.5	96.8 ± 1.6	96.7 ± 1.6	-
Test 3							
REDTA	55.7 ± 4.6	89.0 ± 2.6	-	93.3 ± 1.6a	92.4 ± 1.8a	92.0 ± 2.0a	85.1 ± 4.6
GRNTA	50.9 ± 5.9	82.3 ± 3.6	-	88.1 ± 2.2ab	90.0 ± 1.9ab	89.7 ± 1.9ab	85.5 ± 2.9
GPA	45.5 ± 7.2	83.5 ± 3.2	-	86.8 ± 2.3b	86.0 ± 2.4b	86.2 ± 2.5b	80.8 ± 3.6

[1]Obtained by subtracting the mean mortality in untreated aphids from mortality in aphids inoculated with *P. neoaphidis*.

[2]Days after inoculation.

[3]CTRED, GARED and VARED are red morphs of the tobacco aphid, *M. nicotianae*, from Windsor, CT, Tifton, GA and Blackstone, VA, respectively.
VAGRN and NCGRN are green morphs of the tobacco aphid, *M. nicotianae*, from Blackstone, VA and Duplin, NC, respectively.
VAGPA is the green peach aphid, *M. persicae* from Glade Spring, VA.

[4]Means followed by the same or no letter within each column are not significantly different (Waller-Duncan K-ratio T test; K ratio = 100).

Discussion:

Yu et al. (1995) reported that North Carolina isolate of *P. neoaphidis* was more virulent to red tobacco aphid than the Kentucky isolate. However, in a preliminary study conducted at the SPAREC, no difference was seen in the virulence of Virginia and Kentucky isolates of *P. neoaphidis* to different biotypes of red and green tobacco aphid and green peach aphid. Cameron and Milner (1981) reported that *P. neoaphidis* is more prevalent in pea aphid than in blue-green aphid, *A. kondoi* Shinji on alfalfa in New Zealand. They attributed this to both differences in susceptibility of aphid species and specificity of the pathogen strain occurring in New Zealand to pea aphid. Milner (1982 and 1985) also identified two biotypes of pea aphid that are distinctly different in their susceptibility to an isolate of *P. neoaphidis*, two isolates of the pathogen that were infective to the resistant biotype of pea aphid, and studied their geographic distribution in Australia. Differences were also found in the epizootics of *P. neoaphidis* in mixed infections with several fungal pathogens in cereal aphids infesting irrigated wheat, barley, and corn (Dean and Wilding 1971 and 1973, Feng et al. 1990, Feng et al. 1991). The pathogen was more abundant in the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) and the rose-grain aphid, *Metopolophium dirhodum* (Walker) than in the bird-cherry oat aphid, *Rhopalosiphum padi* (L.), the corn leaf aphid, *R. maidis* (Fitch) and the English grain aphid, *Sitobion avenae* (F.). Similarly, Dean and Wilding (1973) found that

epizootics of *P. neoaphidis* were more prevalent in the fescue aphid, *M. festucae* (Theobald), than in *M. dirhodum* and *S. avenae* on wheat in UK.

The incubation period for *P. neoaphidis* is 3-4 d, and cadavers of aphids with symptoms of infection should be seen after that period (Butt et al. 1990 and personal observation). Several aphids died without showing symptoms of infection during 1-2 DAI, before the counts were made. When these aphids were observed under stereo microscope, white growth of fungus, presumably from conidial germination, could be seen on the bodies of some aphids. This indicates that the pathogen is capable of killing the aphids without internal development. Fungal growth on the body and appendages might have interfered with the feeding and movement of aphids. Formation of several penetration pegs (the structures that penetrate host cuticle) all over the body could have also killed the aphids before the pathogen invaded body tissues. Since it takes only 3-4 DAI for the fungus to kill the aphids, it is possible that infection during later parts of observation period could be the result of secondary inoculum. Sporulating aphids infected by inoculation at the beginning of the experiment could have served as secondary source of inoculum for aphids that were not infected from the original inoculation of fungal cultures.

During most of the observation period (i.e., on a total of 17 out of 19 dates from three tests), *P. neoaphidis* was equally virulent to the biotypes from different geographic locations. Comparison of red and green morphs of tobacco aphid and

green peach aphid also gave similar results. If counts during 1-2 DAI had been available, they could have provided more insight on pathogen induced mortality early after inoculation.

Comparing the virulence of different isolates of *P. neoaphidis* from different tobacco growing regions would be of further interest. Knowledge on the virulence of different isolates of the pathogen to different biotypes of the tobacco aphid would be useful to identify the most virulent pathogen that could be used effectively against the susceptible biotype.

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CHAPTER IX

Influence of temperature and substrate type on the developmental morphology of *Pandora neoaphidis* (Zygomycetes: Entomophthorales), a pathogen of the tobacco aphid (Homoptera: Aphididae)

Abstract:

Developmental morphology of *Pandora neoaphidis* (Remaudière & Hennebert) Humber was observed on the surfaces of tobacco aphid, *Myzus nicotianae* Blackman, tobacco leaf (*Nicotianae tabacum* L.), and glass coverslip at 13 and 20^{0}C, and 12 and 24 h post-inoculation in the dark at $\geq$ 98% relative humidity. *P. neoaphidis* germinated on all substrates by giving rise to either a germ tube, an appressorium, or a secondary conidium. The response of *P. neoaphidis* to the substrate was similar on the surfaces of the two living substrates, but was different from that on the inert surface of coverslip. The proportion of ellipsoid conidia (includes both primary and secondary conidia) was similar on all substrates. Higher proportion of appressoria and lower proportions of round secondary conidia and germinating conidia (giving rise to germ tube or secondary/tertiary conidia or both) were seen on aphid and leaf surfaces, while the opposite was observed on coverslips. On the two living substrates, appressorial

formation predominated over round secondary conidia and germinating conidia at 20^0C, and the reverse was true at 13^0C, and the proportion of ellipsoid conidia remained the same at both temperatures. Time had no influence on these structures at 20^0C on the two living substrates. However, more germinating conidia (which would in turn have to produce appressoria for infection) and fewer of infective appressoria were observed within 12 h post-inoculation at 13^0C, though the difference was significant only on tobacco leaf. On coverslips, while the proportions of appressoria and round secondary conidia remained constant at both temperatures, higher germinating conidia at 13^0C and higher ellipsoid conidia at 20^0C were found. Significantly lower proportion of ellipsoid conidia was seen 24 h post-inoculation at 20^0C. The round secondary conidia were more at 24 h post-inoculation at 20^0C on coverslip.

Key words: *Pandora neoaphidis*, *Myzus nicotianae*, substrate, temperature, germination.

Introduction:

The host range of *Pandora neoaphidis* (Remaudière & Hennebert) Humber includes several species of aphids and a few species of hemipterans (Wilding and Brady 1984). Great reductions in populations of *Aphis fabae* Scopoli were resulted by introducing *P.(Erynia) neoaphidis* (Wilding et al. 1986).

Conidial germination of another entomophthoralean, *Zoophthora radicans* (Brefeld) Batko was dependent on temperature in vitro and in vivo (Van-Roermund et al. 1984, McGuire et al. 1987, Magalhaes et al. 1991, Wraight et al. 1992). More recent investigations elucidated the influence of temperature on the growth of *P. neoaphidis*, germination and type of its conidia in vitro, and on production of conidia in vivo (Morgan et al. 1995, Yu et al. 1995). Optimal temperatures for conidial germination of *P. neoaphidis* appeared to be between 18 and 23^{0}C (Morgan et al. 1992, Morgan et al. 1995), while maximum conidial production occurred between 10 and 25^{0}C (Yu et al. 1995).

The fate of conidia after landing on the host surface determines the success of invasion by the pathogen. The invasive and developmental processes of *P. neoaphidis* on *Acyrthosiphon pisum* at 20^{0}C in continuous light (Harris) was studied by Brobyn and Wilding (1977) and Butt et al. (1990). The primary conidium of *P. neoaphidis* germinates about 4 h post-inoculation and forms either a germ tube, or an appressorium, or a secondary conidium (Brobyn and Wilding 1977, Butt et al. 1990). Differential response of *P. neoaphidis* in conidial

germination on coverslips and leaves of field beans (Brobyn 1987) and of *Aschersonia aleyrodis*, another entomopathogen, on cucumber leaves and cuticle of *Trialeurodes vaporariorum* (Westwood) (Fransen 1995) was previously known. Mode of germination and infection process could also vary on different body regions of the host in response probably in response to the thickness of the cuticle and topography of its surface (David 1967, Pekrul and Grula 1979, Wraight et al. 1990, St. Leger et al. 1991). Information on the response of the pathogen to the type of substrate would help understand the fate of inoculum on different surfaces in the environment.

This research was aimed at examining the developmental morphology of conidia inoculated on three surfaces and incubated at two temperatures with observations at two time intervals.

Materials and Methods:

Rearing aphids: Aphids were collected from field tobacco and reared, in a growth chamber at 22^{0}C 16L:8D photophase, on tobacco plants growing in sterile potting medium. Newly born aphids were collected from these plants with a camel's hair brush and transferred to excised greenhouse tobacco leaves rinsed with deionized water. These leaves were placed in Styrofoam® cups (710 ml) by inserting the petioles in 1% water agar (Fisher Scientific, New Jersey, NJ) in the bottom of cups and lids were placed on cups to keep aphids inside. Aphids were

transferred to new leaves after 5 or 6 d. When they reach 9-d age, aphids were again transferred to new leaves in new cups and allowed to larviposit for 6-8 h. After larviposition, viviparae were gently removed using moist camel's hair brush and neonate nymphs were retained in the cups. The nymphs were used for the experiment when they became 5-d old.

Culturing *P. neoaphidis*: *P. neoaphidis* was isolated from field collected tobacco aphids as per the method described by G.L. Nordin (personal communication; see Appendix 12). The fungus was grown on modified SEMA (Sabouraud maltose-egg-milk-agar) (Yu et al. 1995; see Appendix 12). Ingredients of SEMA included 74.6% fresh yolk from unwashed eggs, 14.4% vitamin D milk, 11% Sbouraud maltose agar (Difco Laboratories, Detroit, MI), 0.1% gentamicin (Sigma Chemical Co., St. Louis, MO) and 0.05% penicillin (Agri Laboratories Ltd., St. Joseph, MO). These cultures were incubated at 20^0C and 14L:10D photophase. The pathogen used for the experiment had been subcultured four times in vitro after it was isolated from the aphid host. Two to 3 week old cultures were used for inoculating aphids. Sporulating cultures were selected for the experiment based on the conidial deposition on the lids of Petri plates.

Inoculation of aphids: On the day of experiment, 25 aphids were placed on a separate tobacco leaf disc (about 55 mm dia) in each of four small Petri plates (60 mm dia by 15 mm). Before the discs were cut, tobacco leaves from greenhouse plants were rinsed with deionized water. A filter paper (Whatman No. 1, 55 mm

circle) was placed on the bottom and a similar one was attached to the lid of each Petri plate with non-toxic Permanent Glue Stic (Avery Dennison, Framingham, MA). These filter papers were moistened to maintain high humidity ($\geq$ 98%) in the plate that is required for conidial germination (Wilding 1971, Carruthers and Hural 1990, Yu et al. 1995). In each Petri plate, 5 small pieces of coverslip (about 40 mm^2) were placed, one in the center and the rest on four sides of it along the wall of the plate.

Aphids were inoculated by inverting the large Petri plates (100 mm dia by 15 mm) over the small plates to expose aphids in the small plates to conidial showers from the cultures. These plates were covered with moist paper towels, placed in a sealed plastic bag and incubated in the dark at 20^0C in the dark. Relative humidity inside the plastic bags was measured using hygrometer (127 mm dia by 32 mm) (Abbeon Cal. Inc., Santa Barbara, CA). Moist paper towels maintained the relative humidity at $\geq$ 98% in the plastic bags. Aphids were exposed to conidial showers for 4 h. Culture plates were rotated 90^0 every quarter of exposure time to promote uniform conidial showers over the exposed area. At the end of the conidial showers, cultures were removed and small plates were covered with their lids. Two plates each were incubated in the dark at 13 and 20^0C. At 12 h and 24 h post-inoculation, one plate each from 13 and 20^0C was taken out from respective incubation chambers. Five small pieces (about 40 mm^2) were cut from tobacco leaf disc in each Petri plate to represent 5 samples of each

treatment.

SEM: Tobacco aphids, pieces of coverslips and tobacco leaf were critical point dried and sputter coated with gold-palladium (10 nm) for scanning electron microscopy (SEM). The conidial germination was observed under SEM (Philips 505) on the surfaces of tobacco aphid, tobacco leaf and coverslip. Observations were made on the entire dorsal region of each tobacco aphid, and at five random locations (area of each location about 0.2 mm^2) on each piece of tobacco leaf and coverslip.

Statistical analysis: A factorial experiment was conducted with substrates as main effects and temperature and time as subeffects. The proportions of different categories of structures found on three substrates were calculated. Data were arcsine-transformed and analyzed by PROC GLM of SAS software (SAS Institute 1987). Significant means were separated by Waller-Duncan K-ratio T test (K-ratio = 100).

Results:

Conidial germination was observed on all substrates by means of a germ tube, an appressorium, or a secondary conidium. Structures found on the three substrates were placed in the following categories: ellipsoid conidium (Fig. 9.1); germinated conidium with collapsed spore and intact germ tube (Fig. 9.2); conidium that has given rise to an appressorium (Fig. 9.3); conidium with

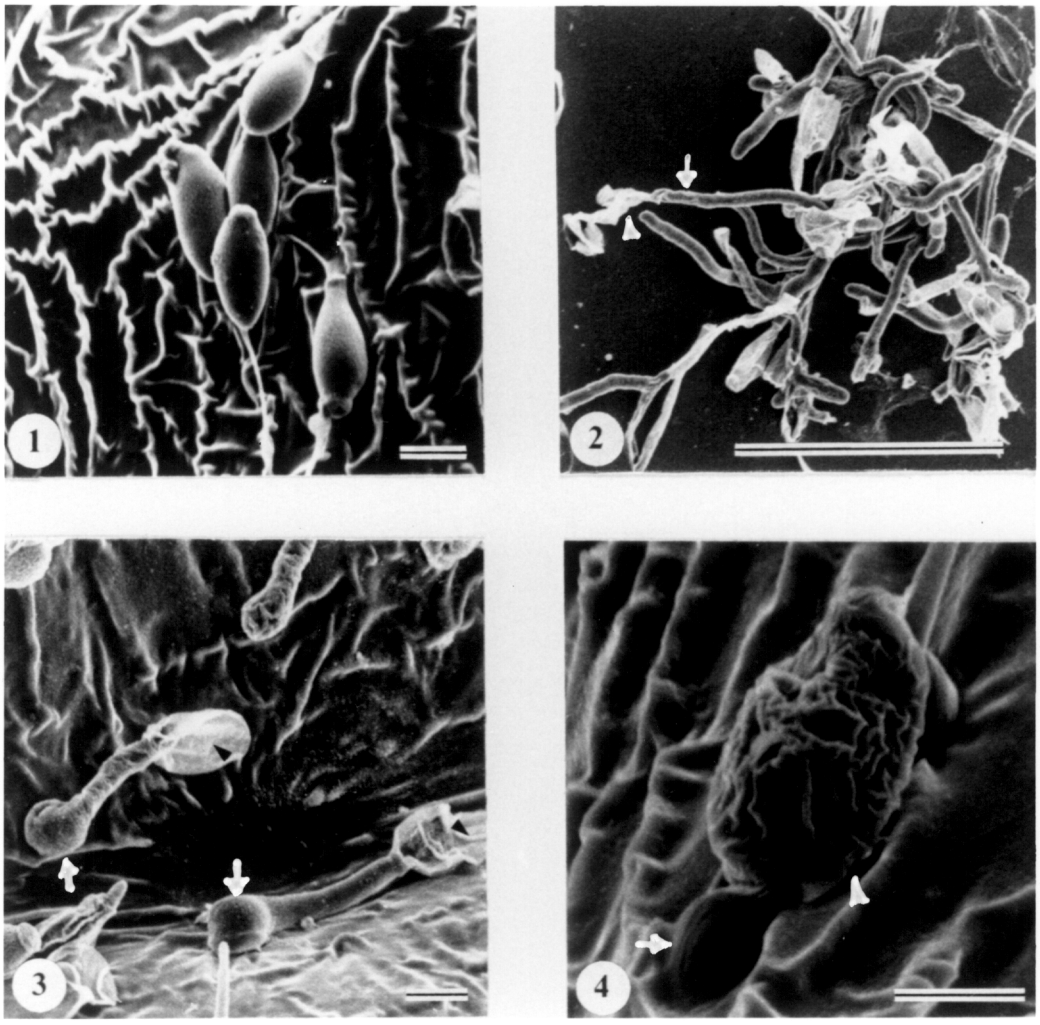
Figures 9. 1-4. Scanning electron micrographs.

Fig. 9. 1. Ellipsoid primary or secondary conidia on aphid cuticle (bar = 10 μm).

Fig. 9. 2. Germinated conidium with collapsed spore (arrowhead) and intact germ tube (arrow) on coverslip (bar = 100 μm).

Fig. 9. 3. Conidium (arrowhead) with globose appressorium (arrow) on aphid cuticle (bar = 10 μm).

Fig. 9. 4. Conidium (arrowhead) with penetration peg (arrow) on the abdomen of aphid (bar = 10 μm).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penetration peg (Fig. 9.4); round secondary conidium (Fig. 9.5); secondary conidium developing on a germ tube (Fig. 9.6); germinating ellipsoid conidium by means of germ tube or initiation of successive (secondary or tertiary) conidium or both (Fig. 9.7); and germinating round secondary conidium by means of germ tube (Fig. 9.8). Primary conidia and secondary conidia that resemble primary were considered as ellipsoid conidia since it was difficult to distinguish them in several instances.

Germination of conidia was influenced by temperature, substrate, and time. The relative proportion of different structures (Figures 9.1 to 9.8) formed in the process of germination varied on the three substrates and at the two temperatures when observed at two intervals (Tables 9.1 and 9.2) ($P \leq 0.05$).

Germ tube: The proportion of germ tubes observed on three substrates was not significantly different. Temperature did not have any influence on germ tubes on aphid and coverslip but, significantly higher proportion of germ tubes were found at 20^0C on leaf. The germ tube was not influenced by time on aphid, but significantly more germ tubes were seen 24 h than 12 h post-inoculation at both temperatures on both leaf and coverslip.

Appressorium: The response of *P. neoaphidis* was similar on aphid and leaf, which are living substrates, in forming appressoria. Appressorial formation was significantly lower on coverslip, an inert substrate. Influence of temperature was seen only on aphid and leaf, where appressorial formation was significantly higher

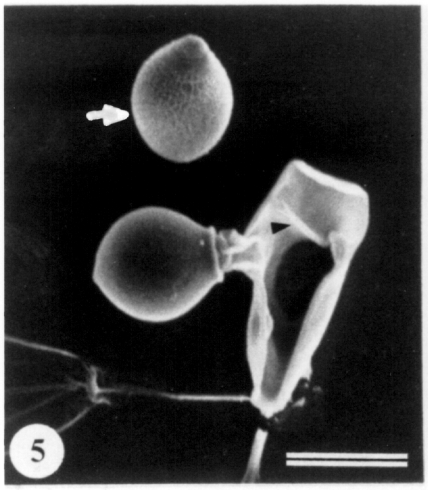
Figures 9. 5-8. Scanning electron micrographs.

Fig. 9. 5. Round secondary conidium (arrow) singly and on collapsed primary conidium (arrowhead) on coverslip bar = 10 μm).

Fig. 9. 6. Secondary conidium (arrow) developing on a germ tube (arrowhead) on coverslip (bar = 10 μm).

Fig. 9. 7. Germinating ellipsoid conidia. A. Ellipsoid conidia (arrowhead) with different stages of development of secondary/tertiary conidia (arrow), (bar = 10 μm); B. Ellipsoid conidium with germ tube (bar = μm); C. Ellipsoid conidium with initiation of secondary/tertiary conidium and germ tube (bar = μm) on aphid cuticle.

Fig. 9. 8. Germinating round secondary conidium, on coverslip (bar = 100 μm). Round secondary conidium (arrowhead) with a germ tube (arrow) on a collapsed primary conidium.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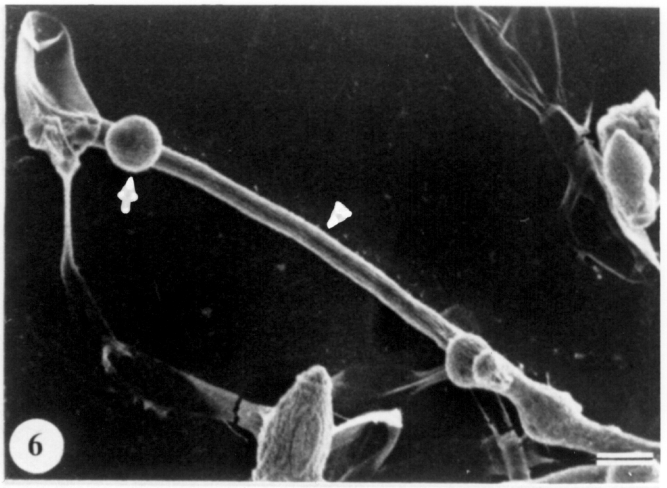
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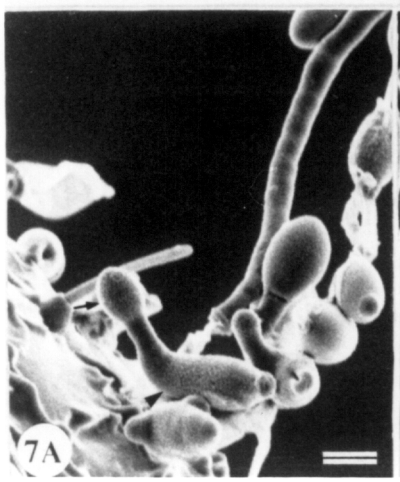
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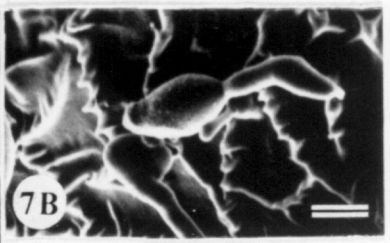
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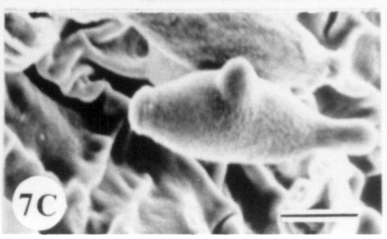
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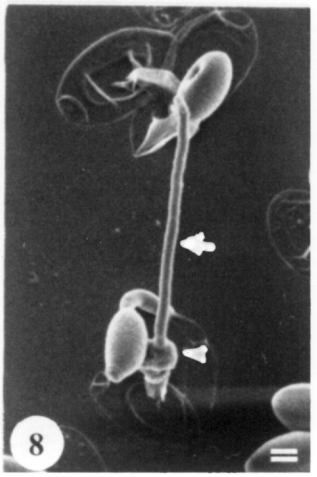
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Table 9. 1. Relative proportion of different structures found on the surfaces of the tobacco aphid, tobacco leaf and coverslip at two temperatures and at two post-inoculation intervals.

Substrate	Temp.	Time	Mean ± SEM							
	(°C)		GT[1]	AP	P	EC	RSC	GEC	GRSC	SCGT
Aphid	13	12	15.0 ± 3.2	5.8 ± 1.8	0.0 ± 0.0	43.0 ± 1.9	12.0 ± 3.5	24.3 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
Aphid	13	24	19.0 ± 6.1	17.0 ± 4.3	0.0 ± 0.0	43.2 ± 12.5	10.4 ± 2.5	10.4 ± 3.3	0.0 ± 0.0	0.0 ± 0.0
Aphid	20	12	18.7 ± 5.9	28.5 ± 7.4	0.3 ± 0.2	51.2 ± 13.4	1.0 ± 0.5	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0
Aphid	20	24	17.4 ± 3.2	27.8 ± 3.1	0.0 ± 0.0	47.6 ± 4.2	7.2 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Coverslip	13	12	1.9 ± 1.2	0.2 ± 0.1	0.0 ± 0.0	38.8 ± 1.9	17.6 ± 1.8	41.5 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
Coverslip	13	24	40.5 ± 5.2	5.0 ± 0.9	0.0 ± 0.0	25.7 ± 4.3	8.2 ± 2.6	17.6 ± 3.2	0.2 ± 0.2	2.8 ± 1.2
Coverslip	20	12	4.0 ± 2.0	0.9 ± 0.5	0.0 ± 0.0	70.0 ± 3.7	7.1 ± 2.0	18.1 ± 3.6	0.0 ± 0.0	0.1 ± 0.1
Coverslip	20	24	31.0 ± 5.9	5.8 ± 2.8	0.0 ± 0.0	31.3 ± 2.0	17.4 ± 3.7	12.2 ± 3.5	1.7 ± 0.5	0.6 ± 0.4
Leaf	13	12	1.6 ± 0.5	0.9 ± 0.2	0.0 ± 0.0	60.8 ± 5.0	10.9 ± 2.4	25.9 ± 2.7	0.0 ± 0.0	0.0 ± 0.0
Leaf	13	24	13.0 ± 2.2	19.4 ± 3.3	0.0 ± 0.0	38.5 ± 2.7	19.2 ± 3.2	9.9 ± 2.1	0.0 ± 0.0	0.0 ± 0.0
Leaf	20	12	16.0 ± 1.8	28.4 ± 7.4	0.0 ± 0.0	50.8 ± 6.4	0.3 ± 0.2	4.4 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Leaf	20	24	29.7 ± 2.7	22.2 ± 3.6	0.0 ± 0.0	41.7 ± 3.9	0.7 ± 0.3	5.3 ± 1.0	0.1 ± 0.1	0.3 ± 0.1

[1]GT, collapsed conidium with intact germ tube; AP, conidium with appressorium; P, conidium with penetration peg; EC, ellipsoid primary or secondary conidium; RSC, round secondary conidium; GEC, EC germinating by means of germ tube, secondary/tertiary conidium, or both; GRSC, RSC germinating by means of germ tube; SCGT, secondary conidium produced on germ tube.

Table 9. 2. Probability > *F* values from ANOVA for relative proportions of different structures found on the surfaces of the tobacco aphid, tobacco leaf and coverslip at two temperatures and at two post-inoculation intervals.

Factor	GT[1]	AP	P	EC	RSC	GEC	GRSC	SCGT
Substrate	0.1429	0.0001	0.0007	0.2454	0.0149	0.0001	0.0011	0.0049
Temperature: Aphid	0.4703	0.0013	1.0687	0.4185	0.0735	0.0006	-	-
Coverslip	0.3033	0.6195	-	0.0002	0.8291	0.0002	0.0245	0.0930
Leaf	0.0001	0.0050	-	0.4777	0.0001	0.0001	0.3370	0.0307
Time: Aphid-13 ^{0}C	0.4768	0.0715	-	0.9058	0.7526	0.0576	-	-
-20 ^{0}C	0.8303	0.8753	0.1849	0.7061	0.0582	0.4226	-	-
Coverslip-13 ^{0}C	0.0021	0.0093	-	0.0566	0.0497	0.0044	0.3739	0.0804
-20 ^{0}C	0.0069	0.0993	-	0.0017	0.0130	0.2867	0.0332	0.1867
Leaf-13 ^{0}C	0.0062	0.0053	-	0.0039	0.0405	0.0085	-	-
-20 ^{0}C	0.0282	0.5138	-	0.2261	0.2145	0.6310	0.3739	0.0705

[1]GT, collapsed conidium with intact germ tube; AP, conidium with appressorium; P, conidium with penetration peg; EC, ellipsoid primary or secondary conidium; RSC, round secondary conidium; GEC, EC germinating by means of germ tube, secondary/tertiary conidium, or both; GRSC, RSC germinating by means of germ tube; SCGT, secondary conidium produced on germ tube.

at 20^0C. Time had no effect at both temperatures on aphid and at 20^0C on coverslip and leaf. However, significantly higher proportions of appressoria were found 24 h than 12 h post-inoculation at 13^0C on coverslip and leaf.

Penetration peg: Only one penetration was seen at 20^0C on the abdomen of aphid within 12 h post-inoculation. Penetration was not seen 24 h post-inoculation at 20^0C or at both time intervals at 13^0C.

Ellipsoid conidium: The proportion of ellipsoid conidia was identical on all substrates. They seemed to be influenced by temperature on coverslip where their proportion was significantly higher at 20^0C. No influence of time was noticed on ellipsoid conidia at both temperatures on aphid, at 13^0C on coverslip, and at 20^0C on leaf. But, at 20^0C on coverslip and at 13^0C on leaf, the proportion of ellipsoid conidia significantly decreased 24 h post-inoculation.

Round secondary conidium: The majority of round secondary conidia were still attached to the collapsed primary conidia, they were produced on, in all instances. The production of round secondary conidia was significantly higher on coverslip. It was not influenced by temperature on aphid and coverslip. On leaf, significantly lower proportion of round secondary conidia was seen at 20 than at 13^0C. A significantly higher proportion of round secondary conidia was seen 24 h than 12 h post-inoculation at 20^0C on coverslip, and at 13^0C on leaf.

Germinating ellipsoid conidium: Type of substrate, temperature on all substrates, and time at 13^0C on coverslip and leaf were found to influence

germinating ellipsoid conidia. A significantly higher proportion of germinating ellipsoid conidia was seen on coverslip than on aphid and leaf, and at 13 than at 20^0C on all substrates. The proportion of germinating ellipsoid conidia was higher at 12 h than 24 h post-inoculation on all substrates at 13^0C, however, the difference was not significant on aphid. Similar proportions of germinating ellipsoid conidia were seen at both time intervals at 20^0C on all substrates.

Germinating round secondary conidium: Germinating round secondary conidia were found only 24 h post-inoculation at both temperatures on coverslip and at 20^0C on leaf. They were not seen on aphid.

Secondary conidium on germ tube: Germ tubes bearing secondary conidia were not seen on aphid. They were seen on coverslip at both temperatures and on leaf at 20^0C. However, they were not observed within 12 h post-inoculation on coverslip and leaf at 13 and 20^0C, respectively.

The ungerminated and the germinated conidia were compared on different substrates, considering ellipsoid conidia as ungerminated and the rest as germinated (Table 9.3). The proportion of each group was not influenced by the type of substrate and by temperature on aphid and leaf. But, on coverslip, significantly higher proportion conidia germinated at 13 than at 20^0C ($P \leq 0.001$). Post-inoculation time also did not have any impact on germinated and ungerminated conidia at both temperatures on aphid, at 13^0C on coverslip, and at 20^0C on leaf. However, significantly higher proportion of germinated conidia

Table 9. 3. Comparison of mean proportions of ungerminated and germinated conidia .

Factor	Temp. (^{0}C)	Time	Ungerminated	Germinated
Substrate[1]:				
Aphid			45.6 a	54.4 a
Coverslip			41.4 a	58.6 a
Leaf			48.9 a	52.1 a
Temperature[2]:				
Aphid	13		43.1NS	56.9NS
	20		49.4	50.6
Coverslip	13		32.2 ***	67.8 ***
	20		50.6	49.4
Leaf	13		49.7NS	50.3NS
	20		46.2	53.8
Time[3]:				
Aphid	13	12	42.9NS	57.1NS
Aphid	13	24	43.2	56.8
Aphid	20	12	51.2NS	48.8NS
Aphid	20	24	47.6	52.4
Coverslip	13	12	38.8NS	61.2NS
Coverslip	13	24	25.7	74.3
Coverslip	20	12	70.0 **	30.0 **
Coverslip	20	24	31.3	68.7
Leaf	13	12	60.8 **	39.2 **
Leaf	13	24	38.5	61.5
Leaf	20	12	50.8NS	49.2NS
Leaf	20	24	41.7	58.3

[1] Means followed by same letter within the column are not significantly different (Waller-Duncan K-ratio T test, K=100).

[2] Means separation for temperature within each substrate. NS, not significantly different ($P \geq 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; H_0, coefficient, 0.

[3] Means separation for time within each temperature and substrate. NS, not significantly different ($P \geq 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; H_0, coefficient, 0.

were found 24 h than 12 h post-inoculation, at 20^0C on coverslip and 13^0C on leaf.

Since conidia collapsed and no signs of successive development was seen in germ tubes, the remaining structures were regrouped, without germ tubes, into four categories viz., appressoria (including penetration peg), ellipsoid conidia, round secondary conidia, and germinating conidia (including germinating ellipsoid and round secondary conidia and conidia on germ tubes), and compared (Table 9.4).

Discussion:

Influence of substrate: Stimulus perceived from the substrate induces formation of appressorium in entomophathogenic fungi (St. Leger 1993). It is evident from my observations that cues from the living substrates triggered formation of appressoria, since their proportion was significantly lower on coverslip. Production of appressoria is the response of *P. neoaphidis* recognizing the substrate as suitable for infection, since the formation of appressoria precedes the penetration of host cuticle (Butt et al. 1990). However, several other factors also induce appressorial formation. Sensitivity of appressorial formation to nutritional and physical stimuli was previously known from other entomopathogenic fungi. Different levels of nitrogenous nutrients, glucose, and hard, hydrophobic surfaces induced in vitro formation of appressoria in *Metarhizium anisopliae* (Metsch.) Sorok. (St. Leger et al. 1989, St. Leger 1993). Appressorial formation of *Z.*

Table 9. 4. Relative proportion of appressoria, ellipsoid conidia, round secondary conidia, and germinating conidia.

Effect	Temp. (°C)	Time	Mean + SEM			
			APP[1]	EC	RSC	GC
Substrate[2]:						
Aphid			22.6 ± 4.3a	54.3 ± 3.9a	9.9 ± 1.9b	13.3 ± 3.5b
Coverslip			4.6 ± 1.4b	50.2 ± 3.4a	16.4 ± 2.2a	28.9 ± 2.7a
Leaf			22.0 ± 3.8a	56.6 ± 3.0a	8.7 ± 2.3b	12.8 ± 2.1b
Temperature[3]:						
Aphid	13		14.0 ± 4.1**	50.9 ± 5.1NS	13.2 ± 2.3**	22.0 ± 3.4***
	20		35.5 ± 5.7	59.3 ± 5.9	5.0 ± 1.7	0.2 ± 0.2
Coverslip	13		4.5 ± 1.7NS	40.8 ± 2.0***	16.5 ± 2.6NS	38.3 ± 2.2***
	20		4.7 ± 2.3	59.6 ± 5.1	16.2 ± 3.7	19.5 ± 2.5
Leaf	13		11.5 ± 3.9**	53.1 ± 4.0NS	16.7 ± 2.8***	18.8 ± 3.0***
	20		32.5 ± 4.5	60.1 ± 4.4	0.7 ± 0.2	6.8 ± 1.1
Time[4]:						
Aphid	13	12	7.2 ± 2.5NS	50.7 ± 2.5NS	13.6 ± 3.7NS	28.5 ± 1.6NS
Aphid	13	24	22.4 ± 6.8	51.1 ± 12.0	12.6 ± 3.1	13.9 ± 4.9
Aphid	20	12	37.1 ± 11.8NS	61.2 ± 12.5NS	1.3 ± 0.7NS	0.4 ± 0.4NS
Aphid	20	24	33.9 ± 4.5	57.5 ± 3.8	8.6 ± 0.9	0.0 ± 0.0
Coverslip	13	12	0.2 ± 0.1*	39.5 ± 1.8NS	17.8 ± 1.6NS	42.4 ± 2.4NS
Coverslip	13	24	8.7 ± 1.8	42.0 ± 3.6	15.2 ± 5.2	34.1 ± 2.6
Coverslip	20	12	0.9 ± 0.5NS	72.9 ± 3.9**	7.4 ± 2.1**	18.8 ± 3.5NS
Coverslip	20	24	8.6 ± 3.9	46.3 ± 3.6	25.0 ± 4.4	20.2 ± 3.8
Leaf	13	12	0.9 ± 0.2**	61.8 ± 5.0*	11.1 ± 2.4*	26.3 ± 2.9*
Leaf	13	24	22.1 ± 3.4	44.4 ± 3.4	22.3 ± 3.7	11.2 ± 2.2
Leaf	20	12	33.7 ± 8.4NS	60.7 ± 7.6NS	0.4 ± 0.2NS	5.2 ± 1.2NS
Leaf	20	24	31.3 ± 4.5	59.4 ± 5.5	0.9 ± 0.4	8.3 ± 1.7

[1]APP, appressorium + penetration; EC, ellipsoid primary or secondary conidium; RSC, round secondary conidium; GC, germinating conidia - germinating EC and RSC, and secondary conidium on germ tube.

[2]Means followed by same letter within the column are not significantly different (Waller-Duncan K-ratio T test, K=100)

[3]Means separation for temperature within each substrate. NS, not significantly different ($P \geq 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; H_0, coefficient, 0.

[4]Means separation for time within each temperature and substrate. NS, not significantly different ($P \geq 0.05$); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; H_0, coefficient, 0.

radicans was severely depressed in vitro in the absence of nitrogen and carbon sources (Magalhaes et al. 1991). The fungus continues to produce successive stages of conidia, until the nutrients are depleted, when the conditions are not congenial for infection. *P. neoaphidis* expressed this behavior by producing significantly higher proportions of appressoria and less of round secondary conidia and germinating conidia on aphid and leaf surfaces. In contract, on coverslip, an inert substrate, the pathogen produced more of successive stages of development and less of infective appressoria, while the proportion of ellipsoid conidia remained constant on all substrates. Apparently *P. neoaphidis* did not receive the signal on coverslip to immediately produce infective structures and continued to germinate by other means. Brobyn et al. (1987) reported that conidia of *P. neoaphidis* rapidly lost their infectivity at 100% relative humidity on coverslips due to exhaustion of energy reserves for production of successive generations of secondary conidia in a short period. Thickness of the cuticle is another factor that influences formation of infective structures and subsequent infection of the host (David 1967, Pekrul and Grula 1979). Response of the pathogen may differ even on various regions within the host body. Infection process of *Z. radicans* varied on head, thorax and abdomen of *Empoasca fabae* (Wraight et al. 1990). In the case of another entomopathogenic fungus, *A. aleyrodis* Webber, the germination of conidia was much less on the surface of cucumber leaf than on the cuticle of fourth-instar larvae of greenhouse whitefly, *T. vaporariorum* (Fransen 1995). This

was attributed to lack of stimulation or presence of inhibitory factors on cucumber leaves. However, germination of *P. neoaphidis* conidia was slower on leaves of field beans (*Vicia faba* L.) than on coverslips at 70-77% relative humidity (Brobyn et al. 1987). The factors that have stimulated appressorial formation or those that have simulated aphid cuticle, on tobacco leaf surface, are unknown. Similarity in the convoluted topography of aphid cuticle and tobacco leaf surfaces is one possible reason for similar response of *P. neoaphidis* on those two surfaces. Role of topographical stimuli on the appressorial formation of *M. anisopliae* on *Manduca sexta* (L.) was reported by St. Leger et al. (1991). The leaf surface chemicals and pH on the tobacco leaf might also have made that surface similar to aphid cuticle. Observing the developmental morphology on coverslips applied with tobacco leaf surface extracts might provide further insight on this aspect.

Decline in the virulence after successive subculturing in vitro has been reported in case of some species of entomopathogenic fungi including *P. neoaphidis* (Rockwood 1950, Morrow et al. 1989, Hajek et al. 1990). Occurrence of only one penetration in this entire study might be the result of loss of virulence of *P. neoaphidis* due to in vitro subculturing.

Influence of temperature: Increase in the germination of primary conidia of *Z. phalloides* Batko on coverslip was observed with increasing temperature within the range of 10-20^{0}C (Glare et al. 1986). In case of *P. neoaphidis*, the time taken to kill blue-green aphid, *Acyrthosiphon kondoi* Shinji, decreased with increase in

temperature within the range of 8-20^{0}C (Milner and Bourne 1983). The temperature optima for in vitro germination of primary and secondary conidia of *P. neoaphidis* were found to be between 18 and 23^{0}C (Morgan et al. 1992, Morgan et al. 1995). In the present investigation, temperature had no effect on the proportion of germinated conidia on aphid and leaf while a significantly higher proportion of germinated conidia was found at 13^{0}C on coverslip (Table 9.3). Since ellipsoid secondary conidia were grouped with primary conidia, the proportion of primary conidia that had germinated and produced those secondary conidia was overlooked in these comparisons. However, 20^{0}C seemed to be more favorable for infection since appressorial formation was significantly higher at that temperature on aphid and leaf. Correspondingly, round secondary conidia and germinating conidia, representing the successive stages of development, were less at 20^{0}C on these two surfaces, but the difference was not significant on aphid. Morgan et al. (1995) also reported the influence of temperature on the production of type of secondary conidia. Within a temperature range between 5 and 30^{0}C, the proportion of ellipsoid secondary conidia decreased while that of round secondary conidia increased with rising temperature. In the current study such comparison between ellipsoid conidia and round secondary conidia was not made, but the proportion of round secondary conidia significantly decreased on aphid and leaf when the temperature was increased. No influence on round secondary conidia was seen on coverslip.

Influence of time: Since 20^{0}C seemed to be more favorable for infection, appressorial formation was as high 12 h as 24 h post-inoculation on all surfaces at this temperature. At 13^{0}C, *P. neoaphidis* took longer to produce appressoria as evidenced by higher proportion of germinating conidia and lower proportion of appressoria 12 h than 24 h post-inoculation. Some of the germinating conidia might have given rise to appressoria by 24 h post-inoculation resulting in lower proportion of germinating conidia and higher proportion of appressoria at that time. Increase in time increased the proportion of round secondary conidia to ellipsoid secondary conidia produced at four temperatures between 10 and 30^{0}C for *P. neoaphidis* in vitro (Morgan et al. 1995). In my study, the proportion of round secondary conidia, among other structures found, significantly increased with increase in time only at 20^{0}C on coverslip and 13^{0}C on leaf. The proportion of ellipsoid conidia significantly decreased at corresponding time and temperatures on those substrates. However, it should be remembered that my comparison was among various structures and not between ellipsoid conidia and round secondary conidia.

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APPENDICES

Appendix 1: Figure 3.1

Date	Percent infection		
	Field 1	Field 2	Field 3
		Mean	
July 8	4.64	3.87	1.76
17	1.57	3.27	-
21	0.12	1.24	0.03
24	1.54	0.41	0.75
28	1.43	2.83	4.83
31	13.10	3.19	-
August 4	19.80	1.25	3.36
7	34.40	14.48	14.77
11	10.76	9.40	1.20
14	18.23	6.56	2.66
19	11.93	9.49	3.86
		SEM	
July 8	0.48	0.53	0.39
17	0.28	0.26	-
21	0.05	0.15	0.03
24	0.31	0.13	0.23
28	0.24	0.24	0.74
31	1.06	0.33	-
August 4	2.14	0.20	0.64
7	3.23	2.00	2.41
11	1.54	0.99	0.23
14	2.53	0.80	0.88
19	3.00	0.68	0.64

Date	Temperature (^{0}C)			Daily rainfall
	Minimum	Mean	Maximum	
July 1	22.80	26.40	30.00	2.50
2	21.10	24.70	28.30	0.00
3	18.30	23.90	29.40	0.00
4	20.00	25.60	31.10	6.40
5	19.40	25.00	30.60	0.00
6	21.10	24.70	28.30	1.30
7	17.80	23.10	28.30	0.00
8	16.70	23.30	30.00	0.00
9	25.00	29.70	34.40	0.00
10	26.70	31.40	36.10	0.00
11	23.90	29.70	35.60	0.00
12	23.90	29.70	35.60	0.00
13	25.00	31.40	37.80	0.00
14	22.80	30.30	37.80	0.00
15	23.30	29.20	35.00	17.80
16	22.80	28.30	33.90	14.00
17	22.80	27.50	32.20	0.00
18	22.80	26.70	30.60	5.10
19	21.70	27.20	32.80	0.00
20	20.60	27.50	34.40	0.00
21	22.80	28.90	35.00	0.00
22	21.70	26.10	30.60	5.10
23	21.70	26.10	30.60	0.00
24	22.80	26.70	30.60	15.20
25	21.70	26.10	30.60	0.00
26	21.10	26.10	31.10	0.00
27	22.20	28.30	34.40	21.60
28	20.00	25.00	30.00	0.00
29	18.30	25.00	31.70	0.00
30	20.60	26.90	33.30	0.00
31	21.10	27.20	33.30	8.90
August 1	15.60	21.10	26.70	0.00
2	13.90	20.30	26.70	0.00
3	16.70	24.40	32.20	14.00
4	20.60	26.10	31.70	0.00
5	17.80	23.10	28.30	0.00
6	18.30	20.80	23.30	8.90
7	18.90	22.20	25.60	2.50
8	18.30	23.60	28.90	0.00
9	21.10	26.40	31.70	0.00
10	18.90	26.70	34.40	0.00
11	21.70	27.80	33.90	0.00
12	20.00	23.30	26.70	5.10
13	17.20	19.20	21.10	45.70
14	17.20	20.00	22.80	3.80
15	16.70	17.50	18.30	7.60
16	16.70	18.30	20.00	5.10
17	18.30	20.60	22.80	3.80
18	19.40	24.20	28.90	0.00
19	20.00	25.00	30.00	0.00

Appendix 2: Figure 3.2

Date	1992		
	Percent infection		
	Field 1	Field 2	Field 3
		Mean	
July 28	65.90	60.60	23.40
31	68.60	51.40	16.80
August 4	49.20	32.40	2.90
7	54.70	35.10	12.80
11	39.30	42.00	8.70
14	27.80	36.40	19.80
19	7.60	6.66	36.10
		SEM	
July 28	4.10	5.00	4.50
31	5.00	4.90	5.10
August 4	5.70	5.10	0.80
7	5.30	4.60	2.70
11	6.10	4.60	1.80
14	6.30	4.30	3.30
19	1.30	1.20	17.00

Date	1994			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 18	761.60	59.60	2.48	1.48
22	608.00	22.70	11.23	2.94
25	429.30	41.90	23.80	5.83
29	339.80	19.60	35.95	5.37
August 2	326.80	24.70	31.75	2.95
5	340.40	20.70	96.88	0.71
9	137.00	11.70	59.54	5.96
18	296.20	20.40	55.37	4.94
24	114.80	22.12	80.56	4.14
28	76.20	11.80	43.95	5.62
September 3	0.00	0.00	0.00	0.00

Date	1995			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 10	405.40	61.60	6.88	1.88
13	354.40	45.70	2.71	0.91
17	772.70	102.50	8.67	1.50
20	784.40	87.04	7.65	1.08
24	504.20	65.70	2.74	0.79
28	253.20	40.40	4.50	1.30
31	51.20	13.12	1.10	0.47
August 3	1.48	0.45	0.00	0.00

Appendix 3: Figure 3.3

Date	1993			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
August 30	121.44	15.50	7.55	3.85
September 1	81.32	14.40	6.11	4.02
3	108.10	17.70	3.03	1.08
7	41.40	7.20	4.04	1.68
8	54.13	6.40	6.40	1.91
9	131.20	24.40	6.50	1.78
12	143.30	37.20	14.30	4.02
17	82.44	7.30	8.60	2.38
20	165.10	29.36	7.40	2.20
23	90.00	12.20	28.33	7.60
27	266.50	45.40	2.50	1.30
October 1	203.5	42.1	18.2	4.63
4	227.24	40.9	16.6	5.84
6	103.8	10.2	25.8	7.35
11	420	45.15	5.8	3.2
14	258.5	28.7	5.11	2.3
18	181.2	17.2	12.4	4.7
21	287.2	27.2	23.8	5.97
26	290.24	53.7	23.84	6.35
28	91.5	19.1	29.6	7.05
November 1	142.6	27.8	21.5	5.68

Appendix 4: Figure 3.4

Date	1994- Field 1			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 7	2018.3	196.60	0.0023	0.0023
11	3316.3	224.50	0.0003	0.0003
14	5988.6	347.20	0.0039	0.0037
18	8709.2	394.10	0.0036	0.0035
21	6493.9	270.10	0.31	0.10
25	4128.5	271.30	13.67	3.00
29	422.9	58.10	17.20	2.90
August 1	57.2	9.90	20.24	2.60
4	30.1	6.10	3.89	0.70
8	34.0	3.70	6.70	1.50
11	61.2	7.50	1.20	0.39
16	317.7	36.70	0.40	0.10

Date	1994- Field 2			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 27	5085.3	568.80	3.00	1.00
August 1	4694.5	325.70	12.10	2.06
4	1613.6	124.70	35.10	4.20
7	1288.1	125.40	81.70	3.95
11	192.0	37.80	90.70	3.14
14	177.1	26.40	65.30	4.90
18	65.2	15.90	4.90	1.50

Appendix 5: Figure 3.5

Date	Temperature (^{0}C)			Relative	Leaf	Daily	Solar
	aximu	Mean	Minimum	humidity	wetness	rainfall	radiation
July 1	33.80	24.70	17.10	87.30	36.90	0.25	1009.90
2	33.80	26.00	19.20	81.80	37.90	0.00	1003.90
3	33.90	26.10	20.70	82.00	37.40	27.94	985.20
4	33.00	24.70	19.20	93.00	38.60	8.89	716.00
5	33.90	25.90	19.60	88.30	41.00	0.51	933.10
6	35.00	27.70	21.20	83.40	41.20	0.00	1065.20
7	34.60	27.90	22.70	81.30	14.40	0.00	993.50
8	33.20	27.90	22.80	79.10	0.34	0.00	1004.50
9	34.50	28.30	21.30	73.50	13.90	0.25	1092.20
10	34.60	25.60	20.80	83.80	44.00	2.03	730.90
11	31.20	24.00	19.20	82.30	32.00	0.00	637.90
12	32.20	25.90	20.00	84.80	33.70	0.00	561.70
13	32.50	25.60	20.90	84.40	16.70	35.31	618.20
14	33.40	26.60	21.20	90.30	24.50	0.25	991.00
15	34.90	27.70	22.10	86.00	27.90	0.00	924.20
16	33.30	26.80	21.60	85.00	38.40	0.00	861.10
17	34.40	25.90	20.50	85.50	51.70	3.30	781.20
18	34.20	26.00	21.00	87.80	39.00	0.00	667.20
19	34.30	26.10	21.20	85.70	23.80	0.00	614.10
20	35.10	25.30	20.10	87.30	55.10	3.30	824.50
21	31.00	24.70	21.50	92.30	48.20	7.87	648.90
22	31.40	25.30	21.80	92.50	34.90	8.38	695.10
23	31.20	24.60	22.30	91.80	33.70	1.27	527.40
24	34.10	26.20	21.30	87.00	32.90	0.00	883.50
25	33.80	26.20	21.60	84.70	43.10	0.00	752.40
26	31.00	23.90	20.40	89.20	68.20	8.38	519.20
27	29.20	23.30	20.20	92.60	50.40	6.60	521.40
28	28.60	23.00	20.20	96.50	57.20	8.13	394.00
29	30.70	23.00	20.00	95.10	58.30	24.89	384.80
30	32.10	24.30	19.40	92.20	32.50	0.00	660.50
31	32.20	24.70	21.10	90.30	40.10	0.76	621.70
August 1	32.10	24.70	19.90	89.50	37.20	0.25	799.90
2	32.80	26.00	20.10	85.60	33.90	0.00	726.10
3	33.40	25.70	20.30	84.00	43.50	0.00	749.70
4	33.10	26.50	22.00	80.60	21.80	0.00	774.90
5	30.50	23.80	18.80	88.50	46.50	2.03	516.00
6	26.60	18.90	12.70	76.20	5.50	0.00	971.30
7	28.10	19.40	11.60	81.90	34.90	0.00	914.50
8	30.00	20.30	12.00	82.30	33.10	0.00	972.60
9	31.60	22.80	14.00	82.00	18.20	0.00	895.80
10	33.50	24.40	17.30	78.90	12.70	0.00	773.50
11	33.10	25.60	19.40	77.40	13.60	0.00	733.10
12	32.40	26.20	21.30	81.00	5.00	0.25	789.60
13	33.70	27.10	21.70	75.00	2.20	0.00	924.80
14	32.10	26.60	21.50	78.70	18.10	9.65	791.50
15	24.90	21.00	17.80	89.20	33.70	6.35	404.50
16	24.60	20.20	17.10	95.60	23.60	10.92	223.80
17	29.90	24.40	21.10	95.90	52.30	27.94	538.90
18	32.70	24.90	19.40	80.40	35.40	0.00	871.60

Appendix 6: Figure 3.6

Date	1995- Field 1-Topped			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 5	1689.6	319.10	0.20	0.09
8	2645.2	391.20	1.34	0.51
11	5688.0	735.30	4.93	1.68
14	7676.0	890.50	8.57	2.39
18	18619.6	2112.70	2.05	0.89
21	13792.1	1454.30	2.81	1.92
24	3938.0	303.40	3.18	0.61
27	153.6	31.20	0.18	0.11
31	16.9	3.20	0.19	0.19
August 3	3.8	1.00	0.00	0.00

Date	1995- Field 2-Topped			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 5	1066.8	216.40	0.59	0.21
8	2258.0	326.80	1.00	0.37
11	3500.0	387.60	3.20	1.23
14	5620.0	533.80	6.00	1.29
18	10543.4	1241.80	0.85	0.12
21	5885.5	988.40	1.01	0.39
24	1412.6	344.70	0.56	0.24
27	42.4	12.50	0.04	0.04
31	8.4	2.64	0.00	0.00
August 3	1.0	0.36	0.00	0.00

Date	1995- Field 1-Untopped			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 5	1500.6	419.00	0.38	0.15
8	2796.4	531.00	1.81	0.64
11	3880.8	536.70	6.12	1.75
14	6002.0	748.10	7.81	1.80
18	11569.6	1155.80	3.90	1.37
21	10589.0	1910.70	2.44	0.68
24	4420.0	509.40	2.89	0.55
27	2689.6	342.00	3.84	0.99
31	628.2	173.30	2.01	0.40
August 3	214.1	57.00	0.33	0.18

Date	1995- Field 2-Untopped			
	Aphids		Percent infection	
	Mean	SEM	Mean	SEM
July 5	1692.2	430.60	0.68	0.17
8	3110.9	603.40	0.99	0.24
11	3534.8	346.20	7.16	2.10
14	4843.5	407.70	14.50	4.00
18	8781.5	643.90	2.20	0.95
21	8529.9	1247.10	3.36	1.80
24	2415.2	375.20	2.33	0.40
27	1521.7	288.70	2.36	0.40
31	490.4	110.50	1.36	0.40
August 3	201.3	60.10	0.31	0.15

Appendix 7: Figure 3.7

Date	Temperature (^{0}C)			Relative	Leaf	Daily	Solar
	Maximu	Mean	Minimum	humidity	wetness	rainfall	radiation
July 1	32.10	24.70	19.70	88.40	36.90	33.80	761.20
2	29.70	23.30	18.10	87.00	40.80	4.32	810.50
3	31.30	22.90	16.60	83.50	33.30	0.00	673.20
4	32.90	24.40	19.60	89.30	44.60	7.87	654.90
5	32.80	25.30	18.90	88.80	33.70	0.25	816.70
6	32.90	24.60	20.00	87.90	43.20	15.70	601.30
7	33.50	24.50	18.70	88.70	36.10	0.25	775.20
8	31.10	25.00	18.60	81.70	38.20	0.00	927.20
9	30.50	22.70	14.80	80.90	37.20	0.00	828.20
10	32.20	24.30	16.00	81.20	33.30	0.00	772.10
11	33.50	25.70	18.40	87.50	28.80	47.00	840.70
12	34.20	25.90	19.00	84.10	43.90	0.00	822.80
13	33.30	25.90	18.30	84.60	42.40	0.00	897.80
14	34.10	27.00	20.20	83.00	17.80	0.00	919.10
15	36.90	29.20	22.20	84.10	7.30	0.00	916.20
16	36.20	27.70	22.10	84.90	30.30	1.02	584.10
17	34.90	27.80	21.30	85.80	33.50	0.25	895.10
18	36.60	28.80	21.70	83.50	6.90	0.00	947.60
19	35.40	27.30	19.30	77.90	25.40	0.00	902.30
20	35.00	26.80	17.10	75.30	33.70	0.00	883.30
21	34.60	27.60	23.10	83.00	22.70	2.29	520.20
22	35.90	27.10	21.70	86.30	45.40	0.25	644.20
23	36.50	28.90	22.40	79.40	36.00	0.00	876.10
24	37.30	28.30	22.50	81.90	22.20	0.51	813.00
25	36.90	28.30	21.60	82.20	11.20	0.00	908.90
26	35.20	27.70	21.10	83.60	31.10	2.03	904.60
27	35.90	27.00	17.30	82.20	45.10	22.10	734.30
28	34.60	25.30	16.50	78.20	31.90	0.25	786.90
29	36.60	28.50	22.30	77.20	29.00	0.00	905.20
30	37.00	28.90	22.30	77.30	41.20	0.00	841.10
31	37.10	28.60	20.60	75.00	55.60	0.00	887.40
August 1	37.80	28.50	20.30	73.90	50.30	0.00	826.50
2	36.00	28.40	21.90	72.40	43.10	0.00	832.20
3	35.60	28.10	20.50	71.70	35.90	0.00	897.20

Appendix 8: Figure 4.1

Host plant	1993			Host plant	1994		
	22 Oct.- 10 Nov.	17 Nov.- 8 Dec.	22 Oct.- 8 Dec.		13 Oct.- 3 Nov.	16 Nov.- 5 Dec.	13 Oct.-. 5-Dec
Broccoli	46.9	37.4	42.1	Broccoli	8.7	3.6	6.2
Cabbage	60.3	56.3	58.3	Cabbage	64.8	84.4	74.6
Collards	64.8	72.7	68.7	Collards	54.1	64.6	59.4
Kale	34.6	32.6	33.6	Kale	5.6	8.5	7.0
Mustard	3.9	21.6	12.8	Lettuce	0.4	2.6	1.5
Rape	52.4	74.9	63.6	Mustard	0.3	11.8	6.0
Spin. Mustard	4.8	24.0	14.4	Radish	1.3	8.6	4.9
Turnip (ptop.)	17.4	52.8	35.1	Rape	9.2	11.5	10.3
Turnip (7top.)	10.3	56.6	33.5	Rutabaga	4.6	13.1	8.9
				Spinach	0.3	8.7	4.5
				Spin. Mustard	0.7	6.6	3.6
				Turnip	0.9	7.6	4.3

Appendix 9: Figure 4.2

Host plant	1993			Host plant	1994		
	22 Oct.- 10 Nov.	17 Nov.- 8 Dec.	22 Oct.- 8 Dec.		13 Oct.- 3 Nov.	16 Nov.- 5 Dec.	13 Oct.-. 5-Dec
Broccoli	15.6	10.4	13.0	Broccoli	2.4	9.9	6.1
Cabbage	15.5	9.8	12.6	Cabbage	2.0	13.9	8.0
Collards	19.1	15.5	17.3	Collards	1.9	7.2	4.6
Kale	23.0	12.7	17.8	Kale	1.5	7.5	4.5
Mustard	36.1	7.6	22.3	Lettuce	0.0	7.8	3.9
Rape	15.3	21.4	18.3	Mustard	0.0	11.4	5.7
Spin. Mustard	21.0	11.5	16.2	Radish	0.0	16.2	8.1
Turnip (ptop.)	26.4	16.3	21.4	Rape	1.7	7.4	4.6
Turnip (7top.)	20.2	19.9	20.0	Rutabaga	3.1	13.5	8.3
				Spinach	0.0	0.0	0.0
				Spin. Mustard	0.0	13.5	6.8
				Turnip	3.1	19.2	11.1

Appendix 10: Figure 4.3

Date	Temperature (^{0}C)	Relative humidity	Leaf wetness	Total rainfall
1993				
October 22	17.2	81.4	33.6	17.5
27	13.3	74.7	25.4	21.3
November 4	7.5	67.9	27.0	28.7
10	7.9	69.9	31.9	21.6
17	15.3	68.3	6.7	5.3
24	9.4	60.2	8.9	0.3
December 3	7.2	68.5	27.1	88.9
8	8.4	75.0	29.4	30.7
1994				
October 13	13.7	75.6	38.4	2.6
21	13.8	73.1	34.1	1.8
27	13.1	73.0	38.5	38.4
November 3	13.9	66.6	29.5	6.9
16	10.7	65.8	28.1	10.2
23	11.5	62.0	33.4	61.7
30	7.3	61.5	21.8	14.2
December 5	10.9	72.4	27.3	4.8

Appendix 12:

Preparation of Sabouraud maltose Egg Milk Agar (SEMA) medium

Ingredients: 6.5 g Sabouraud maltose agar (SMA)

8.5 ml cow's milk (whole milk)

80 ml deionized water

2 unwashed fresh eggs

1. Sterilize fresh unwashed eggs in 70% ethyl alcohol for at least 30 min.
2. Take SMA and half the quantity of water in one beaker and milk with remaining half of the water in another beaker. Autoclave the two beakers with an empty third beaker (for separating egg yolk) and a stir bar at 110^0C for 20-25 min.
3. Add egg yolks to SMA, mix thoroughly, and pour contents into milk while mixing with stir bar. Add gentamicin (0.1% by vol.) and penicillin (0.05% by vol.) to the medium and pour into Petri plates (100 by 15 mm) (about 20 ml/plate) after thorough mixing.

Isolation of *Pandora neoaphidis*:

Surface sterilize living-infected or recently fungus-killed aphid cadavers by soaking them in 5-10% bleach (sodium hypoclorite 5.25%) solution and 0.01% gentamicin solution for 30-60 sec in each. Add a drop or two of ethyl alcohol in the bleach solution to break surface tension. Blot the aphids dry with autoclaved filter paper. Transfer the aphids onto the SEMA medium in Petri plates.

Subculturing *P. neoaphidis*:

Cut a small disc (about 5 mm dia) from the regenerating portion of a culture and transfer onto SEMA in a Petri plate. If the cultures are to be used to inoculate aphids, place 2-3 discs in each Petri plate to obtain large area of sporulating cultures in short time.

Appendix 11: Figure 5.1

Date	1994				Date	1995			
	Temperature (^{0}C)			Daily		Temperature (^{0}C)			Daily
	Minimum	Mean	Maximu	rainfall		Minimum	Mean	Maximu	rainfall
July 7	22.7	27.9	34.6	0.0	June 24	19.6	24.2	31.1	16.3
8	22.8	27.9	33.2	0.0	25	19.1	24.7	32.6	0.0
9	21.3	28.3	34.5	0.3	26	20.0	23.3	31.7	24.4
10	20.8	25.5	34.6	2.0	27	19.9	23.3	31.1	8.4
11	19.2	24.0	31.2	0.0	28	20.7	23.1	29.7	6.9
12	20.0	25.9	32.2	0.0	29	20.0	23.1	30.4	14.2
13	20.9	25.5	32.5	35.3	30	20.1	24.0	30.7	3.8
14	21.2	26.5	33.4	0.3	July 1	19.7	24.7	32.1	33.8
15	22.1	27.6	34.9	0.0	2	18.1	23.3	29.7	4.3
16	21.6	26.8	33.3	0.0	3	16.6	22.9	31.3	0.0
17	20.5	26.0	34.4	3.3	4	19.6	24.4	32.9	7.9
18	21.0	26.0	34.2	0.0	5	18.9	25.3	32.8	0.3
19	21.2	26.0	34.3	0.0	6	20.0	24.6	32.9	15.7
20	20.1	25.3	35.1	3.3	7	18.7	24.5	33.5	0.3
21	21.5	24.7	31.0	7.9	8	18.6	25.0	31.1	0.0
22	21.8	25.3	31.4	8.4	9	14.8	22.7	30.5	0.0
23	22.3	24.6	31.2	1.3	10	16.0	24.3	32.2	0.0
24	21.3	26.2	34.1	0.0	11	18.4	25.7	33.5	47.0
25	21.6	26.2	33.8	0.0	12	19.0	25.9	34.2	0.0
26	20.4	23.9	31.0	8.4	13	18.3	25.9	33.3	0.0
27	20.2	23.3	29.2	6.6	14	20.2	27.0	34.1	0.0
28	20.2	23.0	28.6	8.1	15	22.2	29.2	36.9	0.0
29	20.0	23.0	30.7	24.9	16	22.1	27.7	36.2	1.0
30	19.4	24.3	32.1	0.0	17	21.3	27.8	34.9	0.3
31	21.1	24.7	32.2	0.8	18	21.7	28.8	36.6	0.0
August 1	19.9	24.6	32.1	0.3	19	19.3	27.3	35.4	0.0
2	20.1	26.0	32.8	0.0	20	17.1	26.8	35.0	0.0
3	20.3	25.7	33.4	0.0	21	23.1	27.6	34.6	2.3
4	22.0	26.5	33.1	0.0	22	21.7	27.1	35.9	0.3
5	18.8	23.8	30.5	2.0	23	22.4	28.9	36.5	0.0
6	12.7	18.9	26.6	0.0	24	22.5	28.3	37.3	0.5
7	11.6	19.4	28.1	0.0	25	21.6	28.3	36.9	0.0
8	12.0	20.3	30.0	0.0	26	21.1	27.7	35.2	2.0
9	14.0	22.8	31.6	0.0	27	17.3	27.0	35.9	22.1
10	17.3	24.4	33.5	0.0	28	16.5	25.3	34.6	0.3
11	19.4	25.5	33.1	0.0	29	22.3	28.5	36.6	0.0
12	21.3	26.1	32.4	0.3	30	22.3	28.9	37.0	0.0
13	21.7	27.1	33.7	0.0	31	20.6	28.6	37.1	0.0
14	21.5	26.6	32.1	20.3	August 1	20.3	28.5	37.8	0.0
15	17.8	21.0	24.9	6.4	2	21.9	28.4	36.0	0.0

Appendix 13: Figure 7.1

Date	Total number of aphids/plant				Percent infection			
	Living-infected	Fungus-killed + Living-infected	Triturated fungus-killed	Untreated control	Living-infected	Fungus-killed + Living-infected	Triturated fungus-killed	Untreated control
August 14	615	613	542	441	0.00	0.00	0.00	0.00
17	769	670	642	549	0.06	0.76	0.06	0.67
18	923	810	748	698	1.40	1.04	0.19	0.93
19	994	973	906	846	2.60	2.90	0.36	0.90
20	1183	1098	1088	952	0.59	0.48	0.07	0.36
23	555	646	671	606	13.20	9.30	2.12	1.40
25	440	455	564	443	14.03	15.00	3.00	1.56
27	435	285	457	352	19.00	11.93	3.65	2.17
30	124	64	181	243	37.43	33.20	6.35	1.62
September 2	46	30	58	41	27.42	11.90	5.60	0.56

Appendix 14: Figure 7.2

Date	Temperature (^{0}C)			Relative humidity	Leaf wetness	Daily rainfall
	Maximu	Mean	Minimum			
August 14	34.20	25.50	18.90	73.20	32.20	0.00
15	35.50	26.60	18.00	72.20	26.50	0.00
16	36.60	27.20	19.30	72.70	26.50	0.00
17	31.10	26.20	22.10	81.70	6.90	1.02
18	31.10	24.70	20.10	86.30	27.80	0.00
19	33.70	24.20	18.30	81.50	40.70	0.00
20	35.20	26.30	19.60	78.00	21.60	0.00
21	33.50	26.40	20.30	72.50	17.60	0.00
22	32.60	24.40	17.80	74.20	16.30	0.00
23	33.10	24.10	16.60	74.10	23.50	0.00
24	34.90	25.40	18.00	69.20	2.00	0.00
25	37.00	27.10	20.30	70.10	10.30	0.00
26	36.00	27.10	21.80	83.60	40.10	0.25
27	35.30	26.80	20.50	78.10	32.40	0.00
28	36.70	27.90	21.10	75.40	18.40	0.00
29	39.30	29.10	21.10	73.40	5.60	0.00
30	36.70	27.40	21.90	73.60	29.40	0.00
31	34.50	25.50	19.30	80.70	34.00	0.00
September 1	38.80	28.90	19.60	77.60	54.50	5.33
2	36.70	27.60	21.50	77.20	34.20	0.51

Appendix 15: Figure 7.3

Days after treatment	Total number of aphids/plant				Percent infection			
	Living-infected	Fungus-killed	Untreated control	Orthene	Living-infected	Fungus-killed	Untreated control	Orthene
0	3250.0	2975.0	2975.0	2875.0	0.00	0.00	0.00	0.00
4	2330.0	1830.0	2100.0	0.3	1.48	1.48	2.48	0.00
7	1665.0	1250.0	1280.0	0.0	7.61	12.55	2.19	0.00
9	3175.0	2450.0	3225.0	1.5	5.05	7.15	0.99	0.00
14	3275.0	3225.0	3275.0	2.5	20.62	21.33	6.73	0.00
19	5663.0	5994.0	5300.0	81.0	19.90	16.20	10.10	0.00

VITA

Surendra Kumar Dara was born to Subba Rao and Sakuntala Dara on 20 September, 1968 in Peddapuram, Andhra Pradesh, India. He attended Lutheran High School and graduated with Secondary School Certificate in April 1983. He enrolled in Intermediate at Sree Raja Vatsavaayi Butchi Seetayamma Jagapati Bahaddur Maha Rani College and graduated in April, 1985 with first rank in Biology major.

He went to The Agricultural College, Bapatla of Andhra Pradesh Agricultural University in September, 1985 and graduated with B.Sc. (Ag) in August, 1989. He continued at The Agricultural College, Bapatla and started M.Sc. (Ag) program in Entomology in October, 1989. He worked on age-specific life- and fertility-tables, and stable age-distribution of *Spodoptera litura* F. with Dr. V. Deva Prasad. He graduated in October, 1991 with first rank in Entomology and third rank in The Agricultural College in M.Sc. (Ag).

He came to the United States in July, 1992 and enrolled in Graduate School of Virginia Polytechnic Institute and State University to pursue Ph.D. in Entomology. He was awarded a scholarship by R. J. Reynolds Tobacco Company to work on the control of tobacco aphid. He was also awarded graduate research scholarship at VPI & SU. Under the guidance of Dr. Paul. J. Semtner at the Southern Piedmont Agricultural Research and Extension Center, he worked on

evaluating the potential of a fungal pathogen for the control of the tobacco aphid.

He served on the Students Awards Committee, and Admissions and Standards Committee of the Department of Entomology, VPI & SU, as student representative. He was selected for the membership of Phi Sigma Biological Honor Society at VPI & SU. He was also a member of Entomological Society of America and Society for Invertebrate Pathology.

He was selected for the post-doctoral insect pathologist position at International Institute of Tropical Agriculture, Cotonou, Republic of Benin. He will assume his new position in March, 1996 to work on the control of cassava green mite.