Chapter V-1

Documentation of naturally occurring pathogens and their impact in agroecosystems

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1 Introduction

Microbial pathogens play a vital role in the natural regulation of many arthropod populations in agricultural systems. Their importance has been underemphasized compared to parasitoids and predators because fewer specialists work on pathogens, and pathogens are generally less noticeable than parasitic and predaceous arthropods.

Because of the disturbed nature of agroecosystems and the need for rapid control of arthropod pests in crops, many entomologists are of the opinion that naturally occurring pathogens have limited value in integrated pest management (IPM). This assumption is inaccurate. Ecologists have become increasingly aware of the role microorganisms play in population regulation. May (1988) argued that the significance of pathogens has generally been underestimated in ecology. Loehle (1995) stated that the idea that pathogens are important regulators only in overly large, stressed populations is incorrect. Invertebrate pathologists have long been aware that microbial pathogens play a major role in regulation of arthropod populations. The eminent pathologist E. A. Steinhaus stated that a "*... knowledge of insect diseases (whether or not it has to do with control) is of fundamental and far-reaching importance in the study of insect ecology*" (Steinhaus, 1949). A worthwhile goal for pest managers is to develop methods to take advantage of the natural control provided by pathogens of pests.

According to Stern *et al.* (1959) in their seminal paper on IPM, *"Chemical control should be used only when the economic threshold is reached and when the natural mortality factors present in the environment are incapable of preventing the pest population from reaching the economic-injury level."* Yet, this central concept of IPM, "natural mortality," especially that provided by pathogens, has rarely been considered in making treatment recommendations in most agroecosystems. Whitten (1992) stated that *"our overriding philosophy is to reduce mankind's dependence on chemical pesticides by identifying other methods which are economic, environmentally sound and lasting in effectiveness. Seeking these alternatives is the exciting challenge."* Natural control of pests by pathogens should play a more prominent role in IPM.

There have been many efforts to predict the prevalence of plant disease (Cook, 1949; Van der Plank, 1963; Waggoner and Horsfall, 1969) and plant pathogen diagnostic laboratories are common, but prior to the work of Kish and Allen (1978), there were no attempts to predict the natural prevalence of disease in insect pest populations. Since then there have been several attempts to develop models or systems to predict the levels of control provided by pathogens to reduce unnecessary pesticide usage. The main factors that need to be determined are: (1) the principal pathogen(s) involved and (2) the effects of these pathogens on pest population density (Kish and Allen, 1978). Ideally such studies

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will be coordinated with similar studies on the effects of predators and parasitoids on the host population and population models of the pest that take into account oviposition, hatching, pupation, and environmental effects on the pest. The insect disease prediction systems proposed by Kish and Allen (1978), Steinkraus and Hollingsworth (1994) and Hollingsworth *et al*. (1995) are first steps in the incorporation of natural control by pathogens into IPM systems.

Documentation of arthropod pathogens in agroecosystems is important in four ways. First, identification and isolation of pathogens from specific arthropod pests are important initial steps in development of new classical biological control agents or microbial pesticides, as described below in the section on the entomopathogenic nematode *Steinernema riobrave*. Techniques for identification and isolation of pathogens are covered in appropriate chapters in Lacey (1997) and will not be covered further here. Second, our understanding of the population dynamics of arthropod pests is enhanced by documentation of the role played by pathogens. Pathogens are often found to be as important or more important in regulation of arthropod numbers as predators and parasitoids. Examples of this are described below. Third, naturally occurring epizootics are sometimes a valuable natural resource for harvesting pathogens for research or use in biological control (Moscardi, 1999; Steinkraus and Boys, 2005). Fourth, in situations where pathogen-induced epizootics regularly reduce pest populations, it may be possible to develop sampling programs or models to predict pathogen-induced pest declines. Such predictive sampling programs can lead to reduced inputs of chemical pesticides. Two case histories of this approach are described below.

2 General considerations

There are a number of variables that need to be considered when documenting the impact of naturally occurring entomopathogens in agroecosystems. These can be divided into two main interrelated categories: first, factors related to the environment a pathogen encounters and, second, factors integral to the pathogen. The first category provides background information for applied epizootiological studies and may require collection of data on temperature, humidity, rainfall, light intensity and duration, soil properties, crop varieties involved, plant stage and architecture, planting dates, cultural practices, as well as pesticide, herbicide, fertilizer and other chemical inputs. In addition, the arthropod host(s) for a pathogen constitutes a portion of the environment experienced by the pathogen. Therefore, the host's biology and stage(s) of importance need to be well understood by the investigator. The second category involves laboratory studies to determine the optimum conditions for growth and survival of the pathogen. More specific requirements are considered below.

A Pathogen prevalence

A major objective is to determine prevalence of the pathogen(s) in the pest population. Prevalence is the most commonly used measure of pathogen impact on a host population and is defined as the number or proportion of hosts infected by a specific pathogen at a given point in time (Fuxa and Tanada, 1987). To accurately determine prevalence, the investigator must not bias his sample towards or against healthy or diseased individuals; in short, samples must be truly random. However, to do this requires knowledge of the life cycle of the pest species. For example, larvae of the corn earworm, *Helicoverpa zea*, develop in corn ears, but when mature, leave the corn to pupate in the soil. A sampling procedure for nucleopolyhedrovirus (NPV) or microsporidium that sampled corn ears after most healthy earworms had left the plants to pupate would be heavily biased in favor of infected larvae, resulting in an overestimate of prevalence, and therefore, impact of the pathogen(s). Almost always, prevalence determinations require periodic sampling of the host population at intervals determined by the biology of the host.

Accurate prevalence requires collecting live arthropod hosts as well as dead hosts that may have been killed by the pathogen. Living hosts collected in the field must survive in the laboratory long enough for the pathogen to become evident. The hosts require suitable food,

temperature, light, and relative humidity. Ideally, live hosts should be held individually to prevent the possibility of transmission of pathogens during the collection and handling procedures, which could increase the apparent prevalence, or contamination by pathogens, resulting in erroneous diagnoses. Excellent examples of methods utilizing these principles are found in Ruano-Rossil *et al*. (2002), Feng *et al*. (2004), and Nielsen and Hajek (2005).

B Identification of pathogens

Host morphology is an important factor in choosing the methods used to diagnose diseased arthropods and identify pathogens. For instance, it is possible to squash entire small arthropods, like tetranychid mites (Carner, 1976; Klubertanz *et al*., 1991) or aphids (Steinkraus *et al*., 1991), then directly examine them on a microscope slide for the microscopic signs of a pathogen. But squashing an entire acridid, or mature noctuid larva, would not be feasible. Specific methods for preparing specimens for diagnosis are presented in Lacey and Brooks (1997). Many other sophisticated diagnostic techniques are available and are discussed in chapters in Section IV. Each pathogen group (viruses, bacteria, protozoa, fungi and nematodes) requires somewhat different handling of hosts collected from the field and for pathogen identification. Novices in insect pathology are strongly encouraged to develop cooperative efforts with skilled diagnosticians or taxonomists of the appropriate pathogen groups. It is also advisable to prepare permanent voucher specimens of the pathogens involved and deposit these in suitable museums or other repositories such as the USDA-ARS Collection of Entomopathogenic Fungal Cultures (Ithaca, NY).

3 Control of pests by naturally occurring pathogens

The concept that the activity of naturally occurring pathogens against pests is important is not new. Steinhaus (1949) stated "…one definite fact stands out clear and indisputable: Entomogenous fungi, in nature and without any help from man, cause a regular and tremendous mortality of many insect pests in many parts of the world and do, in fact, constitute an efficient and extremely important natural control factor. Accordingly, entomogenous fungi are of great economic importance in insect control, even though man has not yet learned how to use them artificially in most instances."

There are many examples of the importance of documenting the occurrence of naturally-occurring pathogens in pest populations. Speare (1922) studied the natural control of the citrus mealybug in Florida by *Neozygites fumosa*, and stated that "entomogenous fungi are worth millions of dollars to the citrus industry. Owing to their excellent work, oranges and grapefruit are grown at a profit in many parts of the State where no money whatsoever is spent on artificial remedial measures." In studies on tetranychid spider mites, Klubertanz *et al*. (1991) found that a *Neozygites* sp. reduced a two-spotted spider mite, *Tetranychus urticae*, population 95% over a 6-day period on soybean. Smitley *et al*. (1986) found that *N. floridana* was the most important cause of population declines in *T. urticae* populations in field corn. Similar results have been reported on the importance of *Neozygites* spp. in natural control of mites on cotton (Carner and Canerday, 1968), lima beans (Brandenburg and Kennedy, 1983), and peanuts (Boykin *et al*., 1984).

Valuable work has been done on the importance of entomopathogenic fungi in regulating aphid populations on potato in Maine. These studies have shown that 5 species of Entomophthorales are the most important natural control agents of several aphid species (Patch, 1907; Shands *et al*., 1972). An intensive survey of potato-aphid pathogens made between 1952–1962 and 1963–1969 showed that fungal activity was the major cause of aphid declines in most years (Shands *et al*., 1972).

The importance of naturally occurring pathogens may be revealed during careful studies of the effect of pesticides, such as fungicides, on pest populations. For instance, Ruano-Rossil *et al*. (2002) conducted research on the green peach aphid, *Myzus persicae*, on potato in Minnesota. They found that fungicides disrupted control by naturally occurring entomopathogenic fungi. When fungicides were applied extremely high aphid populations resulted. The three

most important fungal species involved were *Pandora neoaphidis* (=*Erynia*), *Entomophthora planchoniana*, and *Conidiobolus obscurus*. Their research clearly indicated the importance of entomopathogenic fungi in controlling early season aphids when the fungi were keeping the aphids below the economic injury level. The Ruano-Rossil *et al*. (2002) study shows that entomopathogens are important in regulating low as well as high pest populations.

Nielsen and Hajek (2005) evaluated the diversity and prevalence of naturally occurring entomopathogenic fungi on the invasive soybean aphid, *Aphis glycines*, in New York State. They found seven species of fungi attacking the aphid, with *P. neoaphidis* the most abundant. In an outbreak of soybean aphids, *P. neoaphidis* caused infection in 84% of the aphids and the population crashed.

Mirid bugs in the genus *Lygus* are important pests of many crops. McGuire (2003) surveyed populations of *L. hesperus* from alfalfa fields in the San Joaquin Valley of California and found that the fungus *Beauveria bassiana* was found infecting *L. hesperus* in every county sampled and was present at almost every sampling date. In one field, the prevalence of *B. bassiana* reached 50%. Steinkraus and Tugwell (1997) and Leland and Snodgrass (2004) also reported on natural *B. bassiana* infections of *Lygus lineolaris*. More information on *B. bassiana* and mirids is presented in Chapter VII-6.

While entomopathogenic fungi often are responsible for dramatic, easily-noticed epizootics, other pathogens, particularly viruses and nematodes also provide control of agricultural pests. For instance, naturally occurring epizootics of *Trichoplusia ni* nucleopolyhedrovirus were recognized by Semel (1956) as important in controlling cabbage looper populations. Two selected examples of the importance of naturally occurring pathogens are discussed in detail below.

A *Natural occurrence of* Steinernema riobrave *in* Helicoverpa zea *in corn*

Noctuids in the genera, *Helicoverpa, Heliothis*, and *Spodoptera*, are serious crop pests worldwide (King, 1994). Raulston *et al*. (1992) isolated a new species of steinernematid nematode, *Steinernema riobrave* (described by Cabanillas *et al*., 1994), infecting prepupae and pupae of corn earworm, *H. zea*, and fall armyworm, *S. frugiperda*, from cornfields in the Lower Rio Grande Valley in Texas and northern Mexico. The 5-year study found that 34 and 24% of all fields contained infected *H. zea* or *S. frugiperda*, respectively. They further found that *S. riobrave* accounted for 49.4 and 46.1% of the mortality of *H. zea* and *S. frugiperda*, respectively. Not only did this research increase our understanding of the mortality factors acting against two important pests during their pupation stage in the soil, but the new nematode species that was discovered has proven to be useful as a biological control agent and is commercially available. The methods used in this research were as follows.

1 *Characteristics of study area*

The study area was defined in terms of geography, rainfall, irrigation, soil type, crop phenology, and pesticide usage. Such details are important. The *H. zea* mortality caused by *S. riobrave* in Texas and Mexico may not occur in other geographical regions or soil types. For example, in a similar study on pupal mortality of *H. zea* in corn fields in Arkansas, Kring *et al*. (1993) did not find *S. riobrave*.

2 *Sampling procedure and statistical analysis of data*

A sampling procedure was developed for prepupae and pupae of *H. zea* and *S. frugiperda*. Two areas were sampled in each of 90–120 fields/year. The samples were taken 50 and 100 m from the field edges after most larvae had left corn ears to pupate. Each sample was taken by carefully scraping 1 m^2 of the soil surface with a garden trowel to uncover pupal tunnels, then excavating the soil to a depth of 10–15 cm following the tunnels to find the pupal chambers. All extracted insects were placed in individual 20 ml plastic cups. Host species, sex, and developmental stage were recorded, and each pupa that died was examined with a dissecting microscope for the presence of nematodes. Data were analyzed by ANOVA and means separated by computing least square means and testing the hypothesis, Ho: LSM(i) = LSM(j) (Raulston *et al*., 1992).

The nematodes were preliminarily identified to genus with the works of Poinar (1990) and later described as a new species by Cabanillas *et al.* (1994).

3 Crucial factors in this study

First, the study was replicated in many fields over a 5-year period, providing strong evidence that the mortality caused by *S. riobrave* was not an isolated incident. Second, the scientists understood the life cycle of the hosts. In this case, the fact that *H. zea* and *S. frugiperda* pupate in the soil, the average depth of pupation, and the phenology of larval movement from host plant to soil, were important facts in the experimental design. Third, holding the extracted pupae individually in cups prevented contamination of healthy pupae with nematodes which would have resulted in erroneous prevalence rates. Fourth, each dead pupa was examined for presence of nematodes to confirm their diagnosis. Fifth, an expert nematode systematist (G. O. Poinar, Jr.) was enlisted to assist in the identification and description of *S. riobrave*.

B Natural occurrence of Zoophthora phytonomi *in* Hypera postica *populations in alfalfa*

The alfalfa weevil, *Hypera postica*, is an introduced pest of alfalfa in the USA that can cause significant yield losses (Harcourt *et al.*, 1974). Several studies have shown that the entomopathogenic fungus, *Zoophthora phytonomi*, is one of the primary mortality factors operating on the alfalfa weevil. In Ontario, Canada, a 10-year study concluded that the alfalfa weevil had ceased to be an economic problem due to early spring epizootic levels of infection by *Z. phytonomi* (Harcourt *et al.*, 1984). This pathogen is also important in the various parts of the USA, but epizootics may not occur early enough to provide complete control. In Illinois, Morris *et al.* (1996) found that *Z. phytonomi* was responsible for preventing *H. postica* populations in some fields from reaching economic thresholds, but not in others. Methods for documenting the occurrence of *Z. phytonomi* are drawn from DeGooyer *et al.* (1995).

1 Field descriptions and plot design

Four Iowa alfalfa fields were chosen about 40 km apart latitudinally. Field locations, size, management, alfalfa variety, and stand densities were documented. Within each field a study site 50 by 50 m or 25 by 100 m was chosen, and divided into 8 equal 12.5 by 25 m plots.

2 Sampling plan

Larvae were sampled using a 0.1 m^2 sampling frame. Each sample consisted of 32 six-stem sampling units from each site on each sampling date. Fields were sampled twice weekly from 1 April until the first cutting of alfalfa. Larvae were extracted from the stems and placed individually into glass vials, then reared at 24 °C with a photoperiod of 16:8 (LD). Each vial contained a fresh alfalfa terminal that was changed every other day. Larvae were reared until they reached the adult stage or died from *Z. phytonomi* infections.

3 Life table construction

Collected data were used to construct life tables. The authors found that *Z. phytonomi* was the key factor regulating within-generation population trends in 1991, causing high mortality of 3rd and 4th stage larvae. Harcourt *et al.* (1990) developed detailed information on the occurrence of epizootics caused by *Z. phytonomi* in southern Ontario. They found that the first diseased alfalfa weevils occurred 204 degree days (DD) above a base threshold of 9 °C from 1 April. Epizootics began 57 DD later and lasted for 10 to 14 days, killing up to 99% of the larvae. They concluded that *Z. phytonomi* was the principal variable regulating alfalfa weevil populations. This information has obvious implications for alfalfa weevil control in Ontario and for reducing pesticide applications.

4 A model for predicting control of *Anticarsia gemmatalis* populations on soybean by *Nomuraea rileyi*

Soybean, *Glycine max*, is an important crop worldwide as a source of protein and oil for humans and livestock. Severe outbreaks of the

velvetbean caterpillar, *Anticarsia gemmatalis*, can defoliate and damage soybean in the southern USA (Funderburk, 1993), and other locations, such as Brazil. In Florida, under certain conditions, *N. rileyi* can decimate *A. gemmatalis* populations with mortality levels approaching 100% Allen *et al.* (1971). Kish and Allen (1978) formulated ideas on predicting prevalence of the entomopathogenic fungus *N. rileyi* on *A. gemmatalis* on soybean. Their aim was to reduce insecticide use by utilizing the natural control provided by *N. rileyi*. They discussed two problems with biological control with naturally occurring pathogens: (1) humankind's lack of control over the natural environmental conditions that favor development of disease, and (2) epizootics may occur after peak pest populations, when crop damage has already resulted (Kish and Allen, 1978). They noted that the consistency of occurrence and high levels of population control provided by *N. rileyi* made it a candidate to be one of the first pathogens to be incorporated into an IPM program. For this approach to work, systems must be identified in which a pathogen naturally provides regular, predictable, epizootics.

Kish and Allen (1978) developed methods to examine and model each factor affecting the host population. Understanding of the environmental effects on pathogen/host dynamics is essential for developing confidence in a predictive model. The sample protocol below is drawn largely from the work of Kish and Allen (1978).

A Sample protocol to document naturally occurring pathogens and prediction of pest control

1 Identify appropriate crop/pest/pathogen system

The first need is to identify a pest population in a selected crop that has a pathogen acting against it that causes regular epizootics. This can only be achieved by field studies or observations by insect pathologists, or by entomologists working in cooperation with pathologists. It is not uncommon for field trials of pesticides, or ecological studies on predators or parasitoids to fail because of the action of pathogens on the target pest. Field entomologists observing pathogens that interfere with chemical pesticide trials, or other field studies, should consider these interferences as opportunities to conduct research on pathogens.

2 Identification, isolation, and culture of pathogen

It is essential that the pathogen(s) under consideration be identified (see Lacey, 1997) and the identification(s) be confirmed by taxonomic experts in the particular pathogen group. Deposit voucher specimens of the pathogen into an appropriate collection. If possible the pathogen(s) should be isolated and cultured *in vitro* or *in vivo* for studies on pathogen biology in the laboratory, such as effects of photoperiod, temperature, and relative humidity.

3 Determine the pathogen's biology and epizootiology

Determine the basic epizootiological factors governing transmission of the pathogen to hosts, pathogen overwintering, behavior of infected hosts, incubation period, pathogen propagules produced per infected host, density of pathogen propagules in the agroecosystem, and temporal occurrence of the pathogen in the air, water, soil, or on plant surfaces. It is essential to know when the pathogen first appears in the host population and when it peaks. Experiments to determine these facts must be designed differently for each pathogen group (viruses, bacteria, protozoa, fungi, nematodes). For example, fungi may be dispersed by air movements, but aerial dispersal is less important with protozoa.

4 Conduct field epizootiological studies

a Plot studies

The crop must be planted in an appropriate experimental design. A randomized complete block design with at least 4 replications is desirable. The crop variety, row spacing, seeding rate, and other factors should be defined and recorded. Often the cooperation of an agronomist is of great value. More realistic studies can be made on commercial plantings,

but the researcher loses some control over crop management.

b Sampling methods

The pest population must be sampled during the season using methods appropriate to its biology. Kish and Allen (1978) used the shake cloth method to sample *A. gemmatalis* with 61 row m/0.4 ha sampled bi-weekly.

c Prevalence determination

Sub-samples of larvae collected from the field were held in the laboratory for determination of the prevalence of the pathogen in the host population. Larvae were retained for 5 days, but not longer, to ensure that the infections had originated naturally in the field and were not a consequence of contamination during collection or in the laboratory. This point needs to be emphasized. First, a random sample of the pest population must be collected to ensure that infection rates accurately represent the situation in the field. Infected insects may not behave normally. Healthy insects may move more rapidly than moribund infected insects, and escape more often from a sweep net, beat sheet, or other method, resulting in an overrepresentation of infected specimens in a sample. Conversely, infected insects may fall to the ground and/or be removed by scavengers, and therefore be collected less frequently than healthy individuals, resulting in an underestimation of prevalence. Second, during sampling, individual arthropods may become contaminated with pathogens during close contact with other captured individuals. Therefore, care must be taken to prevent such events. The fate of individual infected *A. gemmatalis* in the field was determined by tagging hosts on plants and observing them over time.

d Effect of agrochemicals

The effect of pesticides, fungicides, and other management options on the pathogen/pest interaction should be determined. For instance, certain fungicides, such as benomyl may suppress development of epizootics of *N. rileyi* (Kish and Allen, 1978).

e Analyze and understand the system

Kish and Allen (1978) showed that the general concept held by many workers that entomopathogenic fungi require wet, rainy conditions to cause epizootics had to be qualified. In fact, they found excessive rain may actually impede fungal epizootic development. Dry, windy conditions promoted dispersal of *N. rileyi* conidia, but also retarded germination and infection unless humid conditions prevailed. Once the fungus was within the host, development of the pathogen was independent of weather conditions. Rain, dew, and relative humidity above 70% promoted conidiophore formation and conidiogenesis, while conidia were washed from host cadavers to the ground by rain. An alternation of wet and dry conditions was most effective in dispersing the pathogen, and excessive free water during early stages of the epizootic retarded development of epizootics.

They then analyzed the results statistically and developed a mathematical model to predict the amount of natural control provided by the pathogen. Pesticide use can be avoided when the pathogen is predicted to reduce the pest population. In practice growers usually follow a variation of the following practices: (1) wait until large, damaging pest populations are observed before spraying a pesticide, or (2) spraying on a schedule, regardless of pest populations. Heavy damage may result from the first approach, and the second, may result in unnecessary control, secondary pest outbreaks, reductions of beneficial arthropods, environmental pollution, and excessive input costs (Kish and Allen, 1978). Neither approach takes into account natural control factors that might suppress the pest population at any given time.

f Implement the model

The model was validated with actual field data. The final step was to incorporate the predictive model into practice. Kish and Allen (1978) believed that the ability to monitor the fungal entomopathogen, *N. rileyi*, and predict mortality levels in *A. gemmatalis* populations on a daily basis could lead to increased accuracy of management decisions and reduce use of pesticides for *A. gemmatalis* on soybean by 60% to 100%.

They planned to implement this model via a computer network with a main centralized computer. Extension inputs would be coordinated by a pest management specialist. This specialist would train extension agents, growers, scouts, and private consultants, in Florida counties. Trained personnel at the farm level would provide the basic data on the pest and beneficial insect populations, insect disease prevalence, and environmental conditions that would be placed in the central computer. The model would then be used to predict the control by *N. rileyi*, and this information provided to county level personnel for rapid and accurate decision-making.

The model of Kish and Allen (1978) does not appear to have been implemented due to decreased planting of soybean in Florida, the low value of soybean crops, and the ease of managing lepidopteran pests on soybean with insecticides (J. E. Funderburk, personal communication).

5 Extension-based sampling service for prediction of natural epizootics of *Neozygites fresenii* in *Aphis gossypii* populations on cotton

A Background

The cotton aphid, *Aphis gossypii*, is a serious pest of many crops worldwide. It is a small aphid with a very rapid life cycle; as short as 4 days during the summer (Isely, 1946). Cotton aphids can directly reduce yield and lint quality through photosynthate removal, indirectly reduce photosynthesis due to sooty mold growth on excreted honeydew, and can cause honeydew-contaminated sticky cotton. During the period 1988 through 2006, the aphid has been a serious cotton pest in the USA. The 1996 introduction of transgenic cottons for noctuid pest control in the USA and recent efforts to eradicate the boll weevil, *Anthonomus grandis*, may unpredictably affect the future pest status of cotton aphids.

Our understanding of aphid population levels that cause economic damage in cotton is incomplete. Studies in Oklahoma (Karner *et al.*, 1997) and California (Godfrey *et al.*, 1997; Godfrey and Wood, 1998) showed significant cotton yield losses due to aphids. However, studies in Tennessee (Lentz and Austin, 1998) and Mississippi (Hardee and Adams, 1998) showed no yield losses due to cotton aphids, and no benefit from insecticides applied for aphid control. In 1998, the cotton aphid was ranked the most important pest of cotton in the San Joaquin Valley, California (Williams, 1998); however, its status as a pest varies year by year. An added complication is the resistance aphid populations develop to insecticides (Grafton-Cardwell, 1991; Kerns and Gaylor, 1992; O'Brien *et al.*, 1992; Harris and Furr, 1993). Insecticides may reduce aphid numbers for only a few days, and in some cases may actually result in aphid population increases (Karner *et al.*, 1997). Insecticide application when aphid populations are not causing economic injury, when aphids are resistant, or when natural control will keep aphid numbers beneath the economic threshold, is an unnecessary expenditure of money and effort for cotton growers.

In 1989, cotton entomologists in the midsouthern USA observed major die-offs of cotton aphid populations, presumably caused by a pathogen. In 1990, the causal agent of the aphid declines observed in the midsouth was identified as the entomopathogenic fungus *Neozygites fresenii* (Steinkraus *et al.*, 1991). The importance of *N. fresenii* in the control of the cotton aphid has been subsequently well-documented (Wells *et al.*, 2000; Marti and Olson, 2006). In 1991, reports of epizootics in *A. gossypii* populations on cotton were reported from the USA (Steinkraus *et al.*, 1991) and Africa (Silvie and Papierok, 1991). These epizootics in cotton aphids on cotton may have been a new phenomenon; certainly there were no similar reports prior to 1991. The occurrence of epizootics in a serious pest (*A. gossypii*), that is difficult to control with insecticides, on a valuable crop (cotton) provided the impetus to develop means of utilizing natural control by this pathogen in cotton IPM. The following is an account of the development and implementation of *N. fresenii* into cotton IPM in the USA.

B Pathogen biology

Upon discovery that a pathogen is responsible for a high level of natural control, the first

step is to conduct research on the biology and occurrence of the pathogen. Preliminary data on the effectiveness of the pathogen may help justify funding from various agencies. Funds are necessary for detailed studies. In this case, funding was initially obtained from the USDA-NRI and USDA Southern IPM competitive grants programs, from the Arkansas Agricultural Experiment Station, and from Cotton Incorporated (a cotton industry supported group). Cotton Incorporated has been instrumental in the success of this program by providing steady funding for 14 years.

Information on the pathogen's biology and interactions with environmental conditions are also needed to manipulate the pathogen, accurately diagnose infected hosts, sample for the pathogen, and develop methods to predict its occurrence. The entomopathogenic fungus, *N. fresenii*, infects many aphid species worldwide. It has been reported from Aphidinae, including at least 9 *Aphis* spp., and *Brevicoryne brassicae, Acyrthosiphum pisum, Myzus persicae, Schizaphis graminum, Rhopalosiphum padi*, and occasionally from certain species of Cinarinae and Chaitophorinae (Thoizon, 1970). This fungus infects only aphids and has no effect on humans, plants, or beneficial insects. Since 1991, many aspects of the biology, epizootiology, and aerobiology of this natural enemy of aphids have been elucidated (Steinkraus and Slaymaker, 1994; Steinkraus *et al*., 1995; Vingaard *et al*., 2003).

It is necessary to have viable pathogen material to study its biology. Unfortunately, *N. fresenii* has not yet been cultured *in vitro* in any practical way; therefore, methods were developed to culture the pathogen *in vivo* and store the fungus in desiccated, frozen, aphid mummies (Steinkraus *et al*., 1993). This provided a source of *N. fresenii* material that permitted determination of the average number of primary conidia produced per host cadaver (3,052 conidia/aphid) and where conidia were dispersed after discharge (77% into the air). It also permitted determination of the time from adherence of capilliconidia to a host aphid till death, and the effect of temperature on this process. The time from host contact with an infective capilliconidium to host death and fungal sporulation was as short as 3 days at 30 °C; one reason why *N. fresenii* produces epizootics quickly.

Further biological studies were made on the pathogen to determine the temporal pattern of sporulation, conidial discharge, and formation of capilliconidia, and the effects of relative humidity and temperature on these processes (Steinkraus and Slaymaker, 1994). These studies indicated that sporulation occurred rapidly within a 5 h period and that temperatures above 35 °C or relative humidities below 85% prevented sporulation. Most primary conidia (93%) germinated to form capilliconidia within 6 h at 25 °C and 100% RH.

This fungus has efficient mechanisms for infecting aphids on plants, and for dispersal within and between fields as airborne conidia. Aerial conidia of *N. fresenii* were collected during epizootics in commercial cotton fields in Arkansas using Rotorod and Burkard spore samplers (Steinkraus *et al*., 1996). Discharge of primary conidia showed a clear diel periodicity, with most conidia present in the air between 0100 and 0500 h. Therefore, primary conidia are dispersed through a cotton field when it is dark, relatively humid and cool, maximizing conidial longevity and survival. Forty-eight percent of sentinel *A. gossypii* exposed to air in a commercial cotton field in Louisiana during an epizootic became infected after 8 h exposure (Steinkraus *et al*., 1999). Exposure of sentinel aphids outside the cotton field, at 10 and 100 m downwind, resulted in 34.8% and 24.0% infected aphids, respectively. Aerial primary conidial densities reached 90,437/m^3 at 0015 h on 2 July 1995. The extremely high numbers of conidia in the air and the precise timing of their discharge, play an important role in the rapid development of epizootics in *A. gossypii* populations.

Based on several years of sampling aphid populations throughout the midsouth and southeast, it was determined that aphid populations generally begin a precipitous decline when the prevalence level reaches 15% (Steinkraus and Hollingsworth, 1994; Hollingsworth *et al*., 1995). Diagnostic procedures developed during these research projects made it possible to diagnose aphid fungus levels from individual fields and predict whether natural declines caused by the fungus will occur. This is practical information for consultants, growers, and extension agents making management

decisions. In 1993, an extension-based aphid fungus sampling service was developed to provide Arkansas growers with this information. In the late 1990s the service was expanded to include Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, and South Carolina. In 2006, the service completed its 14th year of operation.

C Diagnosis of diseased aphids

Preliminary work resulted in the development of methods for accurately diagnosing aphids for the pathogen. The cotton aphid is tiny and soft-bodied, making it possible to squash an entire aphid in lactophenol on a microscope slide (Steinkraus *et al.*, 1991). The slide is then scanned at 200x magnification with a phase microscope and the stages of *N. fresenii* are recorded. The following categories of infection status are readily observed: capilliconidia attached to the aphid, protoplasts, hyphal bodies or resting spores present within the aphid, and conidiophores and primary conidia present. This research and service has been greatly simplified by the fact that *A. gossypii* is generally the only aphid species regularly encountered in cotton, and *N. fresenii* is the most common pathogen regularly found infecting *A. gossypii* on cotton. The aphid pathogen, *P. neoaphidis*, is occasionally found in low levels in cotton aphids but is usually easily recognized and not involved in major epizootics in this system (Steinkraus, unpublished data). In systems, such as potato, which are attacked by multiple species of aphids that in turn are infected by many species of entomopathogenic fungi, sampling procedures will be more complicated (Shands *et al.*, 1972).

D Geographical and temporal prevalence

In 1992 and 1993, a study was made to determine how widespread *N. fresenii* was in the states of Arkansas, Louisiana, and Mississippi, when the epizootics occurred, and the speed with which the epizootics reduced aphid populations (Steinkraus *et al.*, 1995). This was done by collecting aphids in 32 (1992) and 35 commercial cotton fields (1993) along major north-south highways in the Mississippi flood plain in eastern Arkansas, western Mississippi, and northern Louisiana. Aphid-infested leaves were collected and stored in 70% ethanol. Randomly chosen subsamples of 30 aphids from each field were diagnosed for *N. fresenii*. This research showed that *N. fresenii* was widespread in Arkansas, Louisiana, and Mississippi fields. A similar study was conducted with cooperating entomologists in 10 cotton growing states in 1995 (Steinkraus *et al.*, 1996). Cooperators collected aphids from cotton fields throughout their states from a total of 47 counties. From each field, 50 randomly chosen aphids were diagnosed for *N. fresenii*. This study showed that *N. fresenii* was present in cotton fields in all 10 states, and in 66% of the samples received. These data indicated that epizootics occurred regularly over wide areas of the USA.

E Methods for predicting epizootics

Based on the understanding of the temporal and geographical occurrence of *N. fresenii* epizootics, prevalence was monitored in *A. gossypii* populations for 3 years to develop sampling strategies for predicting aphid population declines due to *N. fresenii* (Hollingsworth *et al.*, 1995). Regression analysis on average aphid densities per leaf and *N. fresenii* prevalence in 6 fields indicated that aphid populations began to decline when prevalence reached 15%, and declined to a low level 5–16 days later. Declines were more rapid in fields with higher aphid densities, and fungus-infected aphids could be detected 10 days before prevalences reached 15%. A sample of only 4–5 leaves was required to detect fungal-infected aphids when prevalence reached 4–5%.

F Sampling service to predict aphid epizootics

An important key to the success of this program has been developing links between research and cooperative extension personnel. In each state, a cotton extension entomologist or IPM specialist has the responsibility of selecting participants for the program and obtaining their addresses, and phone and FAX numbers. Based on the available laboratory resources of space, microscopes, and labor, and the need for participants in most cotton-growing counties in Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North

Carolina, and South Carolina, 20–30 participants per state were chosen. Participants have been county extension agents, private consultants, growers, or researchers who have responsibility for sampling cotton fields and making aphid management decisions. The number of participants varies each year based on how serious a pest cotton aphids were perceived to be the previous year.

In May, each participant is supplied with sampling kits, instructions on how to sample aphids from the fields, and data sheets. The kits consist of 30 ml vials containing 70% ethanol placed inside padded mailing tubes, and pre-addressed return envelopes. Participants sample aphids from their fields when aphids are perceived to be a problem, then mail them via a 2-day express mail service to our laboratory at the University of Arkansas for processing where laboratory technicians have been trained to mount and diagnose aphids for *N. fresenii*. Fifty aphids are randomly selected and diagnosed from each sample. Each aphid is examined for fungal infection at 200x magnification with a phase microscope. Results, expressed as percentage prevalence, are supplied to participants within 48 h by FAX or telephone. In addition, summaries of diagnostic results are faxed to state coordinators weekly for dissemination of the data within their respective states. In 1997, the service developed an Internet website (http://www.uark.edu/misc/aphid) containing all results. Results from each field are uploaded daily onto the Internet site, making them rapidly available to growers and other interested parties in the 8 state area. At the end of the 1997 season, participants were surveyed to determine the value of the service.

G *Results of the sampling service in 1997*

In 1997, 97 participants received kits and 64% of the participants sent samples to our laboratory. Samples were received from 54 counties: 13 in Arkansas, 11 in Louisiana, and 30 in Mississippi. A total of 469 samples were received; 162 from Arkansas, 109 from Louisiana, and 198 from Mississippi. A total of 13,880 ha were sampled; 3,835 in Arkansas, 3,117 in Louisiana, and 6,928 in Mississippi. Each sample took approximately 2 h to process. The estimated final cost per sample, including labor, slides, shipping charges, and kits, but excluding costs of phase and dissecting microscopes, was approximately $40.

No fungus was present in samples collected from any state in 1997 before 7 June. The first samples containing infected aphids were collected in Franklin parish, Louisiana, on 12 June (4%), Attala County, Mississippi, on 25 June (2%) and Chicot County, Arkansas, on 3 July (2%). The first samples containing 15% or more infected aphids were collected in Franklin parish, Louisiana on 23 June, in Leflore and Sunflower Counties, Mississippi, on 3 July, and in Ashley County, Arkansas, on 7 July. Infection levels of 15% are important because they are usually followed within a few days by aphid population declines. Therefore, the fungus provided natural control of cotton aphids in 1997 in early to mid-July. The timing of the epizootics has varied by 1–4 weeks in different years.

The first samples with infected aphids in Mississippi and Arkansas lagged behind those of Louisiana by ca. 2–3 weeks. This lag time in infection levels among states is indicative of a general south-to-north progression of the fungus which has been observed in previous years. Because epizootics occur first in Louisiana, it may be possible to use the sampling service to locate Louisiana fields in which there are ongoing early epizootics, then collect aphid-infested plants from these fields to inoculate northern Arkansas fields in order to initiate earlier aphid epizootics. This approach was attempted in 1999 with some success.

The range of infection levels and numbers of samples in Arkansas are shown in Figure 1. Daily variations in infection levels from field to field within the same county may be due in part to the effect of wind on conidial dispersal between fields. Sampling frequency and infection levels in Arkansas both peaked the week of 20 July. After this date, few samples were received because aphid populations had declined to negligible levels across the state due to *N. fresenii*. These findings demonstrate that *N. fresenii* usually is most prevalent in hot weather, unlike most fungal pathogens which infect aphids in cooler weather during the spring and fall.

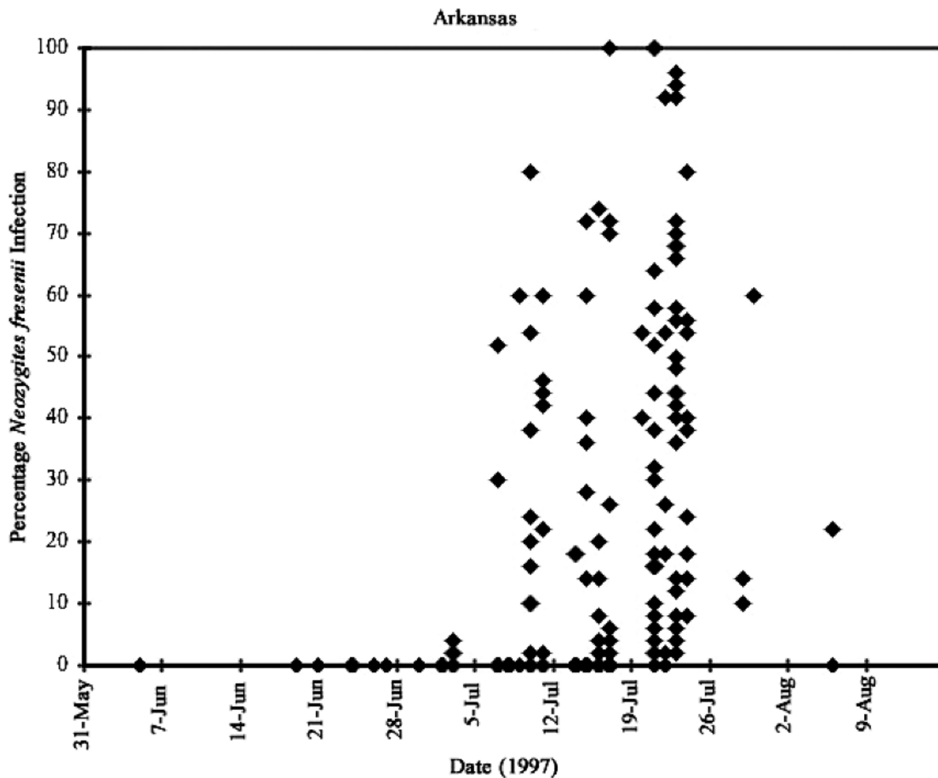

Figure 1. Percentage of aphids infected with *Neozygites fresenii* from Arkansas cotton aphid samples. Further graphs of results of the Cotton Aphid Fungus Sampling Service can be view at the internet website (http://www.uark.edu/misc/aphid)

Because of the time involved in collecting, shipping, and processing samples, it is crucial that samples be shipped in a timely manner in order for diagnostic results to be useful in management decisions, especially if an aphicide treatment is being considered. If there is no fungus present in a field being considered for an insecticide spray for aphids, then a treatment may be in order, though at the risk of reducing beneficial arthropods. On the other hand, if the fungus level in the field is 15% or greater, a treatment may be unnecessary because the aphid populations will decline naturally due to the fungus.

Seventy-one percent of the participants who sent samples in 1997 responded to a follow-up survey. Ninety percent said that the service saved money because they had avoided aphid-insecticide treatments when the fungus was present and 98% said that they used service data in making aphid management decisions. Many positive comments were received during the post-season survey on the service. Eight representative comments are listed below .

1. "*This program was very helpful and saved thousands of dollars in insecticide costs.*"
2. "*We had 5 growers prepared to spray ca. 2500 acres until the survey revealed that the fungus was present. At $7.50/acre, this was a significant savings.*"
3. "*Without it I would have made a follow-up aphid spray. It saved the farmer money.*"
4. "*Detection of fungal infestations before they are observed visually is extremely helpful.*"
5. "*Speed with which you identified percentage aphids having fungus was excellent.*"
6. "*Information gained from the service made decisions easier on many fields not sampled.*"
7. "*Gave the grower and myself and other consultants a way to define what was happening in the field instead of just wondering.*"
8. "*Participating helped my understanding of how/when the fungal disease works & how we can best fit reliance of this disease into our pest management program.*"

6 Summary

Naturally occurring pathogens of arthropod pests in agroecosystems in some cases may be providing important control of arthropod pests. Identification of such situations may permit the development of sampling procedures for determining or predicting the natural control provided. Research in this area can result in increased reliance on natural enemies in the IPM programs on crops, with concomitant reductions in pesticide usage. These are important goals of IPM. Natural control by pathogens is considerable and often underestimated. As Waage (1992) stated, *"It is tempting to see classical biological control as an unusual, one-off event, but this misses an important point: there is no fundamental difference between these successes with exotic natural enemies and the action of indigenous species in our local crops. The striking before and after picture of classical biological control simply isolates a process which is going on around us all the time, and largely undetected. What is revealed is an ecological phenomenon which is relevant to all pest management."*

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