Geographical and temporal dynamics of *Chaetocnema pulicaria* populations and their role in

Stewart's disease of corn in Iowa

by

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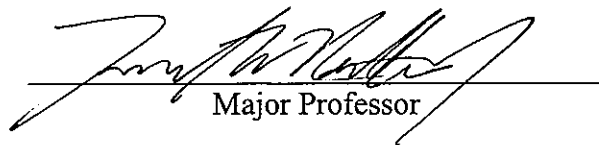
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TABLE OF CONTENTS

v

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CHAPTER 1

INTRODUCTION

Thesis Organization

This thesis is organized into five chapters. Chapter 1 is the introduction and justification, chapters 2 and 3 are journal papers, chapter 4 is a preliminary analysis of winter environmental variables and their use in forecasting for Stewart's disease of corn, and chapter 5 is general conclusions and discussion. References can be found at the end of each chapter, except chapter 5 and are specific to that chapter.

Literature Review

Importance Of Stewart's Disease Of Corn

Stewart's disease of corn, caused by the bacterium *Pantoea stewartii* (syn. *Erwinia stewartii*) has strong worldwide economic implications due to zero tolerance phytosanitary regulations. Pepper (1967) reported that the disease can be found throughout most of the United States and that the disease has a substantial worldwide distribution, including Central America, Europe, USSR, and China. Although Stewart's disease occurs sporadically from season-to-season and location-to-location in the United States, this disease has a tremendous negative impact on the worldwide trade of corn seed (*Zea mays*). The quarantines in place limit the ability of corn seed to be exported if even a single Stewart's disease infected plant is found in a field. Also important is the fact that early season infection of sweet corn by *P. stewartii* can significantly reduce yields in susceptible and moderately susceptible sweet corn hybrids (Pepper, 1967; Suparyono and Pataky, 1989; Pataky et al., 1990; Pataky et al., 1995).

In the 1930's, there was a dramatic increase in the severity of Stewart's disease on sweet corn in the Northeastern U.S., which was the impetus for Neil Stevens (New Haven, Connecticut) to develop a disease forecasting system to predict the severity of Stewart's disease in the Northeastern regions (Stevens, 1934; Elliot, 1935). Since seed corn inspections began in Iowa in 1972, the prevalence of Stewart's disease (the numbers of fields where Stewart's disease is observed divided by the total number of fields inspected X 100) has been quite sporadic. However, in the past five years, there has been an exponential increase in the prevalence of Stewart's disease in Iowa, including new records of 25% prevalence in 1998 and 58% prevalence in 1999 (Nutter et al., 1998; Esker and Nutter, 2000).

Seed Transmission

In his initial paper, Stewart (1897) argued the main form of dissemination *of P. stewartii* was through the bacterium clinging to seed, 'manured' farm implements, or the washing of soil. Dissemination of the pathogen via 'infected' seed was hypothesized based on controlled experiments in which other possible sources of inoculum for corn were supposedly eliminated. From these experiments, it was believed that the presence of diseased corn seedlings could only have been due to seed-to-seedling transmission. Moreover, Smith (1909) was successful in obtaining eight times as many cases of Stewart's disease with plants grown from suspicious seed than from disinfected (mercuric chloride water) seed (Smith, 1909). The incidence of Stewart's disease in the untreated plots was 10% compared to 2% for treated plots, but the effect of residual mercuric chloride on corn flea beetles was not considered.

Rand and Cash (1933), using controlled studies, isolated *P. stewartii* from the endosperm of seed. They also showed from greenhouse studies that seed-to-seedling

transmission efficiency ranged from 2 to 13%. Their studies were reportedly performed in the absence of any insect vectors. The results of these preliminary experiments concerning seed-to-seedling transmission led to the establishment of strict phytosanitary regulations to regulate the sale and exportation of seed corn among countries (Stewart, 1897; Smith, 1909; Rand and Cash, 1933; Frutchey, 1936). Using growout tests, Frutchey (1936) was able to show that *P. stewartii* survived in infested seed and that the bacterium could be isolated from infested seed. Frutchey's studies, however, indicated that transmission of *P. stewartii* to corn was almost zero when potential factors for transmission, such as insects, were absent from the growout studies.

With the development of an enzyme-linked immunosorbent assay (ELISA) to test for the presence/absence of Stewart's disease in corn seed (Lamka et al. 1991), the seed-to-seedling transmission of Stewart's disease was found not to be epidemiologically important (Block et al., 1998). In spite of these findings, zero tolerance phytosanitary regulations remain in place and continue to restrict seed corn producers and companies from exporting seed from fields where Stewart's disease has been found to occur. According to phytosanitary regulations, even a single infected plant in a field would render the seed corn (from that fall) unsaleable for overseas markets. If a country deems a corn seedlot to be unacceptable, the worst-case scenario would be that the infested corn seedlot could only be used domestically as field corn (animal feed), thus, greatly diminishing the value of the crop. Alternatively, a seed corn company could attempt to prove that the seed was not infected by paying for costly growout or ELISA tests to prove that a seedlot was free of Stewart's disease, thereby, allowing for its exportation (Block et al., 1999).

Causative Organism: *Pantoea stewartii*

The causative organism of Stewart's disease, *Pantoea stewartii,* is described morphologically as a short bacillus, usually in pairs, with rounded ends, sporeless, and with the ability to grow readily on nutrient agar, gelatin, or potato agar. According to F. C. Stewart (1897), *P. stewartii* was unable to produce any gases, indicating it was probably an aerobic organism. Smith (1914) later showed the bacterium had no preference for pairing, and had an average size of 1.0 μm in diameter and less than 4.0 μm in length. Moreover, he reported that the bacterium was motile, strictly aerobic, and that the bacterium was readily stained using anilin dyes. Pepper (1967) described *P. stewartii* as a non-motile, non-flagellated, non-spore forming, and capsule-forming gram-negative rod 0.4-0.8 μm by 0.9-2.2 μm. Pepper also showed the bacterium could range from being aerobic to facultatively anaerobic, depending on environmental conditions.

Nomenclature

Pepper (1967) described the long history of taxonomic classification of the causative organism of Stewart's disease, now referred to as *Pantoea stewartii.* In 1997, the one hundred-year anniversary of Stewart's disease was observed, as it was F. C. Stewart who published the first paper recognizing a bacterial wilt disease of corn in 1897. Stewart described the problem of early sweet corn varieties being subjected to a wilt where the fibro-vascular bundles of plants contained numerous bacilli, and therefore, the pathogen that caused the disease was probably bacterial. This new disease was observed in Long Island, New York, although there were reports of a similar disease elsewhere, including Iowa (Stewart, 1897). In his original paper, Stewart left the organism unnamed. *Pseudomonas stewartii* was the name given to the bacterium by Stewart in 1898 (Smith, 1909). In 1914,

the name was changed to *Bacterium stewarti*, which was subsequently changed to

Aplanobacter stewarti in 1918 on the basis that no flagella or motility was observed (Smith,

1914; McCulloch, 1918). In 1920, *Bacillus stewarti* was the new name given to the pathogen

and in 1923, it was changed to *Phytomonas stewarti* (Holland, 1920). In 1938 the name was

again changed, this time to *Xanthomonas stewarti*, and then to *Xanthomonas stewartii*

(Dowson, 1939, 1957). In 1963, *Erwinia stewartii* was the name given to the pathogen (Dye,

1962, 1963). Until recently, this was the name most commonly recognized, and even today,

many people continue to use this name. On the basis of an examination of electropherograms

of soluble proteins in 1993, the name was changed to *Pantoea stewartii* and many scientists

are now beginning to use this name (Mergaert et al., 1993). Therefore, for the rest of this

paper *Pantoea stewartii* will be the name used.

Insect Vector: *Chaetocnema pulicaria*

The corn flea beetle, *Chaetocnema pulicaria* Melsh, is in the Order Coleoptera,

Family Chrysomelidae. The corn flea beetle is believed to be the most important vector to

acquire and transmit *P. stewartii*. The corn flea beetle is native to the Western Hemisphere,

and more specifically, to the eastern-half of the United States (Metcalf et al., 1962; Dill,

1979). The corn flea beetle is a small (1.8-mm), shiny, black beetle with enlarged hind

femurs, allowing for its tremendous jumping ability.

The preferred host of the corn flea beetle is corn, but the insect has been recorded to

feed on a large variety of alternative host plants. Poos (1939, 1955) observed and recorded

corn flea beetle feeding a number of monocot species, including, *Dactylis glomerata* L.

(orchard grass), *Digitaria sanquinalis* (L.) Scrop. (crabgrass), *Pancium dichotomiflorum*

Michx. (fall manicum), *Agrostic alba* L. (redtop), *Panicum capillare* L. (witchgrass), *Poa*

pratenis L. (Kentucky bluegrass), *Sorghum vulgare* var. sudanese (Sudan grass), *Setaria lutescens* (Weigel) Hubb (yellow foxtail), *Setaria faberii* Herrm. (giant foxtail), *Triticum aestivum* L. (wheat), *Hordeum distichon* L. (barley), *Avena sativa* L. (oats), and *Phleum pratense* L. (Timothy).

The corn flea beetle survives the winter months as an adult. Poos (1955) showed that corn flea beetles overwinter at the base of grasses near cornfields. In studies in Indiana, Dill (1979) also observed the corn flea beetle overwintering in foxtails and *Setaria* spp. surrounding corn fields. When the adult corn flea beetles again become active in the spring, the females will begin to lay their eggs at the base of corn plants in the field (Poos, 1955; Dill, 1979). These eggs are yellowish-white and semi-translucent. On average, it takes six days for eggs to hatch. The larval stage of the corn flea beetle occurs almost exclusively in the soil and (on average) lasts approximately two weeks (Dill, 1979).

Injury by *C. pulicaria* can cause economic damage to corn by direct leaf injury due to feeding and by transmitting of the Stewart's disease bacterium. There is a general lack of information concerning the population dynamics of the corn flea beetle during the growing season, which will influence feeding injury, pathogen dissemination, and disease development. This lack of information negatively impacts on the implementation of management strategies (such as avoidance or protection), that could be used to reduce disease risk. Management tactics to support these strategies include the selection of low risk planting sites, altering the time of planting to avoid the overwintering corn flea beetle generation, and the use of insecticidal seed and foliar treatments to reduce corn flea beetle feeding.

Insect Vector Transmission

Rand (1923), and Rand and Cash (1924), were the first to investigate the role of the corn (brassy) flea beetle in the transmission of the bacterium. Rand (1923) documented the role of the corn flea beetle as a carrier to facilitate initial infection and secondary spread of the pathogen throughout the corn growing season. Experiments by Rand and Cash (1924) conclusively proved that soil was not a source of pathogen inoculum for seedling infection, and that the probability of successful seed-to-seedling transmission was also quite low (~2% from their experiments). They hypothesized that early season infection was probably due to an insect vector that had acquired and transmitted the causal bacterium. They also examined the role of insects other than the corn flea beetle, including the 12-spotted cucumber beetle (*Diabrotica duodecimpunctata* Fab.) and another flea beetle (*Chaetocnema denticulata* Ill.) (Rand and Cash, 1933). Results indicated that corn flea beetles transmitted the bacterium more efficiently than the 12-spotted cucumber beetle and the other flea beetle.

Further work by Elliot and Poos conclusively showed that the corn flea beetle was the most important insect vector of *P. stewartii*, in that *P. stewartii* was isolated from a higher percentage of corn flea beetles than any other insect (Poos and Elliot, 1936). Later, Elliot and Poos (1940) examined the overwintering behavior of *C. pulicaria*. They hypothesized that the proportion of insects carrying *P. stewartii* going into dormancy, was related to the proportion of insects that could serve as disease vectors in the spring. This hypothesis, however, was never tested and analyzed statistically.

Dill (1979) attempted to study the survival characteristics of corn flea beetles by placing 25 beetles into 10-cm X 30-cm diameter containers (metal, open-ended cylinder) at two sites in Indiana. These containers were placed in the soil in late fall, left out for the

winter, and examined the following spring for beetle survival. Only four of the original 250

corn flea beetles survived, and therefore, no statistical analysis was performed. Dill

hypothesized that the low number of survivors was probably due to an exceptionally harsh

winter, as evidenced by the extremely low corn flea beetle populations the following spring,

and was not due to poor experimental design.

While there has been a great deal of empirical research concerning the transmission

of *P. stewartii* via insect vectors, there is still much information missing. More definitive

evidence is necessary concerning the incidence (actual percentages) of corn flea beetles that

carry the bacterium with respect to time. Techniques such as ELISA that were used to

quantify seed-to-seedling transmission (Block et al., 1998) could be modified and used to

quantify the proportion of *P. stewartii*-infested corn flea beetles over the course of the

season. This would provide a quick and easy method to identify, quantify, and compare the

epidemiological importance of insect vector populations over time and space. Also, ELISA

could be used to monitor acquisition of the bacterium by corn flea beetles throughout the

growing season (temporal changes throughout the growing season).

Disease Symptoms and Losses

Symptoms of Stewart's disease often occur in two phases: the early season wilt phase

and the late leaf blight phase. There are numerous literature citations describing the

symptoms caused by Stewart's disease, including those by Stewart (1897), Smith (1914),

Rand and Cash (1933), Ivanoff (1933), Frutchey (1936), and Pepper (1967). During the wilt

phase, symptoms often begin at the site of corn flea beetle feeding scars. Linear watersoaked

lesions, followed by stunting and wilting, and direct plant loss can occur during this phase

when the disease is severe (Dill, 1979; Pepper, 1967). The bacterium may also invade the

vascular bundles of corn and it is by this aspect that the pathogen can be identified by the yellow, bacterial ooze that is easily observed when the vascular bundles are cut (Stewart, 1897; Rand and Cash, 1933; Pepper, 1967; Carlton and Munkvold, 1995).

The second phase of pathogenesis is known as the late leaf blight phase. Symptoms during this phase typically begin at the site of corn flea beetle feeding scars (Pepper, 1967; Dill, 1979). Once the bacterium has entered the corn leaf, the bacterium multiplies and causes a yellowish water-soaked lesion or streak that soon becomes necrotic. These streaks elongate and coalesce along the leaf veins of corn leaves and when symptoms are severe, entire plants can be blighted and killed. It is during the late leaf blight stage (usually in early to mid-August) when seed corn fields are inspected for foliar diseases of corn.

Disease Cycle

The disease cycle was first described by Pepper (1967), and is summarized in Figure 1. Stewart's disease is considered to be a polycyclic disease, since there are believed to be several infection cycles per season (Campbell and Madden, 1990; Agrios, 1997). The rate of disease increase is proportional both to the amount of overwintering initial inoculum and the rate that new inoculum is produced throughout the season as a result of secondary spread by two or more summer generations of corn flea beetles. However, the exact number of field generations that can occur in Iowa is not known.

Pantoea stewartii overwinters in the gut of dormant adult corn flea beetles which can be found beneath the soil in grassy areas surrounding corn fields (Poos, 1955; Pepper, 1967; Dill, 1979). Hybrid dent corn, inbred dent corn, and popcorn sweet corn are all susceptible to infection by the pathogen although sweet corn is the most susceptible and hybrid dent corn is the least susceptible (in general). Infection occurs after infested adult corn flea beetles feed

SUMMER
Symptoms expressed in
corn and acquistion of
Pantoea stewartii by corn
flea beetles

Number of
beetle
generations?

Secondary
infection of
corn plants

SPRING
Pantoea stewartii is
transmitted from corn
flea beetles to corn
seedlings

Stewart's Disease of Corn

FALL
Corn harvested
and corn flea
beetles migrate
from senescing
corn to grass

WINTER
Pantoea stewartii
overwinters in dormant
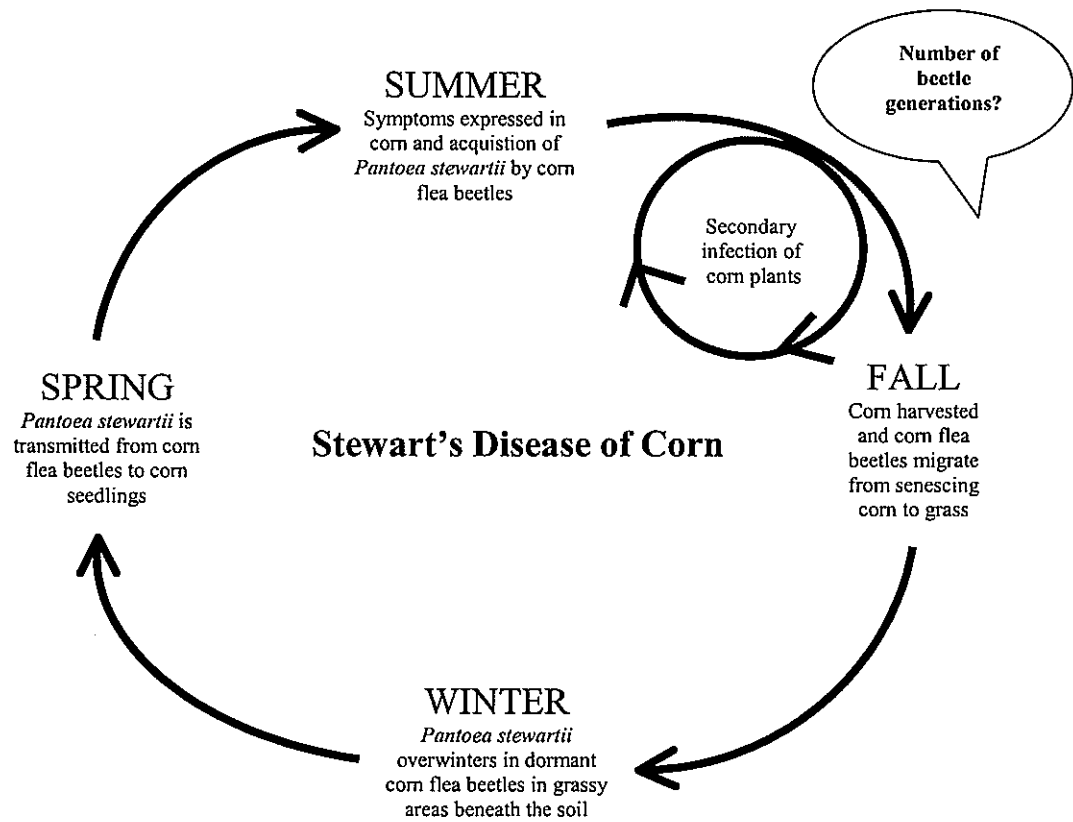corn flea beetles in grassy
areas beneath the soil

Figure 1. Disease cycle of Stewart's disease of corn.

on corn seedlings, thereby, transferring the bacterium to the young plants. Adult corn flea beetles subsequently lay their eggs around the base of the corn plant. It has been reported that newly hatched corn flea beetles are not infested with the bacterium, and therefore, the beetle larvae that hatch are free of the pathogen (Dill, 1979). As the new adults develop, they can acquire the bacterium from infected corn plants. Corn flea beetles that consume the bacterium are carriers of the bacterium for life (approximately 30 to 35 days) (Dill, 1979). As the growing season continues, secondary infection in corn can occur as new adult corn flea beetles emerge and acquire the bacterium by feeding on infected pants and transferring the bacterium to non-infected corn plants.

Rand and Cash (1933) observed that approximately 83% of corn plants became infected when 12 to 50 wilt-fed corn flea beetles were placed into insects cages with an uninfected corn plant. Elliot and Poos (1940) tested for the presence of *P. stewartii* in corn flea beeltes by crushing and plating the material onto agar plates. Approximately 20-30% of the 3800-5600 corn flea beetles tested were infested with the bacterium. Roberts (1955) estimated that 10 to 20% of corn flea beetle populations emerging from dormancy were infested with *P. stewartii*. However, during the midsummer months, the percentage of infested beetles was found to be as high as 75% (Roberts, 1955).

The ability to monitor the temporal changes in percentage of infested corn flea beetles is an extremely important piece of the epidemiological puzzle for this pathosystem. In 1991, an ELISA method was developed to test corn seed for the presence/absence of *P. stewartii*, and this method has been used to show that seed is a rare source of pathogen inoculum (Lamka et al., 1991; Block et al., 1998). Because the corn flea beetle serves as the main dispersal unit for *P. stewartii*, modifying and applying this ELISA method to quantify the

proportion of corn flea beetles that have the pathogen would provide a quick and efficient way to monitor the temporal changes in the proportion of *P. stewartii*-infested corn flea beetles.

Disease Forecasting

The potential risk of Stewart's disease has been predicted using disease forecasting since the early 1930's (Nutter et al., 1998; Boewe, 1948; Stevens, 1934). The application of disease forecasting to a pathosystem requires that the disease be important and sporadic, that the crop be economically important, and that management tactics are readily available to reduce the disease risk. The occurrence of Stewart's disease of corn satisfies all of these requirements.

The Stewart's disease forecasting system developed by Stevens is one of the oldest in existence (Cambell and Madden, 1990). It is a preplant warning system, whereby disease risk (severity) is predicted prior to planting on the basis of estimating the level of initial inoculum (Shrum, 1978). Stevens (1934) stated that two conditions must be met for Stewart's disease epidemics to develop. The first was the presence of a favorable environment for survival of the pathogen (in this case, the vector *Chaetocnema pulicaria* Melsh), and the second was that the environment be favorable for secondary disease development. For example, in 1932 and 1933, severe epidemics of Stewart's disease were observed in and around New Haven, Connecticut. The winter temperatures preceding these growing seasons were unusually warm compared to weather conditions that occurred in previous years, when epidemics of Stewart's disease did not occur. Based upon his empirical observations, Stevens (1934) hypothesized that if the sum of the mean monthly temperatures for December, January, and February were greater than 90°F or -3.3°C, there was a better

chance for corn flea beetle survival, and thus, a higher risk for Stewart's disease to develop during the ensuing growing season. In the two epidemic years of 1932 and 1933, the sum of the mean monthly temperatures was calculated to be greater than 100°F or 1.7°C. Based on these limited observations, Stevens (somewhat arbitrarily) established that the threshold for severe epidemics was >90°F (-3.3°C) and that if the sum of the mean monthly temperatures was less than this, no epidemic would be expected.

In 1949, Boewe (1949) made modifications to the Stevens' system from observations made in Illinois regarding the leaf blight (late summer) stage of Stewart's disease. In Boewe's system, there would be higher incidence (% of plants infected) and severity (% area of the leaf infected) of Stewart's disease when the sum of the mean monthly temperatures for December, January, and February, reached a lower index threshold compared to Stevens' system (Table 1). For example, Boewe's modifications indicated that there would be a moderate to severe leaf blight (Stewart's disease) epidemics when the winter temperature index threshold exceeded just 85°F or -6.1°C. Boewe predicted low disease when the index was between 80 and 85°F (-8.9 and -6.1°C), and that no epidemics would occur when the temperature threshold index was less than 80°F or -8.9°C.

Castor et al. (1975), using the Stevens-Boewe system, created a computer program to aid in the calculations needed for the Stevens-Boewe forecasting system. The program analyzed temperature data and gave a preseason forecast based on weather information obtained from a particular weather station or group of weather stations (county, or region for Pennsylvania). The use of the computer program greatly increased the speed at which forecasts could be made. It is also critical that information regarding disease risk be quickly and effectively communicated to corn producers (Chaube and Singh, 1991).

Table 1. The Stevens-Boewe disease forecasting system for Stewart's disease of corn

(Stevens, 1934; Boewe, 1948).

Index Value (°F and °C)	Stewart's Disease Prediction
100 or More (> 2.2 °C)	Severe
90 to 100 (-3.3 to 2.2 °C)	Severe
85 to 90 (-6.2 to -3.3 °C)	Moderate to Severe
80 to 85 (-8.8 to -6.2 °C)	Light to Moderate
Below 80 (< -8.8 °C)	Trace, at Most

The Iowa Seed Corn Industry is an economically important enterprise for the state. Seed corn inspections handled by the Iowa Department of Agriculture and Land Stewarship and are performed annually to look for foliar corn diseases in corn seed production fields. The main piece of information obtained from the inspections is prevalence, which is the number of fields where a foliar corn disease was found divided by the total number of fields inspected. Forecasting for prevalence of Stewart's disease is not what the Stevens-Boewe system was developed for. Because of this, the potential for economic impact due to Stewart's disease could be increased, as the Stevens-Boewe system may not accurately reflect the risk of prevalence of Stewart's disease.

In the early 1990's, research on modifying the Stevens-Boewe forecasting system to predict disease prevalence (as opposed to severity) began at Iowa State University (Nutter et al., 1998). Because the Stevens-Boewe warning system was not designed to predict the prevalence of Stewart's disease, it is not an appropriate tool for managing the disease in seed

production fields (Nutter et al., 1998). Based upon 26 years of weather and disease data, a new threshold of 24°F (-4.4°C) was proposed for the individual winter months rather than a 3-month index (Nutter et al., 2001; Nutter et al., 1998). Instead of calculating the sum of the average winter temperatures as was done by Stevens and Boewe, the Iowa State Method assigns a risk score based on the number of winter months in which mean monthly temperatures exceed 24°F (-4.4°C) (Table 2). If two or three months exceed this minimum temperature threshold, a moderate to severe risk of high Stewart's disease prevalence would be expected. A low risk would be expected if one month exceeded the temperature threshold, and essentially no risk if zero months were above the 24°F threshold. The Iowa State Method accurately predicted all eight high disease prevalence years (1972 to 1997) when the actual prevalence of Stewart's disease was approximately 9% or higher (Figure 2).

Table 2. The Iowa State Method for disease forecasting for Stewart's disease of corn (Nutter et al., 2001; Nutter et al., 1998).

Number of Months $\geq$ 24°F (-4.4 °C)	Predicted Risk
0	Negligible
1	Low
2	Moderate to High
3	High

Seed Treatment of Corn Seed

Pepper (1967) stated recommendations for use of seed treatment with insecticides and antibiotics were made without adequate knowledge concerning the presence or absence of the corn flea beetle populations. Rich (1956) was able to reduce the severity of wilt symptoms with seed soaks using 2,4,6-trichlorophenoxyacetic acid, Crag 1 herbicide, captan, terramycin, streptomycin, and HD-160 (sodium S-(2-benzothiazolyl)thioglycolate). The use of antibiotic seed treatments was done before substantial work showed that seed-to-seedling transmission of Stewart's disease was extremely low (Block et al., 1998). Natti (1958) found that while providing wilt control, many antibiotic seed treatments caused other injuries to corn seedlings.

While antibiotics did not provide benefits in terms of reducing seed-to-seedling transmission, insecticides applied to reduce corn flea beetle populations have had beneficial impacts on reducing initial disease levels. Munkvold et al. (1996) and Pataky et al. (2000), treated corn seeds with a systemic insecticide, imidacloprid (Gaucho), and observed reductions in corn flea beetle feeding and infection by *P. stewartii* when 3.0 and 6.0 g a.i./kg seed concentrations were used. Gaucho is a part of the new class of insecticides, called chloronicotinyls, and recently was approved for commercial use in corn. Gaucho (1-[(6-Chloro-3-pyridinyl methyl)]-N-nitro-2-imidazolidinimine) is now being recommended and used as a seed treatment to reduce the risk of the seedling wilt phase of Stewart's disease (Munkvold and Rice, 1998).

Foliar Insecticides

There has been little research to examine the efficacy of using foliar insecticides to reduce corn flea beetle populations. Inherently, there are some problems with using foliar

insecticides. These include the lack of quantitative cost-benefit information for establishing economic thresholds for insecticide applications to reduce disease incidence and severity. Also, there are potential health hazards associated with the mixing and application of insecticides as most insecticides are highly toxic to humans, animals, and the environment (Pepper, 1967; Pedigo, 1999). Experimentally, Ayers et al. (1979) were successful with in reducing the severity of Stewart's disease by applying carbofuran (2,3-dihydro-2,2-dimethyl-7-benzo-furanyl methylcarbamate) at a rate of 1.12 kg a.i./ha of in furrow during planting. In 1995, Hoffman et al. showed that foliar-applied insecticides were needed in New York when >6 beetles were found per 100 sweet corn plants. In Iowa, the recommended threshold for hybrid field corn is when 50% or more of corn plants have severe feeding injury, and there are five or more beetles per plant prior to the five leaf stage (V5) (Munkvold and Rice, 1998). For seed corn, the action threshold for insecticides is when 10% of plants have severe feeding injury and two or more beetles per plant are present on susceptible inbreds. A major problem with the injury thresholds is that "severe feeding injury" was not defined operationally.

Scouting for corn flea beetles can be difficult as these insects are 'sensitive' to human movement, and beetles can hop or jump away from a moving object at the slightest disruption of the corn canopy. This may result in underestimating of corn flea beetle populations and recommended action thresholds may not be triggered (Adams and Los, 1986). The use of sticky traps may help to quantify the actual seasonal and spatial distribution of corn flea beetle populations in and around corn fields since these traps rely only on landing rates of corn flea beetles and they are not affected by human or mechanical disruption.

In lieu of scouting or sticky traps, the use of degree-day (DD) models may help in alerting growers as to when corn flea beetle populations are likely to emerge, thus indicating when insecticide applications are needed to reduce disease risk. Dill (1979) indicated a developmental threshold of 16°C (60.8°F), which was used as the basis by Adams and Los (1986), to predict the emergence of the first summer generation of corn flea beetles, which occurred at approximately 150 DD (°C degree-day units) in Connecticut.

Other Control Methods

Insect Biocontrol Dill (1979) observed two potentially important biological control organisms associated with the corn flea beetle. One was a predatory Hymenopteran (parasitic wasp) that fed on the beetle and the second organism was a parasitic nematode. In laboratory studies, Dill found that the parasitic wasp larvae killed 36% of beetles that were exposed to it. The parasitic nematode was observed feeding on the beetle's ovaries, thereby reducing potential for reproduction.

In 1981, Woods et al. isolated a bacteriophage of *P. stewartii*. In their experiments, the phage was limited to 8 of 13 *Erwinia (Pantoea) stewartii* isolates tested. The authors suggested the use of the phage for control of the bacterium, but no further research on this has been conducted. McCammon et al. (1985) isolated avirulent mutants of *P. stewartii* using the bacteriophage Mu pf7701, however, little additional research has been conducted since this study. Other than these limited studies, there is no other published information concerning the use of biological control organisms to control the corn flea beetle or pathogen.

Resistance: Probably the best way to reduce potential risk of Stewart's disease causing economic harm is through the development and use of resistant hybrids and inbreds.

Typically, hybrid dent corn is more resistant than either inbred dent corn or sweet corn

varieties, with sweet corn varieties being the most susceptible. Sweet corn hybrids are bred

for other high value traits, such as taste and quality, and therefore, disease resistance is not

often emphasized in the breeding programs. The first *P. stewartii*-resistant hybrid was

Golden Cross Bantam, and this variety was found to outperform open-pollinated varieties

during the Stewart's disease epidemics of 1932 and 1933 (Smith, 1935).

More recently, Gardner and Wallin (1980) reported the presence of inbred specificity

to *Erwinia (Pantoea) stewartii* as a result of testing of 60 corn lines (58 inbreds). These

lines were selected from the *Maize Research and Breeders Manual*. Pataky et al. (1985,

1990, 1998) at the University of Illinois have extensively studied genetic resistance to

Stewart's disease. Their research has shown that early-maturing hybrids are more susceptible

to *P. stewartii* than mid- or late-maturing hybrids.

<u>Iowa Seed Corn Inspection Database</u>

Beginning in 1972, graduate students from the Department of Plant Pathology at Iowa

State University have been hired by the Iowa Department of Agriculture and Land

Stewardship to conduct inspections of seed corn fields in Iowa. These field inspections are

mandatory if seed corn is to be exported (Forrest W. Nutter, Jr., Personal Communication).

Since 1972, 500 to over 1300 seed corn fields per year have been inspected for Stewart's

disease (as well as for all other foliar corn diseases). The presence of Stewart's disease in a

seed corn field precludes the exportation of that seed from the United States to countries with

phytosanitary restrictions for this disease, unless fields where Stewart's disease has been

observed are then subsequently tested by seed corn companies using laboratory assays (i.e.

growout tests and/or ELISA) to substantiate that a seedlot is free of Stewart's disease.

Justification

Since 1995, there has been a dramatic, exponential increase in the prevalence

Stewart's disease of seed corn in Iowa (Figure 2). Prevalence, defined as the number of

fields where Stewart's disease has been observed divided by the total number of fields

inspected X 100, has increased from 13% in 1995 to 58% in 1999. The prevalence of

Stewart's disease reached a 28-year high of 58% (1317 Iowa fields inspected) in 1999.
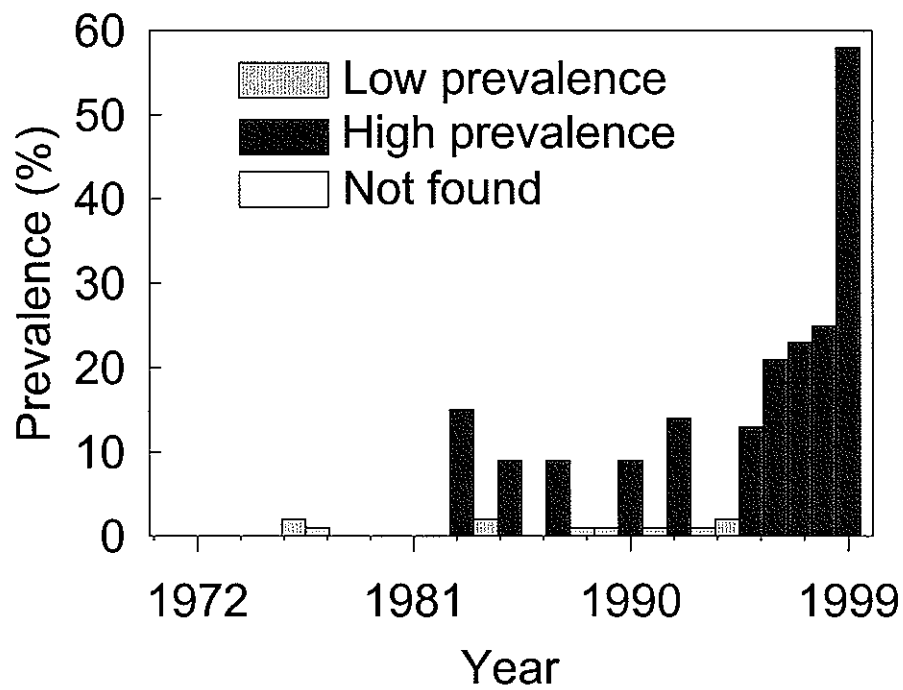

Figure 2. Prevalence of Stewart's disease of corn in Iowa, 1972-1999. Each year of data is

based on 500 to over 1,300 seed corn field inspections.

In order to better understand the Stewart's disease pathosystem, additional knowledge is needed regarding the role of the insect vector, *Chaetoconema pulicaria*, with regards in disease development. At present, there is little information regarding the spatial and temporal distribution of corn flea beetles in corn fields. Heichel et al. (1977) described "two cycles" of corn flea beetle populations (life cycles) during a growing season. Hoffman et al. (1995) evaluated the within- and between-plant distributions of corn flea beetles in small field plots in New York. Adams and Los (1986) used yellow sticky traps to monitor corn flea beetle populations and found that traps at a height of 0.6 m trapped significantly higher numbers of corn flea beetles than traps placed at 1.2 and 1.8 m, respectively. They also observed that yellow sticky traps captured significantly higher numbers of corn flea beetles than traps of other colors. Much of the information from the Adams and Los (1986) research focused on the late season monitoring of corn flea beetle populations and more quantitative information is needed regarding population dynamics of the corn flea beetle throughout the growing season. Critical information is particularly lacking concerning corn flea beetle population dynamics early in the growing season, when the seedling wilt phase of Stewart's disease occurs. Quantitative information concerning the corn flea beetle populations dynamics during the season is needed in order to develop degree-day models that may improve the timing of within-season applications of foliar insecticides. In addition to information regarding the spatial and temporal distribution of corn flea beetles, we need a better understanding of the importance of initial inoculum (proportion of *P. stewartii*-infested corn flea beetles) present before, during, and after the corn growing season. This will provide quantitative information concerning an important risk factor that should help to improve the management of Stewart's disease of corn.

An ELISA procedure developed by Lamka et al. (1991) to test for the presence or absence of *P. stewartii* in corn seed could be modified and used to test for *P. stewartii* in individual corn flea beetles. Such an approach could greatly increase our knowledge of inoculum level dynamics.

The Stevens-Boewe forecasting system is not useful for predicting the prevalence of Stewart's disease in seed production fields in Iowa. The Iowa State Method (developed by Forrest W. Nutter Jr.) has more accurately predicted the prevalence of Stewart's disease in seed production fields and has been more reliable in predicting the seasonal and geographic (county-level) risk for this disease (Nutter et al., 1998). Like the Stevens-Boewe system, the Iowa State Method relies solely on air temperature data and this may not be the best variable to predict the prevalence of Stewart's disease of corn. In addition to the disease risk models based upon the air temperatures, other environmental variables that could affect the survival of the corn flea beetle (such as soil temperature, snowfall, and duration of snowcover) may potentially be better variables to predict the occurrence of Stewart's disease in Iowa, and additional research is needed in this area.

To address the lack of knowledge regarding the Stewart's disease pathosystem, it is necessary to study corn flea beetle populations with regards to quantifying changes in corn flea beetle populations with respect to time, as well as quantifying changes in the percentage of corn beetles that are found to carry the bacterium. It is also necessary to begin to examine the epidemiological importance of other environmental variables that may provide a better means to predict corn flea beetle survival and development, and therefore, Stewart's disease of corn. Therefore, the objectives of this study are to:

1.   Obtain spatial and temporal information regarding the population dynamics of *Chaetocnema pulicaria* using yellow sticky cards and sweep nets

2.   Obtain temporal information regarding the percentage of *Chaetocnema pulicaria* infested with *Pantoea stewartii* using enzyme-linked immunosorbent assay (ELISA)

3.   Begin preliminary analyses of winter environmental weather variables in addition to air temperature to work on developing a more refined forecasting system for Stewart's disease of corn

References

Adams , R.G. and Los, L. M. 1986. Monitoring adult corn flea beetles (Coleoptera: Chrysomelidae) in sweet corn fields with color sticky traps. Environ. Entomol. 15: 867-873.

Agrios, G. N. 1997. Plant Pathology, 4th Ed. Academic Press, San Diego.

Ayers, J.E., MacNab, A.A., Tetrault, R.C., and Yocum, J.O. 1979. The influence of selected insecticides on yield and the incidence of Stewart's disease in sweet corn. Plant Dis. Reptr. 63: 634-638.

Block, C.C., Hill, J.H., and McGee, D.C. 1999. Relationship between late-season severity of Stewart's bacterial wilt and seed infection in maize. Plant Dis. 83: 527-530.

Block, C.C., Hill, J.H., and McGee, D.C. 1998. Seed transmission of *Pantoea stewartii* in field and sweet corn. Plant Dis. 82: 775-780.

Boewe, G.H. 1949. Late season incidence of Stewart's disease on sweet corn and winter temperatures in Illinois, 1994-1948. Plant Dis. Reptr. 33: 192-194.

Campbell, C.L. and Madden, L.V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.

Carlton, W.M. and Munkvold, G.P. 1995. Corn Stewart's Disease. Iowa State University, Extension Bulletin. Ames, IA. Pm-1627.

Castor, L.L., Ayers, J.E., MacNab, A.A., and Krause, R.A. 1975. Computerized forecasting system for Stewart's bacterial disease on corn. Plant Dis. Reptr. 59: 533-536.

Chaube, H.S. and Singh, U.S. 1991. Plant Disease Management: Principles and Practices. CRC Press, Boca Raton, FL.

Dill, J.F. 1979. Biology and Management of the Corn Flea Beelte, *Chaetocnema pulicaria* Melshheimer, Relative to the Incidence of Stewart's Disease in Corn. Ph.D. Thesis, Purdue University, West Lafeyette, IN. 167 Pages.

Dowson, W.J. 1957. Plant Diseases Due to Bacteria. Cambridge University Press, London.

Dowson, W.J. 1939. On the systematic position and generic names of the gram negative bacertial plant pathogens. Zent. Bakt. II. 100: 17193.

Dye, D.W. 1963. A taxonomic study of the genus *Erwinia*. New Zealand J. Science 12: 223-236.

Dye, D. W. 1962. The inadequacy of the usual determinative tests for the identification of *Xanthomonas* spp. New Zealand J. Science 5: 393-416.

Elliot, C. 1935. Dissemination of bacterial wilt of corn. Iowa State College J. Science 9: 461-480.

Elliot, C., and Poos, F.W. 1940. Seasonal development, insect vectors, and host range of bacterial wilt of sweet corn. J. Agric. Res. 60: 645-686.

Esker, P.D. and Nutter, F.W., Jr. 2000. Monitoring the dynamics of corn flea beetles infested with *Pantoea stewartii* by ELISA. Phytopathology 90:S22.

Frutchey, C.W. 1936. A study of Stewart's disease of sweet corn caused by *Phytomonas stewarti*. Mich. Agric. Exp. Stn. Tech. Bull. 152: 2-25.

Gardner, C.A.C., and Wallin, J.R. 1980. Response of selected maize inbreds to *Erwinia stewartii* and *E. zeae*. Plant Dis. 64: 168-169.

Heichel, G. H., Sands, D. C., and Kring, J. B. 1977. Seasonal patterns and reduction by carbofuran of Stewart's bacterial wilt of sweet corn. Plant Dis. Reptr. 61: 149-153.

Hoffman, M.P., Kirkwyland, J.J., and Davis, P.M. 1995. Spatial distribution of adult corn flea beetles, *Chaetocnema pulicaria* (Coleoptera: Chyrsomelidae), in sweet corn and development of a sampling plan. J. Econ. Entomol. 8: 1324-1331.

Holland, D. F. 1920. Generic index of the commoner forms of bacteria. J. Bacteriol. 5: 191-229.

Ivanoff, S.S. 1993. Stewart's wilt disease of corn, with emphasis on the life history of *Phytomonas stewarti* in relation to pathogenesis. J. Agric. Res. 47: 749-770.

Lamka, G.L., Hill, J.H., McGee, D.C., and Braun, E.J. 1991. Development of an immunosorbent assay for seedborne *Erwinia stewartii* in corn seeds. Phytopathology 81: 839-846.

Mergaert, J., Verdonck, L., and Kersters, K. 1993. Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. indologenes subsp. nov. Int. J. Syst. Bacteriol. 43: 162-173.

McCammon, S.L., Coplin, D.L., and Rowan, R.G. 1985. Isolation of avirulent mutants of

Erwinia stewartii using bacteriophage Mu Pf7701. J. Gen. Micro. 131: 2993-3000.

McCulloch, L. 1918. A morphological and cultural note on the organism causing Stewart's

disease of sweet corn. Phytopathology 8: 440-442.

Metcalf, C. L., Flint, W.P., and Metcalf, R.L. 1962. Destructive and Useful Insects, Their

Habits and Control, 3rd Ed. McGraw-Hill Book Co., New York.

Munkvold, G.P. and Rice, M.E. 1998. Plant disease risk of Stewart's disease high in 1998.

Integrated Crop Management, Iowa State University, University Extension. Ames,

IA. IC-480(5).

Munkvold, G. P., McGee, D.C., and Iles, A. 1996. Effects of imidacloprid seed treatment

of corn on foliar feeding and *Erwinia stewartii* transmission by the corn flea beetle.

Plant Dis. 80: 747-749.

Natti, J.J. 1958. Effect of seed treatments with antibiotics on bacterial wilt of corn. Plant

Dis. Reptr. 42: 953-958.

Nutter, F.W., Jr. Personal Communication. Department of Plant Pathology. Iowa State

University. Ames, IA.

Nutter, F.W., Jr., Rubsam, R.R., Taylor, S.E., Harri, J.A., and Esker, P.D. 2001.

Geospatially referenced disease and weather data to improve site-specific forecasts

for Stewart's disease of corn in the U.S. corn belt. Computers and Electronics in

Agriculture (In press).

Nutter, F.W., Jr., Wegulo, S.N., Rubsam, R.R., Taylor, S.E., and Harri, J.A. 1998. Use

of geospatially-referenced disease and weather data to improve site-specific forecasts

for Stewart's disease of corn. Phytopathology 88: S68.

Pataky, J.K., du Toit, L.J., Freeman, N.D. 2000. Stewart's wilt reduction of an international collection *Zea mays* germ plasm inoculated with *Erwinia stewartii*. Plant Dis. 84: 901-906.

Pataky, J.K., du Toit, L.J., Revilla, P., and Tracy, W.F. 1998. Reactions of open-pollinated sweet corn cultivars to Stewart's wilt, common rust, northern leaf blight, and southern leaf blight. Plant Dis. 82: 939-944.

Pataky, J.K., du Toit, L.J., Kunkel, T.E., and Schmitt, R.A. 1995. Severe Stewart's wilt in central Illinois on sweet corn hybrids moderately resistant to *Erwinia stewartii*. Plant Dis. 79:104.

Pataky, J.K., Suprayono, Hawk, J.A., Gardiner, M.L., and Pauly, M.H. 1990. Associations between Stewart's wilt ratings and maturity of sweet corn hybrids. Plant Dis. 74: 792-796.

Pataky, J.K. 1985. Relationships among reactions of sweet corn hybrids to Goss' wilt, Stewart's bacterial wilt, and northern corn leaf blight. Plant Dis. 69: 845-848.

Pedigo, L.P. 1999. Entomology and Pest Management. 3rd Ed. Prentice Hall, Upper Saddle River, NJ.

Pepper, E.H. 1967. Stewart's Bacterial Wilt of Corn, 4th Ed. Heffernan Press, Worchester, MA.

Poos, F.W. 1955. Studies of certain species of *Chaetocnema*. J. Econ Entomol. 48: 555-563.

Poos, F.W. 1939. Host plants harboring *Aplanobacter stewarti* without showing external symptoms after inoculation by *Chaetocnema pulicaria*. J. Econ. Entomol. 32: 881-882.

Poos, F.W., and Elliot, C. 1936. Certain insect vectors of *Aplanobacter stewarti*. J. Agric. Res. 52: 585-608.

Rand, F.V. 1923. Bacterial wilt or Stewart's disease of corn. The Canner 56:164-166.

Rand, F.V., and Cash, L.C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362: 1-22.

Rand, F.V., and Cash, L.C. 1924. Further evidence of insect dissemination of bacterial wilt of corn. Science 59: 67-69.

Rich, S. 1956. Seed treatments to protect corn seedlings against Stewart's wilt. Plant Dis. Reptr. 40: 417-420.

Roberts, A.L. 1955. Bacterial wilt and Stewart's leaf blight of corn. U.S. Department of Agriculture Farmers' Bulletin. 2092:13.

Shrum, R.D. 1978. Forecasting of epidemics. Pages 223-238 in: Plant Disease an Advanced Treatise Volume II, How Disease Develops in Populations. J.G. Horsfall and E.B. Cowling eds. Academic Press, Inc., New York.

Smith, E.F. 1914. Stewart's disease of sweet corn (maize). Pages 89-147 in: Bacteria in Relation to Plant Diseases. Carnegie Institute of Washington, Washington, D.C.

Smith, E.F. 1909. Seed corn as a means of dissemination of bacterium. Science 30: 223-224.

Smith, G.M. 1935. Golden Cross Bantam sweet corn: its development and distribution. Canning Age 16:171-173.

Stevens, N.E. 1934. Stewart's disease in relation to winter temperatures. Plant Dis. Reptr. 18: 141-149.

Stewart, F.C. 1897. A Bacterial Disease of Sweet Corn. N.Y. Agric. Exp. Stn. Bull. 130: 422-439.

Suparyono and Pataky J.K. 1989. Influence of host resistance and growth stage at the time

of inoculation on Stewart's wilt and Goss's wilt development and sweet corn hybrid yield. Plant Dis. 73: 339-345.

Woods, T.L., Israel, H.W., and Sherf, A.F. 1981. Isolation and partial characterization of a bacteriophage of *Erwinia stewartii* from the corn flea beetle, *Chaetocnema pulicaria*. Protection Ecology 3: 229-236.

CHAPTER 2

THE TEMPORAL DISTRIBUTION OF *CHAETOCNEMA PULICARIA*

(COLEOPTERA: CHRYSOMELIDAE) POPULATIONS IN IOWA

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Abstract

Knowledge is needed to improve management recommendations for reducing
Stewart's disease of corn, caused by the bacterium *Pantoea stewartii*, and the vector,
Chaetocnema pulicaria Melsh. In 1999 and 2000, yellow sticky cards and sweep netting
were used to document the occurrence of an overwintering, first, and second generation of *C.
pulicaria* in Iowa. The overwintering adult generation emerged as early as the middle of
April and continued until early June in both years, with populations as high as 45 ± 7.9 *C.
pulicaria* per ten sweeps (mean ± standard error). The first generation of *C. pulicaria* was
observed between the third week in June through the end of July, with populations ranging
from 0.10 ± 0.10 to 16.70 ± 1.42 *C. pulicaria* per 10 sweeps in corn fields. The second
generation of *C. pulicaria* was observed between the first week of August through late
September and for some locations, into early October, with populations ranging from $0.02 \pm
0.04$ to 27.80 ± 2.76 *C. pulicaria* per 10 sweeps. Orthogonal contrasts indicated that, at the
end of the growing season, there were greater numbers of *C. pulicaria* on yellow sticky cards
located along the grass borders compared to yellow sticky cards located within corn fields (F-
values: 6.04 to 73.50, p-values: 0.02 to < 0.0001), indicating the movement of *C. pulicaria*
from the corn field back into the grasses at the end of the growing season. This new

quantitative information will be useful for developing management recommendations for both *C. pulicaria* and Stewart's disease of corn. For example, time of planting could be altered to avoid the overwintering generation of *C. pulicaria*. In addition, knowledge concerning the seasonalities of the first and second generation of *C. pulicaria* during the corn growing season can be used to recommend the optimal timing for foliar-applied insecticide applications to control these generations.

Introduction

Chaetocnema pulicaria Melsh (Coleoptera: Chrysomelidae), is an economically important pest in the U.S. Corn Belt (*Zea mays*) (Dill, 1979). *Chaetocnema pulicaria* can damage the corn plant directly or indirectly. Although this pest can cause severe damage by directly feeding on corn leaves, it is also economically important because of its role in transmitting the bacterium, *Pantoea stewartii*, which causes Stewart's disease of corn (Rand and Cash, 1933; Elliot and Poos, 1940; Pepper, 1967). If population densities of *C. pulicaria* are sufficiently high early in the growing season, plant death due to extensive feeding damage is possible (Dill, 1979). However, the primary epidemiological importance of *C. pulicaria* feeding is because of the risk of transmission of *P. stewartii* from *C. pulicaria* to corn (Pepper, 1967).

Chaetocnema pulicaria feed on corn leaves throughout the growing season; in addition, they feed on numerous other grass species (Poos, 1955; Dill, 1979). Stewart's disease of corn is globally important since there are zero-tolerance, phytosanitary regulations in place that restrict the import of seed corn from the United States into other countries. These regulations can go into effect even if a single plant with Stewart's disease is found in a seed corn field. This is especially important considering that Stewart's disease was found in

25% of Iowa production fields in 1998 and in 58% of the fields in both 1999 and 2000

(Nutter et al., 1998; Esker and Nutter, 2000). Significant yield losses due to Stewart's

disease have also been observed in sweet corn in Illinois (Pataky, 1985; Suparyono and

Pataky, 1989).

Symptoms of Stewart's disease often occur in two phases, the early-season wilt phase

and the late leaf blight phase (Pepper, 1967). Symptoms of the wilt phase include elongated,

linear, water-soaked lesions, stunting, wilting, and plant death (Smith, 1914; Rand and Cash,

1933; Pepper, 1967; Dill, 1979). In Iowa, the late leaf blight phase of Stewart's disease is

typically observed in August, which coincides with the time that phytosanitary seed corn

inspections are performed in Iowa. Pathogenesis during this period is associated with *C.*

pulicaria feeding scars (Pepper, 1967; Dill, 1979; Carlton and Munkvold, 1995). Once the

bacterium has been introduced into the corn leaf by *C. pulicaria*, the pathogen multiplies and

a yellowish, watersoaked lesion or streak occurs that is followed by necrosis. These streaks

elongate and coalesce along the veins of the corn leaf, and if the disease is severe, entire

leaves (and plants) may be blighted blighted and killed (Pepper, 1967).

Insecticide seed treatments to reduce *C. pulicaria* populations have been investigated

(Munkvold et al., 1995; Pataky et al., 2000). The systemic insecticide imidacloprid

(Gaucho®, Gustafson Inc., Dallas, TX), which has been approved for commercial use in corn

fields, is a chloronicotinyl insecticidal treatment that prevent *C. pulicaria* populations from

feeding on corn leaves early in the growing season. Foliar insecticide applications can also

help to prevent *C. pulicaria* feeding throughout the corn growing season, although most

recommendations for spraying are made prior to the fifth leaf stage (V5) of corn (Carlton and

Munkvold, 1995). No recommendations are currently available for reducing *C. pulicaria*

populations mid to late in the growing season as a means to reduce the spread of *P. stewartii* because information on the population dynamics of *C. pulicaria* is lacking. This information would be extremely useful in reducing the late season blight phase of Stewart's disease.

In general, biological information concerning the population dynamics of *C. pulicaria* is limited (Heichel et al., 1977; Adams and Los, 1986). In Connecticut, Adams and Los (1986) studied changes in *C. pulicaria* populations using yellow sticky traps and observed a first summer generation of *C. pulicaria* that occurred about the first of August and a 'potential' second generation of *C. pulicaria* that occurred in September. They did not document an overwintering generation of *C. pulicaria*. Previously, Heichel et al. (1977) observed "two cycles" (two generations) of beetle activity that resulted in the transmission of *P. stewartii* in sweet corn in Connecticut. To date, the seasonality and number of *C. pulicaria* generations in Iowa have not been elucidated, and this is an obstacle to effective control recommendations. This information is necessary to make more timely recommendations regarding applications of foliar insecticides, both early and late in the corn growing season. The number of *C. pulicaria* generations needs to be clearly defined to better understand which generations are economically important and to establish economic action thresholds to improve disease management tactics. To elucidate this missing information, the objective of this research was to characterize the geographic and temporal distribution of *C. pulicaria* populations in Iowa using yellow sticky cards and sweep netting.

Materials and Methods

Yellow Sticky Card Sampling

The geographic and temporal patterns of *Chaetocnema pulicaria* populations in Iowa were monitored using yellow Sticky Strip™ insect cards (16 cm by 16 cm) (Olson Products,

Medina, OH) on traps at a height of 0.6 m (2 ft) (Adams and Los, 1986). Traps for holding

sticky cards were constructed using a modified design described by Adams and Los (1986).

Sticky cards were attached to 16 by 16 by 1.3 cm pieces of plywood placed on stakes 0.9 m

above the soil surface.

To quantify the spatial and temporal dynamics of *C. pulicaria* populations, transects

(traps placed at a uniform distance into a corn field) consisting of 30 traps (3 replications

with 10 traps per replication) were placed at the Iowa State University (ISU) Been Farm in

Ames, IA (1999), the ISU Hinds Farm in Ames, IA (2000), the ISU Northwest Research

Farm in Sutherland, IA (1999 and 2000), and the ISU Southeast Research Farm in

Crawfordsville, IA (1999 and 2000). Collectively, these locations form a diagonal across the

state of Iowa from the northwestern corner (Sutherland) to the southeastern corner

(Crawfordsville). The selection criteria for field locations for the traps was based on size, as

each field needed to be greater than 46 m (150 ft) in length (width of the field was variable at

each location), and each field was required to be bordered on one side by grass, and on the

other by soybeans.

Traps were placed in transects into the field after corn had been planted. In 1999,

traps were placed in the field on day of year (DOY) 126 (06-May) in Sutherland, DOY 138

(18-May) in Crawfordsville, and DOY 162 (11-June) in Ames. In 2000, traps were placed in

the field on DOY 132 (11-May) in Sutherland, DOY 137 (16-May) in Crawfordsville, and

DOY 141 (20-May) in Ames. Three transects or replications consisting of five traps

originating from the grass border and from the soybean border into the corn field were

established at each location. Traps were placed along the transects at near-equidistant

intervals (0 m, 3 m, 6 m, 9 m, and 18 m) from both grass and soybean borders. Sticky cards

were changed every 7 to 10 days and the number of *C. pulicaria* per card were counted on each trap before securing a new 16 X 16 cm yellow sticky card onto each trap. On occasion, traps containing the yellow sticky cards had to be removed from the field for minimal time periods (< 1 day) to allow for general field maintenance practices (i.e. pesticide applications, tillage etc.). Traps were maintained in the field as close to harvest time as possible, or until late in the fall when the number of *C. pulicaria* per card was less than one for two consecutive sampling periods. In 1999, traps were removed on DOY 262 (19-September) at Sutherland and DOY 266 (23-September) at Crawfordsville. The traps at the Ames location were removed on DOY 252 (09-September) as this field was harvested for silage. In 2000, traps were removed on DOY 262 (18-September) at both Ames and Crawfordsville and on DOY 278 (4-October) at Sutherland.

Sweep Net Samples

Sweep netting was performed as an additional method to quantify *C. pulicaria* populations in Iowa in 1999 and 2000. The number of *C. pulicaria* over time was monitored at each of six locations during the 1999 and 2000 growing seasons using a 38.1 cm diameter (15 in) super heavy-duty sweep net (Gempler's, Belleville, WI). Sweep netting was performed at the ISU Curtiss and Been Farm in Ames, IA (1999), the ISU Bruner Research Farm in Ames, IA (2000), the ISU McNay Research Farm in Chariton, IA (1999 and 2000), the ISU Northeast Research Farm in Nashua, IA (1999 and 2000), the ISU Northern Research Farm in Kanawha, IA (1999 and 2000), the ISU Northwest Research Farm in Sutherland, IA (1999 and 2000), and the ISU Southeast Research Farm in Crawfordsville, IA (1999 and 2000).

Prior to growth stage V5, when sweep samples could be safely taken in corn fields without adversely affecting (injuring) corn plants, sweep netting was performed only along the grass borders of each corn field. The sweep netting began in mid-to late April along the corn field edges. After the V5 stage was reached, sweeping netting was performed within corn fields. The fields sampled were the same as those that also had yellow sticky cards (Ames, Crawfordsville, and Sutherland). Samples were collected from each corn field well after corn had senesced and continued until the number of *C. pulicaria* were close to zero on two consecutive dates (late October into early November). Ten replications (samples) were obtained at each location per sampling date. The areas within the field for *C. pulicaria* sweep netting were arbitrarily selected. A single replication was obtained by swinging the sweep net 10 times over an approximately 6 m (20 feet) linear transect. *Chaetocnema pulicaria* were collected and placed into 16.8 X 14.9 cm (6 5/8 X 5 7/8 in) zip-lock freezer bags. Following collection, samples were transported to the Epidemiology Laboratory at Iowa State University, and placed into a -17.8°C (0°F) freezer for approximately 24 hours. Quantification of corn flea beetle populations was done by dispensing the contents from the freezer bags onto a flat surface. *Chaetocnema pulicaria* were then separated from other insects and plant debris, and the number of *C. pulicaria* per bag was determined.

Based on observations made in 1999 concerning *C. pulicaria* populations in corn versus grass borders, an additional set of ten replications per sampling period were collected from the grass borders of corn plots in 2000, beginning (approximately) in the middle of July (during the 1st generation of *C. pulicaria*) at all six locations. This was done to examine and compare corn flea beetle populations in corn versus grass borders from mid-season onwards. Samples were collected in a manner identical to both the early season grass collection and

within-season corn field collections. Samples were collected from the grass borders until *C. pulicaria* were no longer found (usually after several hard freezes).

Data Analysis

The mean number of *C. pulicaria* per yellow sticky card and also per 10 sweeps (one replication) using sweep netting was determined for each sampling date. *Chaetocnema pulicaria* populations (means ± SE) were plotted with respect to time (day of year) to determine both the number and the seasonal patterns of *C. pulicaria* generations in Iowa. These data were also used to determine relative population levels among locations as well as when population peaks occurred for each generation and location.

Orthogonal contrasts were performed to compare the border yellow sticky cards (grass and non-grass) with all other yellow sticky cards in transects, the grass border (0 m) cards with all other cards (including non-grass border), and the grass border (0 m) cards with the non-grass border (0 m) cards in order to determine if *C. pulicaria* preferred corn or grass borders.

Results

Overwintering, first, and second generation of *C. pulicaria* were observed at Ames, Crawfordsville, and Sutherland using yellow sticky cards in 1999 and 2000 (Figure 1). The end of the overwintering generation was observed at Crawfordsville in 1999 (Figure 1). The first generation was first detected on DOY 193 (12-July) at Ames, DOY 181 (30-June) at Crawfordsville, and DOY 195 (14-July) at Sutherland. Peak numbers of *C. pulicaria* per card were observed on DOY 193 (12-July) at Crawfordsville (7.13 ± 0.41). There was no clearly defined peak at Ames or Sutherland, although at Ames, the highest observed number of *C. pulicaria* per card in the first generation was 2.90 ± 0.83 on DOY 214 (02-August).

Second generation *C. pulicaria* were first observed on DOY 214 (02-August) at

Crawfordsville and DOY 221 (09-August) at Ames. Because the number of *C. pulicaria* was

low at Sutherland, no differentiation of the generations could be observed. The peak number

of *C. pulicaria* in the second generation occurred on DOY 231 (19-August) at Crawfordsville

(14.50 ± 1.63), DOY 245 (02-September) at Ames (11.40 ± 1.60), and DOY 249 (06-

September) at Sutherland (1.40 ± 0.31).

In 2000, the end of the overwintering generation (end of May) was observed at all

three locations (Figure 1). The first generation of *C. pulicaria* was first observed on DOY

179 (27-June) at Crawfordsville, 185 (03-July) at Ames, and 192 (10-July) at Sutherland.

Peak numbers of *C. pulicaria* were observed on DOY 193 (11-July) at Crawfordsville (1.70

± 0.30), DOY 195 (13-July) at Ames (1.23 ± 0.11), and DOY 207 (25-July) at Sutherland

(9.17 ± 1.30). The second generation of *C. pulicaria* was observed on DOY 202 (20-July) at

Ames, DOY 221 (08-August) at Crawfordsville, and DOY 220 07-August) at Sutherland.

The peak number of *C. pulicaria* per strip was observed on DOY 230 (18-August) at Ames

(2.40 ± 0.23), DOY 247 (03-September) at Crawfordsville (2.40 ± 0.41), and DOY 250 (06-

September) at Sutherland (8.87 ± 1.32).

Significantly higher numbers of *C. pulicaria* on the grass border sticky card were

observed on DOY 252 (09-September) (p = 0.0002), DOY 261 (18-September) (p < 0.0001),

and DOY 266 (23-September) (p < 0.0001) than on all other yellow sticky cards. The grass

border card had significantly higher numbers of *C. pulicaria* than the non-grass border card

on DOY 252 (p = 0.0244), DOY 261 (p < 0.0001), and DOY (p < 0.0001).

Using sweep netting in 1999, the overwintering adult generation was followed by a first and second generation of *C. pulicaria* in Iowa (Figure 2). In 1999, the first sign of the overwintering generation was between DOY 120 (30-April) and 138 (18-May) (Table 1). *Chaetocnema pulicaria* were not found using sweep netting at Kanawha, Nashua, and Sutherland. The end of the overwintering generation was observed among locations in Iowa between DOY 151 (31-May) and 165 (14-June). Between the overwintering generation and first sign of the first generation, there was a period of 14 days at Chariton and Crawfordsville, and 32 days at Ames when *C. pulicaria* were not found. The first sign of the first generation of *C. pulicaria* occurred between DOY 172 (21-June) and 190 (09-July). Peak first generation *C. pulicaria* were observed between DOY 193 (12-July) and 210 (29-July). Second generation *C. pulicaria* were first observed between DOY 214 (02-August) and 240 (28-August). Peak second generation *C. pulicaria* were observed between DOY 232 (20-August) and 249 (06-September). *Chaetocnema pulicaria* were no longer found in corn fields from DOY 273 (30-September) to 281 (08-October), although sampling at Ames ended on DOY 252 (09-September) when the field was harvested early.

In 2000, an overwintering, first, and second generations of *C. pulicaria* were found at all six locations where sampling occurred (Figure 2). The first sign of the overwintering generation was observed between DOY 109 (18-April) and 125 (04-May) (Table 1). The end of the overwintering generation was observed between DOY 151 (30-May) and 157 (05-June). The number of days where *C. pulicaria* were not found between the overwintering generation and first generation ranged from 18 days (Chariton) to 25 days (Nashua). First generation *C. pulicaria* were observed between DOY 173 (21-June) and 178 (26-June), peaking between DOY 192 (10-July) and 207 (25-July). Second generation *C. pulicaria*

observing the overwintering generation of *C. pulicaria*. This new information is especially important considering the risk of early planted corn to corn flea beetle feeding and the initiation of the seedling wilt phase of Stewart's disease. For instance, in 2000, corn fields in the southern part of Iowa were planted in very early April, and the time of corn emergence coincided with emergence of the overwintering adult *C. pulicaria* generation resulting in the loss of entire fields in Iowa due to Stewart's disease. Our study will help seed corn producers to be better prepared to scout for and manage corn flea beetle populations with foliar insecticides in early planted corn.

Our study also indicated that there was a significant beetle-free period between the overwintering generation and first sign of the first generation when *C. pulicaria* were not found, and the application of foliar insecticides during this period would be unnecessary. This beetle-free period was anywhere from two to more than four weeks in duration and occurred approximately during the month of June.

In Iowa, the first generation of *C. pulicaria* was observed between late-June into July and this was approximately two weeks earlier then the observations made by Adams and Los (1986) in Connecticut. The timing of the first generation in Iowa is extremely important in relation to when seed corn inspections should be made. Transmission of *P. stewartii* by the first generation will have profound effects on disease levels that will be observed in August when seed corn inspections occur in Iowa (and most other Corn Belt states). Since there are no insecticide spray recommendations for the late leaf blight phase of Stewart's disease in Iowa, our study has provided new information to develop and test insecticide spray programs based on the biology and the insect pest. Moreover, there is the potential to develop a degree-day model that would help to improve the timing of insecticide applicaitons.

Quantitative information concerning the population dynamics of the corn flea beetle coupled with knowledge of the proportion of the corn flea beetle population that has the bacterium will help to predict disease risk in the current season as well as the ensuing season (Esker and Nutter, 2001). This knowledge will ensure that disease management tactics are in place before corn is planted to reduce the risk of *P. stewartii*. These recommendations would include the need for insecticide seed treatments, choice of low-risk planting sites, and advanced warning for where and when foliar-applied insecticide applications are warranted.

The population dynamics of *C. pulicaria* that were found from one year to the next do not discount the notion that there might be some migration by *C. pulicaria*. Evidence for this is the fact that only the second generation of *C. pulicaria* was observed in northern Iowa in late 1999. However, in 2000, the overwintering, first, and second generations were found at relatively high numbers at these northern locations, indicating that migration may have little epidemiological importance as far as the early-season spread compared to *C. pulicaria* populations that actually overwinter.

References

Adams , R.G. and Los, L. M. 1986. Monitoring adult corn flea beetles (Coleoptera: Chrysomelidae) in sweet corn fields with color sticky traps. Environ. Entomol. 15: 867-873.

Carlton, W.M. and Munkvold, G.P. 1995. Corn Stewart's Disease. Iowa State University, Extension Bulletin. Ames, IA. Pm-1627.

Dill, J.F. 1979. Biology and Management of the Corn Flea Beelte, *Chaetocnema pulicaria* Melshheimer, Relative to the Incidence of Stewart's Disease in Corn. Ph.D. Thesis, Purdue University, West Lafeyette, IN. 167 Pages.

Esker, P.D., and Nutter, F.W., Jr. 2001. The temporal dynamics of *Chaetoconema pulicaria* (Coleoptera: Chrysomelidae) populations infested with *Pantoea stewartii*. Phytopathology 91: (xxx-xxx).

Esker, P.D., and Nutter, F.W., Jr. 2000. Monitoring the dynamics of corn flea beetles infested with *Pantoea stewartii*. Phytopathology 90: S22.

Elliot, C., and Poos, F.W. 1940. Seasonal development, insect vectors, and host range of bacterial wilt of sweet corn. J. Agric. Res. 60: 645-686.

Heichel, G. H., Sands, D. C., and Kring, J. B. 1977. Seasonal patterns and reduction by carbofuran of Stewart's bacterial wilt of sweet corn. Plant Dis. Reptr. 61: 149-153.

Munkvold, G. P., McGee, D.C., and Iles, A. 1996. Effects of imidacloprid seed treatment of corn on foliar feeding and *Erwinia stewartii* transmission by the corn flea beetle. Plant Dis. 80: 747-749.

Nutter, F.W., Wegulo, S.N., Rubsam, R.R., Taylor, S.E., and Harri, J.A. 1998. Use of geospatially-referenced disease and weather data to improve site-specific forecasts for Stewart's disease of corn. Phytopathology 88: S68.

Pataky, J.K. 1985. Relationships among reactions of sweet corn hybrids to Goss' wilt, Stewart's bacterial wilt, and northern corn leaf blight. Plant Dis. 69: 845-848.

Pataky, J.K., du Toit, L.J., Freeman, N.D. 2000. Stewart's wilt reduction of an international collection *Zea mays* germ plasm inoculated with *Erwinia stewartii*. Plant Dis. 84: 901-906.

Pepper, E.H. 1967. Stewart's Bacterial Wilt of Corn, 4th Ed. Heffernan Press, Worchester, MA.

Poos, F.W. 1955. Studies of certain species of *Chaetocnema*. J. Econ Entomol. 48: 555-563.

Rand, F.V., and Cash, L.C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362: 1-22.

Smith, E.F. 1914. Stewart's disease of sweet corn (maize). Pages 89-147 in: Bacteria in
Relation to Plant Diseases. Carnegie Institute of Washington, Washington, D.C.

Suparyono and Pataky J.K. 1989. Influence of host resistance and growth stage at the time
of inoculation on Stewart's wilt and Goss's wilt development and sweet corn hybrid
yield. Plant Dis. 73: 339-345.

Table 1. Mean and standard errors for *C. pulicaria* generations at six locations in Iowa during the 1999 and 2000 corn growing seasons.

Location	Year	First Sign[1]	First Generation	Peak First Generation	Second Generation	Peak Second Generation	Last Sampling
Ames	1999	138[2]	190	200	221	238	252
		2.10 ± 0.43	2.50 ± 0.78	11.20 ± 1.58	1.90 ± 0.43	14.40 ± 2.04	Harvest
	2000	109	173	195	222	240	269
		44.90 ± 7.89	0.50 ± 0.22	7.30 ± 0.96	0.60 ± 0.22	2.00 ± 0.70	0.00 ± 0.00[3]
Chariton	1999	120	172	200	214	238	273
		4.50 ± 0.73	0.30 ± 0.15	16.70 ± 1.42	2.80 ± 0.66	9.60 ± 0.90	0.00 ± 0.00
	2000	125[4]	173	196	222	230	262
		10.90 ± 2.26	0.10 ± 0.10	13.30 ± 1.51	1.50 ± 0.50	3.50 ± 0.62	0.00 ± 0.00
Crawfordsville	1999	120	172	193	214	238	273
		18.10 ± 1.67	0.10 ± 0.10	8.50 ± 1.53	6.10 ± 0.88	27.80 ± 2.76	0.00 ± 0.00
	2000	111	173	193	222	230	269
		10.30 ± 1.54	0.20 ± 0.13	9.00 ± 1.54	0.60 ± 0.27	2.10 ± 0.53	0.00 ± 0.00
Kanawha	1999	NA[5]	NA	NA	232	249	281
		...	...	...	0.08 ± 0.15	0.28 ± 0.55	0.00 ± 0.00
	2000	116	178	192	207	220	271
		2.40 ± 0.34	0.10 ± 0.10	3.00 ± 0.79	2.67 ± 0.63	3.10 ± 0.0	0.10 ± 0.10
Nashua	1999	NA	NA	NA	220	232	276
		...	...	...	0.02 ± 0.04	0.10 ± 0.20	0.00 ± 0.00
	2000	116	178	200	250	257	271
		3.00 ± 0.67	0.50 ± 0.17	16.00 ± 1.58	1.70 ± 0.50	6.00 ± 1.42	2.40 ± 0.48
Sutherland	1999	NA	195	210	240	249	276
		...	0.02 ± 0.03	0.20 ± 0.38	0.04 ± 0.07	0.08 ± 0.16	0.00 ± 0.00
	2000	116	174	207	250	257	271
		1.10 ± 0.23	0.10 ± 0.10	15.00 ± 1.20	0.30 ± 0.15	3.00 ± 0.70	0.10 ± 0.10

[1] First sign is the first time *C. pulicaria* were observed at a location.
[2] Number corresponds to day of year (Julian).
[3] 0.00 ± 0.00 indicates the 2nd consecutive sampling date without *C. pulicaria*
[4] The first sample at Chariton on DOY 125 was after inclement weather prevented the two previous sampling attempts.
[5] NA = no *C. pulicaria* were found.

Table 2. Number of *C. pulicaria* per replication (mean ± SE) using sweep netting along the grass borders in 2000. The columns correspond to the major second generation events observed in the corn.

Location	DOY[1]	Second Generation (first sign)	Peak Second Generation	Last Sampling in Corn
Ames	DOY	222	240	269
		3.20 ± 0.47	23.10 ± 3.19	76.67 ± 13.61
Chariton	DOY	222	230	262
		1.10 ± 0.31	3.90 ± 0.59	4.80 ± 1.14
Crawfordsville	DOY	222	230	269
		0.90 ± 0.23	8.50 ± 1.17	34.50 ± 7.04
Kanawha	DOY	207	220	271
		0.20 ± 0.13	2.70 ± 0.58	26.96 ± 8.52
Nashua	DOY	250	257	271
		8.10 ± 1.24	41.90 ± 5.10	87.00 ± 12.42
Sutherland	DOY	250	257	271
		22.10 ± 2.30	12.90 ± 2.35	64.00 ± 8.52

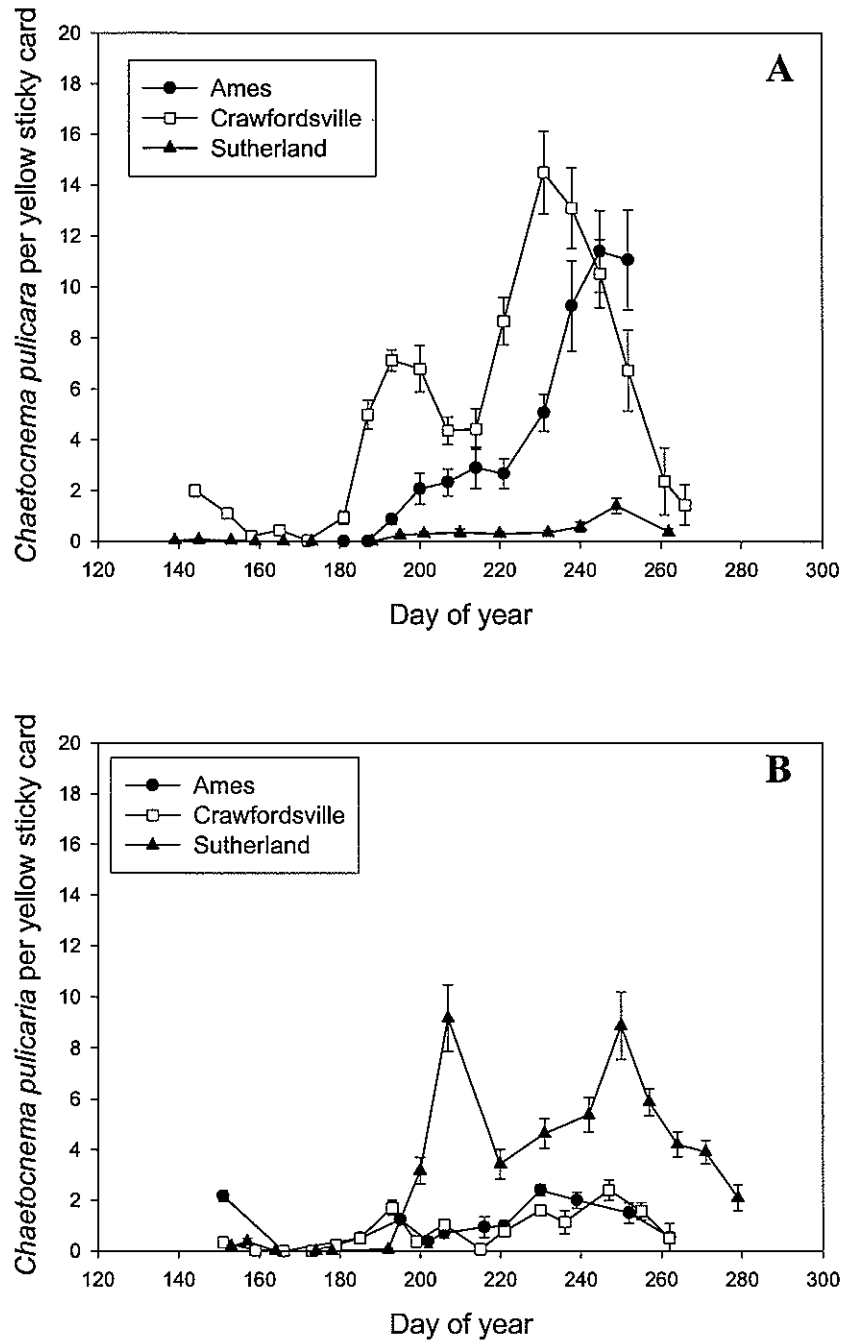[1] Number corresponds to day of year (Julian).

Figure 1. *Chaetocnema pulicaria* per yellow sticky strip (mean ± SE) (n=30) in (A) 1999
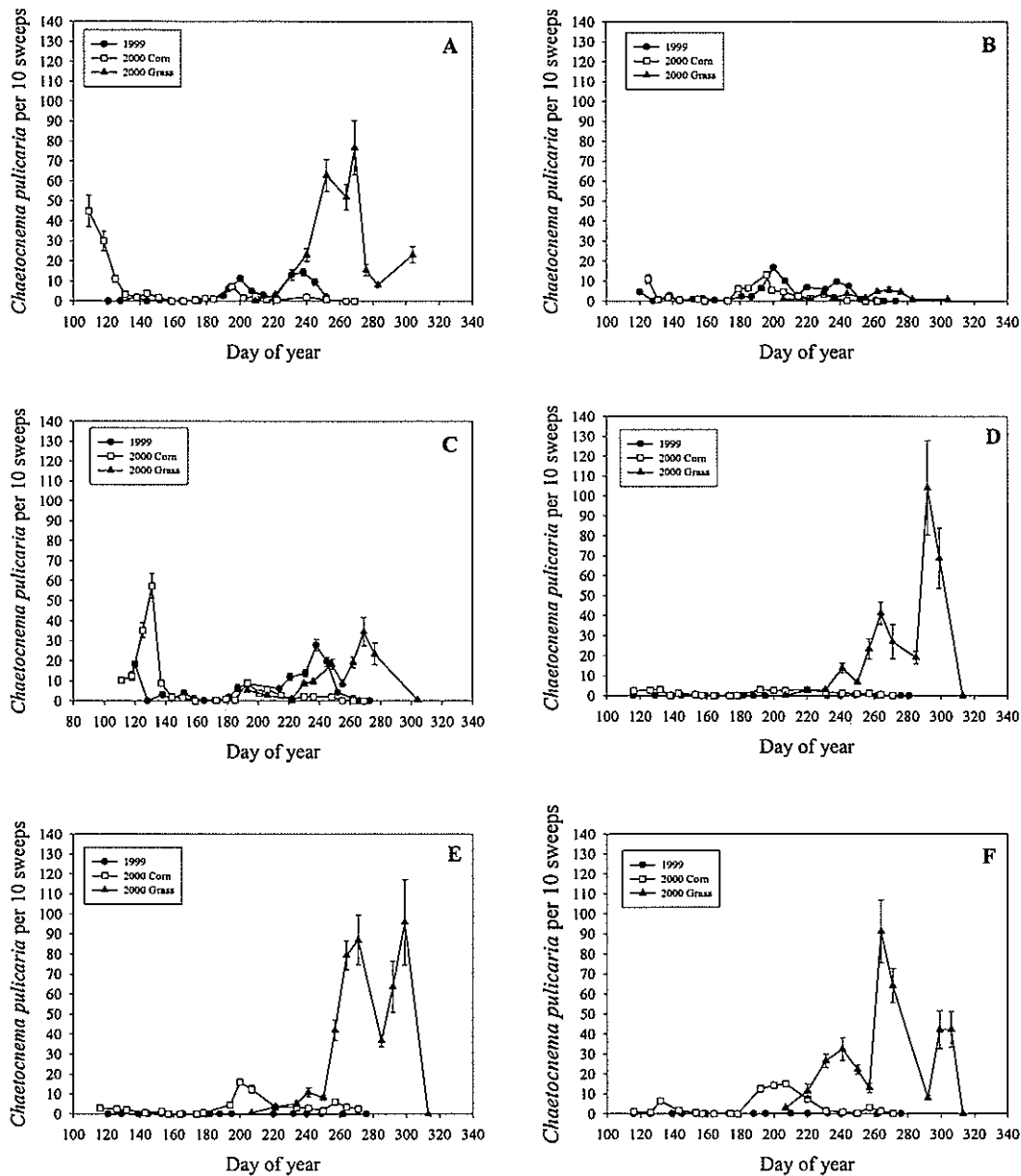
and (B) 2000 in Iowa.

Figure 2. Mean ± SE of *C. pulicaria* per 10 sweeps (replication) from (A) Ames, (B) Chariton, (C) Crawfordsville, (D) Kanawha, (E) Nashua, and (F) Sutherland, Iowa in 1999 and 2000. In 2000, a second set of ten samples was taken simultaneously in the grass borders as in the corn beginning in approximately mid-July.

CHAPTER 3

THE TEMPORAL DYNAMICS OF *CHAETOCNEMA PULICARIA*

(COLEOPTERA: CHRYSOMELIDAE) POPULATIONS INFESTED

WITH *PANTOEA STEWARTII*

Paul D. Esker and Forrest W. Nutter, Jr.

Abstract

Temporal changes in the proportion of *Chaetocnema pulicaria* Melsh populations

(corn flea beetle) infested with the bacterium, *Pantoea stewartii*, which causes Stewart's

disease of corn, were monitored using enzyme-linked immunosorbent assay (ELISA). Using

sweep nets, approximately 90 corn flea beetles were collected each week from six locations

in Iowa beginning in the fall of 1998 through fall of 2000. Individual beetles were tested for

the presence of *P. stewartii* using a compound direct, peroxidase labeled ELISA (with slight

modifications) (Agdia Inc., Elkhart, IN) to determine the proportion of *P. stewartii*-infested

corn flea beetles. The proportion (number of corn flea beetles testing positive divided by the

total number of corn flea beetles tested) of infested corn flea beetles in the end of the season

adult generation ranged from 0.04 to 0.19 in the fall of 1998. In the early spring of 1999, the

proportion of overwintering adult corn flea beetles infested with *P. stewartii* ranged from

0.10 to 0.11 and were not significantly different from the fall 1998. During the 1999

growing season, the proportion of infested corn flea beetles ranged from 0.04 to 0.86, with

the highest observed proportions occurring in early-to late August which coincided with the

second field generation. Going into dormancy in 1999, the proportion of beetles infested

ranged from 0.20 to 0.77. In the early spring of 2000, the proportion of overwintering adult

corn flea beetles infested with *P. stewartii* ranged from 0.08 to 0.30 and these proportions

were significantly less than the fall of 1999 proportions at Ames, Chariton, and Nashua based

on chi-square analysis (p-values: 0.04 to < 0.0001). During the 2000 growing season, the

proportion of *P. stewartii*-infested corn flea beetles ranged from 0.08 to 0.53, and again, the

highest observed proportions occurred in August, which coincided with the second field

generation of corn flea beetles. Corn flea beetle populations sampled just prior to the 2000

winter had proportions of infested beetles ranging from 0.08 to 0.20. This study provides

new quantitative information concerning the population dynamics of *P. stewartii*-infested *C.*

pulicaria populations in hybrid corn that should be useful to predict the seasonal and site-

specific risks associated with Stewart's disease of corn.

Introduction

Stewart's disease of corn (*Zea mays*), caused by the bacterium *Pantoea stewartii*

(Smith, 1914; Elliot and Poos, 1940), is an economically important disease in both sweet

corn and seed corn fields (Pepper, 1967; Pataky, 1985; Carlton and Munkvold, 1995). Direct

yield losses of 10 to 20% have been documented in sweet corn fields (Pataky, 1985;

Suparyono and Pataky, 1989), while in the seed corn industry, there is a zero-tolerance for

Stewart's disease of corn due to phytosanitary regulations that limit the export of corn seed

originating from infested fields (in the United States) to other countries. This disease has

tremendous economical implications considering that the prevalence of Stewart's disease in

Iowa was 25% of seed production fields in 1998 and 58% in both 1999 and 2000 (Nutter et

al., 1998; Esker and Nutter, 2000). This high prevalence would require either costly grow-

out tests or enzyme-linked immunosorbent assays (ELISA) (on seed) be performed by companies to verify that the seed was indeed free of *Pantoea stewartii* (Block et al., 1999).

Historically, the role of seed transmission was considered epidemiologically important (Smith, 1914; Pepper, 1967). However, Block et al. (1998) conducted experiments that clearly showed that seed-to-seedling transmission is well below 1%. In spite of these results, strict quarantine sanctions still remain in effect.

The most important mode of pathogen dissemination from corn plant to corn plant is the adult corn flea beetle (*Chaetocnema pulicaria* Melsh) (Rand and Cash, 1924; Rand and Cash, 1933; Poos and Elliot, 1936; Poos, 1955; Dill, 1979). Corn flea beetles acquire the bacterium after feeding on infected corn plants and the bacterium is then transmitted to healthy plants when infested corn flea beetles feed locally. Depending on the stage of crop development when transmission occurs, two phases of Stewart's disease may develop. The first that may develop is known as the seedling wilt phase. This phase occurs when *P. stewartii*-infested corn flea beetles feed on young corn plants and successfully transmit *P. stewartii* (Pepper, 1967). Following transmission, linear, water-soaked lesions occur, followed by stunting and wilting (Smith, 1914; Rand and Cash, 1933; Pepper, 1967; Dill, 1979). When the disease is severe during this phase, direct seedling loss may occur. The second phase of the disease is known as the leaf blight phase. Symptoms during this period typically begin at the site of corn flea beetle feeding scars (Pepper, 1967; Dill, 1979; Carlton and Munkvold, 1995). Once the bacterium has entered a corn leaf, the bacterium multiplies and causes a yellowish water-soaked lesion or streak that soon becomes necrotic. These streaks elongate and coalesce along the leaf veins of corn leaves and when symptoms are severe, entire leaves can be blighted and killed.

There is little quantitative information concerning the dynamics of *P. stewartii*-infested corn flea beetle populations. By crushing and culturing the contents from the crushed corn flea beetles onto agar plates, Elliot and Poos (1940) estimated approximately 20-30% of the 3800-5600 corn flea beetles tested were found to have the bacterium. Roberts (1955) estimated that 10 to 20% of corn flea beetle populations that emerged from dormancy were infested with *P. stewartii*. However, during midsummer, the percentage of infested beetles was as high as 75% (Roberts, 1955).

Although the above studies have provided some quantitative information concerning the proportions of corn flea beetles infested with the bacterium at single sampling points in time (in sweet corn), there have been no quantitative studies conducted concerning the proportions of adult corn flea beetles that carry the bacterium at the end of the growing season (before overwintering), nor those that carry the bacterium when beetles emerge in the spring that will provide the primary source of initial inoculum. Moreover, there is no quantitative information concerning the within-season temporal dynamics of infested corn flea beetle populations in hybrid corn or sweet corn. The ability to detect and quantify the proportion of *P. stewartii*-infected corn flea beetles using a modified enzyme-linked immunosorbent assay (ELISA) for corn flea beetles could be employed to obtain a better understanding of the seasonal dynamics of *P. stewartii*-infested corn flea beetle populations. Therefore, the objective of this study was to quantify the temporal dynamics of corn flea beetle populations infested the Stewart's disease bacterium by sampling and testing individual corn flea beetles from several locations in Iowa.

Materials and Methods

Corn flea beetles were collected from six locations in Iowa using sweep netting following protocols that have previously been described (Esker et al., 2001). Ten replications of 10 sweeps per replication were taken from either the grasses surrounding corn fields or in corn fields and corn flea beetles were collected and separated from other insects and debris for use in ELISA. Approximately 90 (N=90) corn flea beetles per sampling date per location were collected for ELISA testing (if populations were sufficiently high) using sweep netting, however, sample numbers below 90 were also tested. Corn flea beetle populations were sampled from the ISU Been and Curtiss Farms in Ames, IA (1999), the ISU Bruner Research Farm in Ames, IA (2000), the ISU McNay Research Farm in Chariton, IA (1999 and 2000), the ISU Northeast Research Farm in Nashua, IA (1999 and 2000), the ISU Northern Research Farm in Kanawha, IA (1999 and 2000), the ISU Northwest Research Farm in Sutherland, IA (1999 and 2000), and the ISU Southeast Research Farm in Crawfordsville, IA (1999 and 2000). Sweep netting was performed weekly in the fall of 1998 and throughout the 1999 and 2000 growing seasons. Individual corn flea beetles were tested for the presence of *P. stewartii* using a compound direct, peroxidase labeled ELISA protocol with slight modifications (Agdia Inc., Elkhart, IN).

ELISA Protocol

A 96-well microtiter plate (Nunc-Immuno™ Plate, Denmark) was used for sample preparation. One hundred μl of 1X-PBST buffer (8.0 g NaCl, 1.15 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, 0.5 g Tween-20, 1000 ml dH_2O, pH 7.4) was placed into each well and one corn flea beetle was then placed into each the buffer-filled well, and ground using a

sterile, 5 mm glass rod. After grinding, an additional 175 µl of 1X-PBST was added to each well.

Twelve to twenty-four hours prior to performing an ELISA, microtiter plates were coated with anti-ES antibody. Fifty µl of anti-ES was mixed with 10 ml of coating buffer (1:200 dilution) (1.6 g Na_2CO_3, 2.9 g $NaHCO_3$, 0.2 g NaN_3, 1000 ml dH_2O, pH 9.6, 4°C (40°F)), which was enough to coat one plate. Two-100 µl samples from each beetle were removed from the grinding wells and used during the ELISA (for each ELISA test, individual corn flea beetle contents were tested in duplicate wells). The plate were incubated at 4°C (40°F) overnight in a 100% humidity chamber.

Before wells could be loaded with prepared corn flea beetle samples, the coated plates were washed 6x using 1X-PBST. This was accomplished by filling each well to overflow and then quickly emptying out the contents into a sink. The plates were gently tapped on paper towel to remove excess buffer and air bubbles, and allowed to dry. Two-100 µl of prepared sample from the corn flea beetle were pipetted into the wells. Also, two positive and two negative controls were used for each plate, with two wells left blank. ELISA plate(s) were incubated at 25°C (77°F) for 2 hours, or overnight at 4°C in a 100% humidity chamber.

Ten minutes before the end of the first incubation, enzyme conjugate was prepared; a 1:5 dilution of MRS component and 1X-PBST was prepared (e.g. 2 ml of MRS (1 part) for 8 ml of 1X-PBST (4 parts), which is enough to coat one plate). Fifity µl of bottle A conjugated antibody (1:200 dilution) and 50 µl of bottle B conjugated antibody (1:200 dilution) was added to the solution. Plate(s) were washed as previously described, and then, 100 µl of the

enzyme conjugate was pipetted into each well. The plate(s) were incubated for 2 hours at 25°C in a 100% humidity chamber.

Ten minutes before the end of the 2nd incubation, OPD substrate was prepared. Using a sterile forceps, 1 OPD stick was added to 10 ml of OPD solution (0.4 ml H_2O_2, 7.3 g Na_2HPO_4, 5.1 g $HOCCOOOH(CH_2COOH)_2·H_2O$, 1000 ml dH_2O, pH 5.0, 4°C) (enough to coat one plate). The OPD stick(s) were left in solution for two minutes and then mixed gently in the solution before being discarded. The plates were washed as previously described and 100 μl of OPD substrate was pipetted into each well. The plates were then incubated for 30 minutes at 25°C in a 100% humidity chamber. A 'tentative' positive could be identified through a coloration change in a well from clear to reddish-orange. The reaction was stopped after 30 minutes with the addition of 50 μl of 3M sulfuric acid. Absorbance of each well was recorded at 490 nm using an EL_x800 Universal Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT) and this information was further analyzed to determine the proportion of *Chaetocnema pulicaria* infested with *Pantoea stewartii*.

Data Analysis

A sample was considered positive if the absorbance value was greater than or equal to two times the average negative absorbance and both well samples from a single corn flea beetle were above this threshold. The binomial probability distribution was used to analyze proportional results (Steel et al., 1997). This was based on the principle that the results were either a positive or negative response. Although the goal was to test 90 corn flea beetles (N = 90) per sampling date per location, this was not always possible due to the seasonality of beetle generations and/or the environmental conditions (rain, cool temperatures, lack of sunshine) that would adversely affect corn flea beetle activity when sweep netting was

performed. Each corn flea beetle was considered an experimental unit and differences in sample size were automatically accounted for using the binomial probability distribution, thereby allowing for the testing and statistical analysis of unequal numbers of corn flea beetles per sampling date. The equation for the binomial probability distribution is given as:

$$P(Y = Y_i|n) = \binom{n}{Y_i} p^{Y_i}(1- p)^{n-Y_i}$$

where the probability that the random variable, Y, takes a particular value, Y_i, given n independent trials is the factorial function of n trials given the probability of that random variable will yield a positive/negative result (Steel et al., 1997).

Using the binomial probability distribution equation, the standard error was calculated for each proportion of positive divided by the total tested in each sampling date and location by the equation:

$$StdError = \sqrt{\frac{p(1- p)}{n}}$$

where p is the proportion with the positive result, 1-p is the proportion with the negative result, and n is the number of trials.

The proportion of corn flea beetles infested at the end of the growing season (the last sampling date when corn flea beetles were found in numbers sufficient to test) was compared with the proportion of corn flea beetles found to be infested at the beginning of the next growing season (the first sampling date when the overwintering adult corn flea beetles were first detected and sampled from the different locations). Chi-square analyses were then performed to determine if there was a significant difference between the proportion of P.

stewartii-infested corn flea beetles going into winter with the proportion infested the ensuing spring (prior to seeding corn).

Results

Proportions of corn flea beetles infested with *P. stewartii* in the fall of 1998 had proportions that were not significantly different from those infested in the spring of 1999 at Ames, Chartion, and Crawfordsville (Table 1). No corn flea beetles were found at Nashua in the fall of 1998 or at Kanawha and Sutherland in the spring of 1999. Based on chi-square analysis, significantly fewer corn flea beetles tested positive for *P. stewartii* in the spring of 2000 compared to the fall of 1999 at Ames (p < 0.0001), Chariton (p < 0.0001), and Nashua (p = 0.0365) (Table 1). However, no significant differences were observed between the fall sample and the spring sample at the other three locations (Crawfordsville, Kanawha, and Sutherland).

Corn flea beetle populations that were infested with *P. stewartii* were highly variable temporally during the period 1998 to 2000, but tended to increase with each generation of corn flea beetles (overwintering generation and plus two field generations) (Figure 1). The proportion of *P. stewartii*-infested corn flea beetles during the 1999 growing season ranged from 0.043 ± 0.043 to 0.856 ± 0.037. In general, the highest observed proportions at the six locations occurred in August at all locations (Table 2).

The proportion of corn flea beetles infested with *P. stewartii* in 2000 also was variable (Figure 2) and ranged from 0.078 ± 0.028 to 0.533 ± 0.074 (Table 2). Again, the highest observed proportions occurred during the month of August at all six locations in Iowa.

Entering the period of fall dormancy in 2000, the proportion of *P. stewartii*-infested corn flea beetles ranged from 0.083 ± 0.056 (Chariton) to 0.200 ± 0.042 (Nashua). The proportion of *P. stewartii*-infested corn flea beetles tested at all other locations (n = 90) were 0.189 ± 0.041 at Ames, 0.089 ± 0.030 at Crawfordsville, 0.178 ± 0.040 at Kanawha, and 0.133 ± 0.036 at Sutherland.

Discussion

This is the first study to clearly document the seasonal dynamics of *P. stewartii*-infested corn flea beetle populations with respect to time. While previous studies have determined the proportion of corn flea beetles infested with *P. stewartii* in sweet corn at single points during the season, none have demonstrated the dynamics of this process (Rand and Cash, 1933; Elliot and Poos, 1940; Roberts, 1955). For example, one important finding was there was a dramatic change in the proportion of *P. stewartii*-infested overwintering corn flea beetles before corn had been planted and emerged at several locations. Our hypothesis initially was that this proportion of infested beetles would remain static until corn had emerged, become infected and serve as additional sources for corn flea beetles to acquire the pathogen. This new information lends itself to three alternative hypotheses: (i) that there is an alternative weed host where the bacterium can be acquired and then transmitted to corn, (ii) that corn flea beetles can transmit the bacterium to one another, or (iii) that both (i) and (ii) are occurring, or (iv) that the bacterium multiplies in the corn flea beetles, growing from nondetectable to detectable levels.

It is also important to note that infested corn flea beetles were found two weeks earlier in 2000 than in 1999, which is important for improving the understanding of initial inoculum and when risk for the wilt phase of Stewart's disease is possible. Information

regarding the overwintering generation has important management implications with regards to time of planting since corn flea beetle emergence may coincide with the highest populations of corn flea beetles that are already infested with the bacterium. This would increase the potential risk of Stewart's disease, which could cause severe economic losses to seed corn and sweet corn (Pataky, 1985; Esker and Nutter, 2000). Planting before or after peak inoculum levels may decrease potential disease risk.

Based upon previous research, we assumed that new individuals from the first field generation of corn flea beetles would not have the bacterium because the bacterium is not passed from adults to eggs (Dill, 1979). If this is true, then previously infected corn plants would provide the inoculum for newly emerging corn flea beetles and new adult beetles would change their status from being negative (non-infested) to positive (infested) after acquiring the bacterium. Corn flea beetles would then be able to transmit the bacterium to other corn plants in the field. As time progresses, the proportion of *P. stewartii*-infested corn flea beetles would be predicted to first increase as the older adult corn flea beetle population (that are infested) reproduce and die (are removed from the total beetle population). Based on the principle that corn flea beetles typically survive 30 days (Dill, 1979), this process of new beetles entering the population and acquiring the bacterium and old beetles leaving the population would result in a discrete time function. The temporal patterns of the proportions of corn flea beetles testing positive for *P. stewartii* support Dill's findings that the bacterium is not passed from adult to egg.

Our results also clearly show that the proportion of *P. stewartii*-infested corn flea beetles is always greater than zero. The reason that the proportion of *P. stewartii*-infested corn flea beetles does not approach zero is that the first and second field generations overlap

during this time period (Esker et al., 2001). Also, at the end of the growing season, the proportion of *P. stewartii*-infested corn flea beetles does not go to zero because some proportion of the corn flea beetles population survive the winter in the adult form (Pepper, 1967; Dill, 1979).

Acquisition of the bacterium by new adult corn flea beetles appears to be rather quick. This is similar to human standard SIR epidemic model, where 'S' stands for susceptible, 'I' for infectious, and 'R' for removed (Andersson and Britton, 2000; Daley and Gani, 1999; Hethcote, 1989). These models have been applied to measles and hepatitis epidemics. As the population increases, the number of infected individuals increases until the population declines in this case, because mature beetles eventually die of old age. This change in the proportion of infected individuals (corn flea beetles) is dramatic and quick.

The significance in the reduction in the overwintering proportion of *P. stewartii*-infested corn flea beetles in 2000 is important as it may provide a clue that it is not in the best interest of the corn flea beetle to harbor the bacterium. There may be adverse changes in the metabolic processes of corn flea beetles, reducing survival probabilities. There may also be some form of a supercooling event that freezes and eliminates the bacterium from the beetle. Future research is needed to determine the temperatures at which the bacterium versus corn flea beetles are killed. Similar work was done by Lam and Pedigo (2000) to determine the minimum temperature that bean leaf beetles (*Cerotoma trifurcata*) could survive. It will then be important to try to determine if there is a relationship between cold tolerance temperatures and the proportion of *P. stewartii*-infested corn flea beetles that survive.

This research has conclusively shown that the inoculum-level (proportion of *P. stewartii*-infested corn flea beetles) in the field changes over time and is a function of the

corn flea beetle population dynamics. This is epidemiologically important information to determining the potential risks of Stewart's disease during the growing season that will influence treatment recommendations to effectively reduce corn flea beetle populations.

References

Andersson, H. and Britton, T. 2000. Stochastic Epidemic Models and Their Statistical Analysis. Springer-Verlag, New York.

Block, C.C., Hill, J.H., and McGee, D.C. 1999. Relationship between late-season severity of Stewart's bacterial wilt and seed infection in maize. Plant Dis. 83: 527-530.

Block, C.C., Hill, J.H., and McGee, D.C. 1998. Seed transmission of *Pantoea stewartii* in field and sweet corn. Plant Dis. 82: 775-780.

Carlton, W.M. and Munkvold, G.P. 1995. Corn Stewart's Disease. Iowa State University, Extension Bulletin. Ames, IA. Pm-1627.

Daley, D.J., and Gani, J. 1999. Epidemic Modelling. Cambridge University Press, Cambridge, UK.

Dill, J.F. 1979. Biology and Management of the Corn Flea Beelte, *Chaetocnema pulicaria* Melshheimer, Relative to the Incidence of Stewart's Disease in Corn. Ph.D. Thesis, Purdue University, West Lafeyette, IN. 167 Pages.

Elliot, C., and Poos, F.W. 1940. Seasonal development, insect vectors, and host range of bacterial wilt of sweet corn. J. Agric. Res. 60: 645-686.

Esker, P.D., and Nutter, F.W., Jr. 2000. Monitoring the dynamics of corn flea beetles infested with *Pantoea stewartii*. Phytopathology 90: S22.

Esker, P.D., Obrycki, J., and Nutter, F.W., Jr. 2001. The temporal distribution of *Chaetoconema pulicaria* (Coleoptera: Chrysomelidae) populations in Iowa. J. Econ. Entomol. : (xxx-xxx).

Hethcote, H.W. 1989. Three basic epidemiological models. Pages 119-144 in: Applied Mathematical Ecology. S.A. Levin, T.G. Hallam, and L.J. Gross eds. Springer-Verlag, New York.

Lam, W.F., and Pedigo, L.P. 2000. Cold tolerance of overwintering bean leaf beetles (Coleoptera: Chrysomelidae). Environ. Entomol. 29: 157-163.

Lamka, G.L., Hill, J.H., McGee, D.C., and Braun, E.J. 1991. Development of an immunosorbent assay for seedborne *Erwinia stewartii* in corn seeds. Phytopathology 81: 839-846.

Nutter, F.W., Wegulo, S.N., Rubsam, R.R., Taylor, S.E., and Harri, J.A. 1998. Use of geospatially-referenced disease and weather data to improve site-specific forecasts for Stewart's disease of corn. Phytopathology 88: S68.

Pataky, J.K. 1985. Relationships among reactions of sweet corn hybrids to Goss' wilt, Stewart's bacterial wilt, and northern corn leaf blight. Plant Dis. 69: 845-848.

Pepper, E.H. 1967. Stewart's Bacterial Wilt of Corn, 4th Ed. Heffernan Press, Worchester, MA.

Poos, F.W. 1955. Studies of certain species of *Chaetocnema*. J. Econ Entomol. 48: 555-563.

Poos, F.W., and Elliot, C. 1936. Certain insect vectors of *Aplanobacter stewarti*. J. Agric. Res. 52: 585-608.

Rand, F.V., and Cash, L.C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362: 1-22.

Rand, F.V., and Cash, L.C. 1924. Further evidence of insect dissemination of bacterial wilt of corn. Science 59: 67-69.

Rand, F.V., and Cash, L.C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362: 1-22.

Smith, E.F. 1914. Stewart's disease of sweet corn (maize). Pages 89-147 in: Bacteria in Relation to Plant Diseases. Carnegie Institute of Washington, Washington, D.C.

Steel, R.G.D., Torrie, J.H., and Dickey, D.A. 1997. Principles and Procedures of Statistics, a Biometrical Approach, 3rd Ed. McGraw-Hill, New York.

Suparyono and Pataky J.K. 1989. Influence of host resistance and growth stage at the time of inoculation on Stewart's wilt and Goss's wilt development and sweet corn hybrid yield. Plant Dis. 73: 339-345.

Table 1. Chi-square analysis comparing the testing of corn flea beetles from the last sampling date in the fall of a growing season to the first sampling date the following season.

Location	Comparison[1]	Fall	Spring	$\chi 2$ value	Probability[2]
Ames	F1998 v. S1999	0.067 ± 0.037	0.100 ± 0.067	0.2167	0.6416
	F1999 v. S2000	0.433 ± 0.052	0.156 ± 0.038	16.7137	<0.0001*
Chariton	F1998 v. S1999	0.044 ± 0.031	0.114 ± 0.048	1.4698	0.2254
	F1999 v. S2000	0.733 ± 0.047	0.300 ± 0.048	33.8376	<0.0001*
Crawfordsville	F1998 v. S1999	0.156 ± 0.054	0.111 ± 0.470	0.3846	0.5351
	F1999 v. S2000	0.200 ± 0.034	0.170 ± 0.032	0.3927	0.5309
Kanawha[3]	F1999 v. S2000	0.227 ± 0.089	0.080 ± 0.054	2.0024	0.1571
Nashua	F1999 v. S2000	0.239 ± 0.045	0.089 ± 0.042	4.3742	0.0365*
Sutherland	F1999 v. S2000	0.241 ± 0.040	0.136 ± 0.073	1.1602	0.2814

[1] F = fall sampling and S = spring sampling.
[2] * indicates $\chi 2$ values that are significant at $P \leq 0.05$-level.
[3] No comparisons were made at Kanawha, Nashua, and Sutherland for Fall 1998 and Spring 1999 since no corn flea beetles from the overwintering generation were found in 1999.

Table 2. Proportions of *P. stewartii*-infested corn flea beetles testing positive by ELISA during the 1999 and 2000 growing seasons in Iowa

Location	Year	Minimum observed proportion[1]	Maximum observed proportion
Ames	1999	0.154 ± 0.050[2]	0.700 ± 0.048
	2000	0.078 ± 0.028	0.422 ± 0.074
Chariton	1999	0.311 ± 0.069	0.856 ± 0.037
	2000	0.156 ± 0.054	0.489 ± 0.075
Crawfordsville	1999	0.200 ± 0.034	0.700 ± 0.048
	2000	0.078 ± 0.028	0.533 ± 0.074
Kanawha	1999	0.184 ± 0.063	0.612 ± 0.059
	2000	0.133 ± 0.051	0.378 ± 0.051
Nashua	1999	0.239 ± 0.049	0.803 ± 0.049
	2000	0.100 ± 0.032	0.356 ± 0.050
Sutherland	1999	0.043 ± 0.043	0.511 ± 0.053
	2000	0.100 ± 0.032	0.333 ± 0.050

[1] indicates the minimum or maximum observed proportion of *P. stewartii*-infested corn flea beetles testing positive using ELISA during the corn growing season
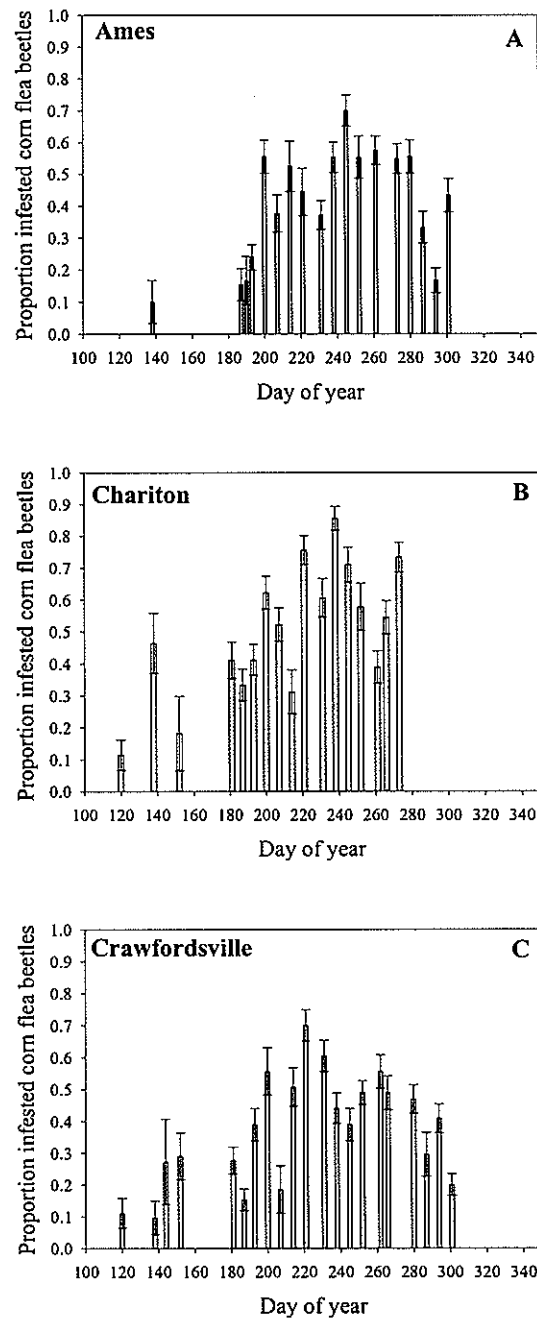[2] mean $\pm$ SE

Figure 1. Temporal dynamics in the proportion of *P. stewartii*-infested corn flea beetles at

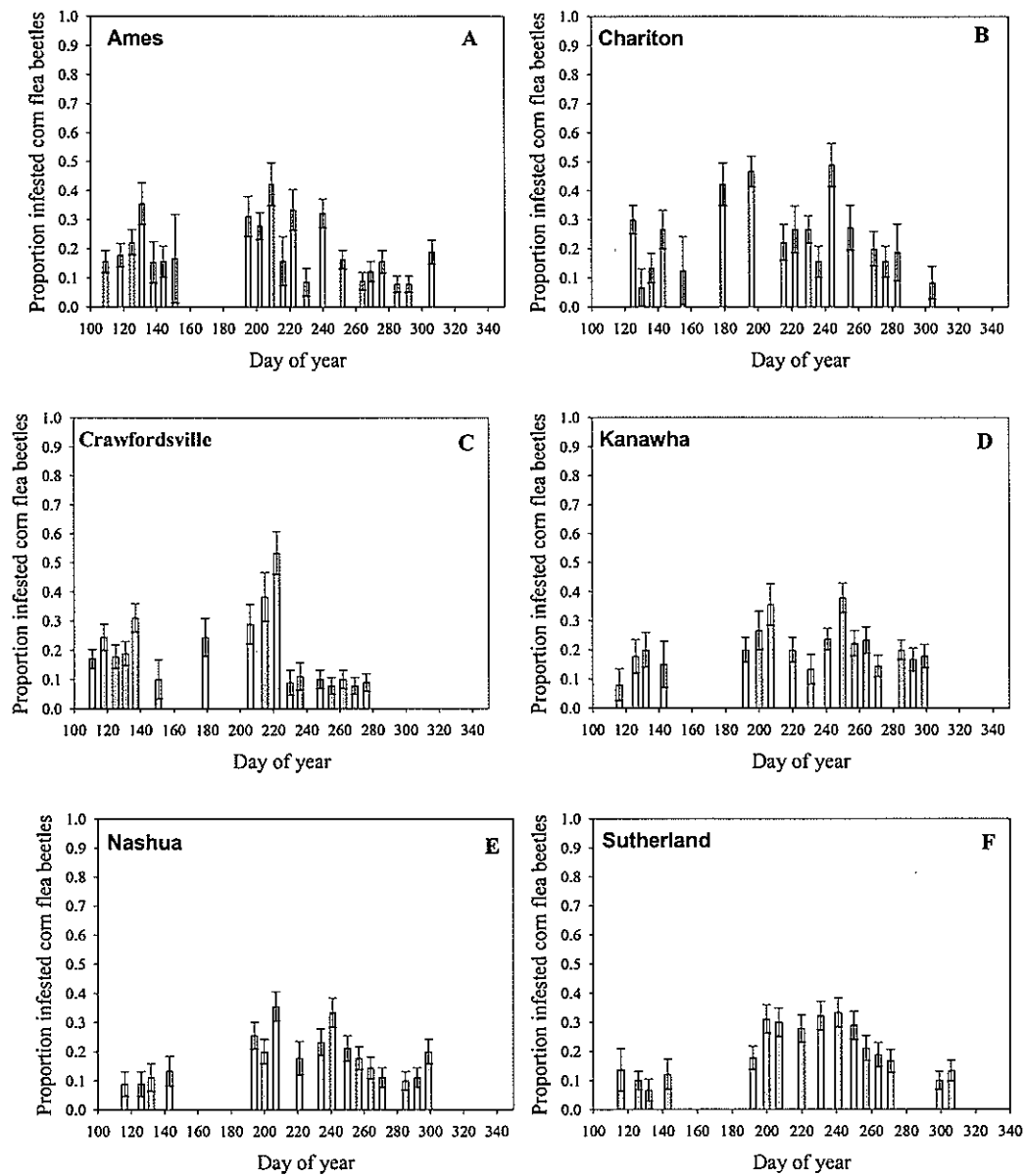(A) Ames, (B) Chariton, and (C) Crawfordsville, Iowa in 1999.

Figure 2. Proportions of *P. stewartii*-infested corn flea beetles at (A) Ames, (B) Chariton, (C) Crawfordsville, (D) Kanawha, (E) Nashua, and (F) Sutherland, Iowa in 2000.

CHAPTER 4

PRELIMINARY ANALYSIS OF WINTER WEATHER VARIABLES TO IMPROVE FORECASTING OF STEWART'S DISEASE OF CORN

Summary

Forecasting for Stewart's disease of corn, caused by the bacterium *Pantoea stewartii*, involves prediction of corn flea beetle (*Chaetocnema pulicaria*) survival based on winter air temperatures for December, January, and February (Stevens-Boewe System and Iowa State Method). Linear regression analysis was used to determine the relationship between air and soil (10 cm) temperature, for nine locations in Iowa, for the months of December, January, and February during 1998-1999 and 1999-2000. Coefficients of determination (R^2) indicated that 46 to 67% of the variability in soil temperature was explained by air temperature in 1998-1999, and 30 to 67% of this variability was explained in 1999-2000. The number of snowfall days during this period ranged from 14 to 33 in 1998-1999, and from 15 to 26 in 1999-2000. The number of snowcover days (days where snow was observed on the ground) ranged from 34 to 60 in 1998-1999 and from 29 to 64 in 1999-2000. The results from these analyses are that while winter air temperature fluctuated greatly during the two years, the soil temperature remained relatively static around −1.1°C.

Introduction

Stewart's disease of corn (*Zea mays*), caused by the bacterium *Pantoea stewartii*, is economically important in both sweet corn and seed corn (Pepper, 1967; Pataky, 1985; Carlton and Munkvold, 1995). The corn flea beetle, *Chaetocnema pulicaria* Melsh, is an important component in this pathosystem, as it is the overwintering habitat for *P. stewartii*,

and as a vector, it provides the primary mode of transmitting the bacterium (Rand and Cash, 1933; Elliot and Poos, 1940; Pepper, 1967; Dill, 1979). In seed corn fields, zero-tolerance phytosanitary regulations limit the export of seed corn from the United States to other countries if Stewart's disease is found in seed corn fields (Block et al., 1999). This is very important to seed corn producers considering seed corn inspections of Iowa corn seed production fields indicated 25% prevalence in 1998 and 58% prevalence in both 1999 and 2000 (Nutter et al., 1998; Esker and Nutter, 2000).

The sporadic nature of Stewart's disease from season-to-season, as shown by disease prevalence in Iowa during the years 1972-1999 (Figure 1), is the main reason that forecasting for Stewart's disease has been attempted since the early 1930's. This is because the winter environment plays a major role on the occurrence of Stewart's disease. Severe epidemics of Stewart's disease occurred in Connecticut in 1932 and 1933 following mild winters and this led Stevens (1934) to hypothesize that the sum of the mean monthly winter temperatures was related to corn flea beetle survival, and thus, related to the risk of Stewart's disease during the ensuing growing season. In the two epidemic years of 1932 and 1933, the sums of the mean monthly temperatures were found to be greater than 1.7°C or 100°F . Based on these limited observations, Stevens (somewhat arbitrarily) established that the threshold for severe epidemics was >-3.3°C or 90°F and that if the sum of the mean monthly temperatures was less than this, no epidemic would be expected.

Boewe (1949) modified the Stevens' system based on observations made in Illinois regarding the leaf blight (late summer) stage of Stewart's disease. In Boewe's system, there would be higher incidence (% of plants with Stewart's disease) and severity (% area of plant infected with Stewart's disease) of Stewart's disease when the sum of the mean monthly

temperatures for December, January, and February, reached a lower index threshold

compared to Stevens' system (Table 1). For example, Boewe's modifications indicated that

there would be moderate to severe Stewart's disease epidemics when the winter temperature

threshold index exceeded just -6.1°C (85°F). Boewe predicted low disease severity when the

index was between -8.9 and -6.1°C (80 and 85°F), and that no epidemics would occur when

the temperature index threshold was less than -8.9°C (80°F).

Castor et al. (1975), using the Stevens-Boewe system, created a computer program to

more quickly calculate the index for the Stevens-Boewe forecasting system. The program

analyzed temperature data and gave a preseason forecast based on weather information

obtained from one or more weather stations (county, or region for Pennsylvania). The use of

the computer program greatly increased the speed at which forecasts could be made and

delivered.

The Iowa seed corn production system is an economically important enterprise for the

state. Seed corn inspections handled by the Iowa Department of Agriculture and Land

Stewardship and are performed annually to look for foliar corn diseases in corn seed

production fields. The main piece of information obtained from the inspections is

prevalence, which is the number of fields where a foliar corn disease was found divided by

the total number of fields inspected. Forecasting for prevalence of Stewart's disease is not

what the Stevens-Boewe system was developed for. Because of this, the potential for

economic impact due to Stewart's disease could be increased, as the Stevens-Boewe system

may not accurately reflect the risk of prevalence of Stewart's disease.

In the early 1990's, research on modifying the Stevens-Boewe forecasting system to

predict disease prevalence (as opposed to severity) began at Iowa State University (Nutter et

al., 1998). Because the Stevens-Boewe warning system was not designed to predict the prevalence of Stewart's disease, it is not an appropriate tool for managing the disease in seed production fields (Nutter et al., 1998). Based upon 26 years of weather and disease data, a new threshold of 24°F (-4.4°C) was proposed for the individual winter months rather than a 3-month index (Nutter et al., 2001; Nutter et al., 1998). Instead of calculating the sum of the average winter temperatures as was done by Stevens and Boewe, the Iowa State Method assigns a risk score based on the number of winter months in which mean monthly temperatures exceed 24°F (-4.4°C) (Table 2). If two or three months exceed this minimum temperature threshold, a moderate to severe risk of high Stewart's disease prevalence would be expected. A low risk would be expected if one month exceeded the temperature threshold, and essentially no risk if zero months were above the 24°F threshold. The Iowa State Method accurately predicted all eight high disease prevalence years (1972 to 1997) when the actual prevalence of Stewart's disease was approximately 9% or higher (Figure 1).

It has been suggested that air temperature alone may not be the best weather variable to forecast the prevalence of Stewart's disease of corn. It is important to determine if other weather variables besides air temperature can better predict the overwintering survival of corn flea beetles. Because corn flea beetles are thought to overwinter in the soil in grassy areas surrounding fields, other variables such as soil temperature, snowfall amount, or snowcover duration may more accurately measure the 'true' environment that corn flea beetles are subjected to rather than air temperature alone. Therefore, the objective of this research was to examine the relationship among weather variables to begin to develop an even more accurate disease forecasting system for Stewart's disease of corn.

Materials and Methods

Mean daily air temperature (°C), snowfall amount (mm), and snowcover (mm) were obtained from nine locations (counties) in Iowa: Ames 5SE (Story County), Onawa 3 NW (Monona County), Cedar Rapids #1 (Linn County), Chariton (Lucas County), Washington (Washington County), Britt (Hancock County), Atlantic 1 NE (Cass County), New Hampton (Chickasaw County), and Primghar (O'Brien County) (Figure 2.3). These data were obtained from the Iowa Climate Review (State Climatologist, Iowa Department of Agriculture & Land Stewardship), a monthly publication of the Iowa Department of Agriculture & Land Stewardship. Soil temperatures (°C) (10 cm depth) for each day were also obtained for the same nine counties using data collected by the ISU Campbell Station Network (Iowa State University, www.pals.iastate.edu/campbell/), which corresponds to nine university-related automated weather stations in Iowa: Ames (Story County), Castana (Monona County), Cedar Rapids (Linn County), Chariton (Lucas County), Crawfordsville (Washington County), Kanawha (Hancock County), Lewis (Cass County), Nashua (Chickasaw County), and Sutherland (O'Brien County).

The predicted risk was determined for each location using the Stevens-Boewe (Table 1) and Iowa State Method (Table 2) using air temperature data for the months of December, January, and February in each of the two winter seasons. Air and soil temperature, snowfall amount and depth, and snowcover duration were collected for the period of December 1 to February 28 (29) in 1998-1999 and 1999-2000, because this is the period that is the basis for the Stevens-Boewe (Stevens 1933 and 1934; Boewe, 1948) and ISU Forecast Methods (Nutter et al., 1998).

Regression analysis was used to quantify the relationship between air temperature and soil temperature to determine the predictive value of air temperature on soil temperature. Mean daily air temperatures were regressed with mean daily soil temperatures. Models were evaluated for goodness of fit based upon the coefficient of determination (R^2), the standard error of the estimate of Y (SEE_y), and the slopes and intercepts were used to test for linear relationships.

Results

In 1999, the predicted risk of Stewart's disease in Iowa ranged from no risk in four counties to a low-to moderate risk in five counties using the Stevens-Boewe system (Table 3). The predicted risk using the ISU Method was moderate-to high in all nine counties. In 2000, the risk of Stewart's disease ranged from no risk in three counties to a low-to moderate risk in two counties, to a moderate-to severe risk in two counties, to a severe risk in one county using the Stevens-Boewe system (Table 3), while the risk was low-to moderate in one county, to a moderate-to high risk in five counties to a high risk in three counties using the ISU Method.

There was a significant linear relationship between soil temperature and air temperature at all nine locations in both winters (December 1 to February 28 in 1998-1999, December 1 to February 29 in 1999-2000). Coefficient of determinations (R^2) ranged from 0.46 to 0.67 in 1999, indicating that only 46 to 67% of the variation in soil temperature was explained by air temperature (Figures 2, 3, 4). All slopes of the regression lines in 1999 were significantly less than one (t-values: 20.1 to 35.3, p < 0.0001), indicating that for each 1° decrease in air temperature, soil temperatures decreased by less than 1°. Intercepts were significantly different from zero (t-values: 3.5 to 8.6, p < 0.0001). Days with measurable

snowfall (> 0.25 mm) ranged from 14 to 33 days, while daily snowcover ranged from 34 to 60 days (Table 4).

Again in 2000, there was a significant linear relationship between soil temperature and air temperature with R^2 values ranging from 0.30 to 0.67, indicating that only 30 to 67% of the variation in soil temperature was explained by air temperature (Figures 5, 6, 7). All slopes were significantly less than one (t-values: 23.4 to 41.2, $p < 0.0001$), indicating that for every 1° decrease in air temperature, soil temperature decreased by less than 1°. Significant differences ($p = 0.05$) in intercepts values from zero were observed at Ames (t = 2.32, p = 0.0226), Cedar Rapids (t = 10.44, $p < 0.0001$), Chariton (t = 9.8, $p < 0.0001$), Crawfordsville (t = 12.22, $p < 0.0001$), Kanawha (t = 3.37 p = 0.0011), Lewis (t = 4.25, $p < 0.0001$), and Nashua (t = 3.35, p = 0.0012). Daily snowfall ranged from 15 to 26 days, while daily snowcover ranged from 29 to 64 days (Table 4).

Discussion

Survival of corn flea beetles is epidemiologically important to the Stewart's disease of corn pathosystems. The corn flea beetle is essential for continuation of the bacterium's lifecycle and transmission of the bacterium to corn plants in the next growing season. To effectively predict potential risks for Stewart's disease, the proper weather variables during the winter need to be examined that provides the best possible information.

Regression analysis indicated that daily mean air temperature can only explain 30 to 67% of the variability in daily mean soil temperature over nine locations during the two winters of 1998-1999 and 1999-2000. The original forecasting system of Stevens was based on using air temperature as a predictor of potential corn flea beetle survival (Stevens, 1934). If corn flea beetles are actually overwintering below the ground, then air temperature may

not be the best variable to use as a predictor, as it is more prone to fluctuations. While air

temperature fluctuated greatly during the winter months (ranging from approximately -20°C

to 15°C), the 10 cm soil temperature stayed relatively static when snowcover was present,

hovering around -1.1°C (30°F). Less extreme temperatures and fluctuations compare to air

temperature was likely due to an insulation effect due to snowcover. Rosenberg et al. (1983)

have reported that as soil depth increases, the soil temperatures remain much more constant,

regardless of surface temperatures. Also, their research compared the impact of a sod layer

versus bare soil and it was observed that there was a damping influence on the sod-covered

soil, with fluctuation in soil temperatures significantly reduced compared to the bare soil.

The same principle seems to be at work here, as the 10 cm soil temperature remains

relatively static, due to an insulating effect from snow.

Refinement of the Iowa State Method by examination of the relationships between

soil temperature, snowfall amount, snowcover duration, and air temperature during the winter

months may help to improve the accuracy of predicting Stewart's disease of corn. Coakley et

al. (1999) have cited numerous examples showing that most research on using weather

information in plant pathology emphasizes the use of a single meteorological variable to

make predictions concerning a specific host-pathogen system, whereas, in nature the

complexity and interactions of numerous variables are multifaceted. Including additional

weather variables, such as soil temperature and snowfall or snowcover may increase the

likelihood of more accurately predicting potential corn flea beetle survival.

Soil temperature may more accurately predict the potential survival of corn flea

beetles, as it is a less variable environmental variable and may more accurately reflect the

environment in which corn flea beetles overwinter. Most insects employ a supercooling

strategy to hibernate, whereby body fluid remains unfrozen, even when the surrounding temperature is below the freezing point (Heinrich, 1996). The supercooled state is unstable and exposure to ice crystals can instantly freeze internal fluids, thereby killing the organism. As long as the overwintering habitat minimizes the exposure risk for the insect (where the microhabitat is kept to near-freezing temperatures), there is a greater probability of that an organism will survive. This concept can be extrapolated from the effects of the environment that the probability an individual will survive or perish to the probability that populations of individuals are likely to survive or perish. High populations of surviving adult corn flea beetles translate into a greater risk for epidemics of Stewart's disease of corn. It is important to determine experimentally the temperature at which corn flea beetles succumb to the environment. For example, Lam and Pedigo (2000) quantified the critical temperature at which the bean leaf beetles (*Cerotoma trifurcata*) died (-5°C and –10°C).

To develop a more accurate risk system for Stewart's disease of corn, more knowledge is needed with respect to the overwintering habits that allow for corn flea beetle survival. This information can then be combined with improved knowledge of the growing season habits of corn flea beetles to provide better pre- and within-season management recommendations with respect to foliar insecticide spraying to reduce the beetle populations.

References

Carlton, W.M., and Munkvold, G.P. 1995. Corn Stewart's Disease. Iowa State University, Extension Bulletin. Ames, IA. Pm-1627.

Castor, L.L., Ayers, J.E., MacNab, A.A., and Krause, R.A. 1975. Computerized forecasting system for Stewart's bacterial disease on corn. Plant Dis. Reptr. 59: 533-536.

Coakley, S.M., Scherm, H., and Chakraborty, S. 1999. Climate change and plant disease management. Annu. Rev. Phytopathol. 37: 399-426.

Dill, J.F. 1979. Biology and Management of the Corn Flea Beelte, *Chaetocnema pulicaria* Melshheimer, Relative to the Incidence of Stewart's Disease in Corn. Ph.D. Thesis, Purdue University, West Lafeyette, IN. 167 Pages.

Elliot, C., and Poos, F.W. 1940. Seasonal development, insect vectors, and host range of bacterial wilt of sweet corn. J. Agric. Res. 60: 645-686.

Esker, P.D., and Nutter, F.W., Jr. 2000. Monitoring the dynamics of corn flea beetles infested with *Pantoea stewartii*. Phytopathology 90: S22.

Heinrich, B. 1996. The Thermal Warriors. Harvard University Press, Cambridge, MA.

Lam, W.F., and Pedigo, L.P. 2000. Cold tolerance of overwintering bean leaf beetles (Coleoptera: Chrysomelidae). Environ. Entomol. 29: 157-163.

Nutter, F.W., Jr., Rubsam, R.R., Taylor, S.E., Harri, J.A., and Esker, P.D. 2001. Geospatially referenced disease and weather data to improve site-specific forecasts for Stewart's disease of corn in the U.S. corn belt. Computers and Electronics in Agriculture (In press).

Nutter, F.W., Wegulo, S.N., Rubsam, R.R., Taylor, S.E., and Harri, J.A. 1998. Use of geospatially-referenced disease and weather data to improve site-specific forecasts for Stewart's disease of corn. Phytopathology 88: S68.

Pataky, J.K. 1985. Relationships among reactions of sweet corn hybrids to Goss= wilt, Stewart's bacterial wilt, and northern corn leaf blight. Plant Dis. 69: 845-848.

Pepper, E.H. 1967. Stewart's Bacterial Wilt of Corn, 4th Ed. Heffernan Press, Worchester, MA.

Rand, F.V., and Cash, L.C. 1933. Bacterial wilt of corn. USDA Tech. Bull. 362: 1-22.

Rosenberg, N.J., Blad, B.L., and Verma, S.B. 1983. Microclimate, The Biological

Environment. John Wiley & Sons, New York.

Stevens, N.E. 1934. Stewart's disease in relation to winter temperatures. Plant Dis. Reptr.

18: 141-149.

Table 1. The Stevens-Boewe disease forecasting system for Stewart's disease of corn

(Stevens, 1934; Boewe, 1948).

Index Value (°F and °C)	Stewart's Disease Prediction
100 or More (> 2.2 °C)	Severe
90 to 100 (-3.3 to 2.2 °C)	Severe
85 to 90 (-6.2 to -3.3 °C)	Moderate to Severe
80 to 85 (-8.8 to -6.2 °C)	Light to Moderate
Below 80 (< -8.8 °C)	Trace, at Most

Table 2. The Iowa State Method for disease forecasting for Stewart's disease of corn (Nutter

et al., 2001; Nutter et al., 1998).

Number of Months $\geq$ 24°F (-4.4 °C)	Predicted Risk
0	Negligible
1	Low to Moderate
2	Moderate to High
3	High

Table 3. Summary of the predicted risk in 1999 and 2000using the Stevens-Boewe system and ISU Method for Stewart's disease of corn. The 1999 and 2000 risks are based on air temperatures during Dec., Jan., and Feb.

Location (County)	1998/ 1999					1999/ 2000				
	Dec	Jan	Feb	SB[1]	ISU[2]	Dec	Jan	Feb	SB	ISU
Cass	29.5	20.6	34.4	LM[3]	MH[4]	27.3	25.4	34.6	MS	H
Chickasaw	27.6	13.5	30.2	NR	MH	25.4	18.1	30.0	NR	MH
Hancock	25.4	12.0	28.3	NR	MH	23.3	16.4	28.1	NR	LM
Linn	32.1	18.3	34.3	LM	MH	29.1	23.4	34.0	MS	MH
Lucas	29.9	19.3	34.7	LM	MH	27.5	24.6	33.9	MS	H
Monona	29.7	19.8	34.5	LM	MH	29.7	25.4	36.0	S	H
O'Brien	26.1	13.4	30.1	NR	MH	25.5	17.4	30.6	NR	MH
Story	29.5	16.3	32.9	NR	MH	26.7	21.4	32.3	LM	MH
Washington	32.7	20.3	34.6	LM	MH	27.4	21.3	33.0	LM	MH

[1] Stevens-Boewe Forecasting System
[2] Iowa State Method
[3] NR = negligible risk, LM = low-to moderate risk, MS = moderate-to severe risk, S = severe risk
[4] LM = low-to moderate risk, MH = moderate-to high risk, H = high risk

Table 4. Determined number of snowfall and snowcover days in 1998-1999 and 1999-2000

for nine counties in Iowa that are part of the ISU Campbell Network (Iowa

Climatological Review).

Location	Year	Snowfall days	Snowcover days
Cass	1998-1999	18	34
	1999-2000	18	29
Chickasaw	1998-1999	17	56
	1999-2000	19	64
Hancock	1998-1999	33	60
	1999-2000	26	62
Linn	1998-1999	29	55
	1999-2000	23	58
Lucas	1998-1999	14	41
	1999-2000	15	39
Monona	1998-1999	20	53
	1999-2000	15	34
O'Brien	1998-1999	19	57
	1999-2000	15	59
Story	1998-1999	28	54
	1999-2000	20	57
Washington	1998-1999	21	43
	1999-2000	17	51

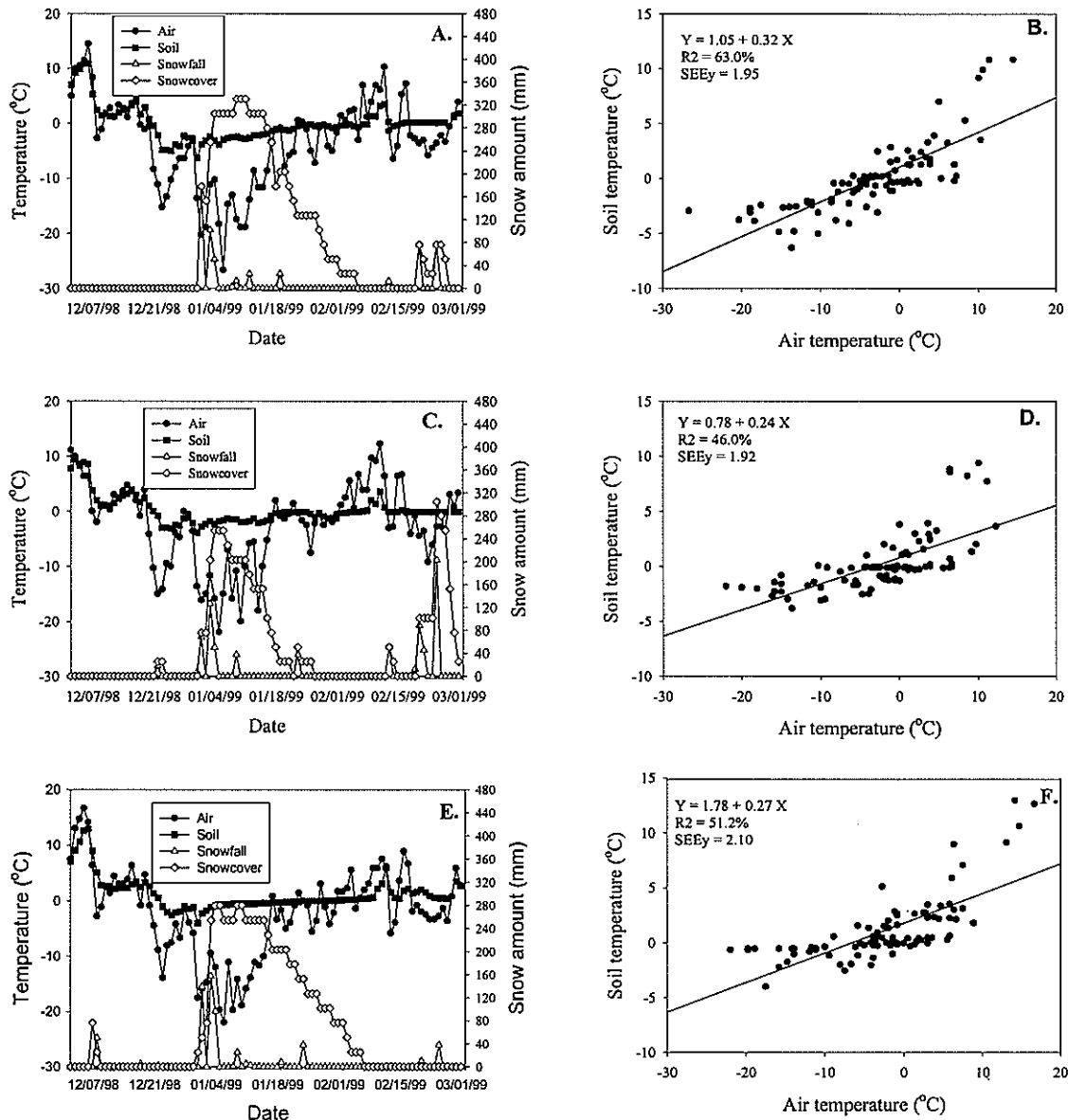**Figure 1.** Prevalence of Stewart's disease of corn in Iowa, 1972-1999.

Figure 2. Daily air temperature, soil temperature, snowfall, and snowcover information for
(A) Ames, (C) Castana, and (E) Cedar Rapids, Iowa in 1998-1999 for the period 1
December through 28 February. Simple linear regression plots for soil
temperature versus air temperature for (B) Ames, (D) Castana, and (F) Cedar
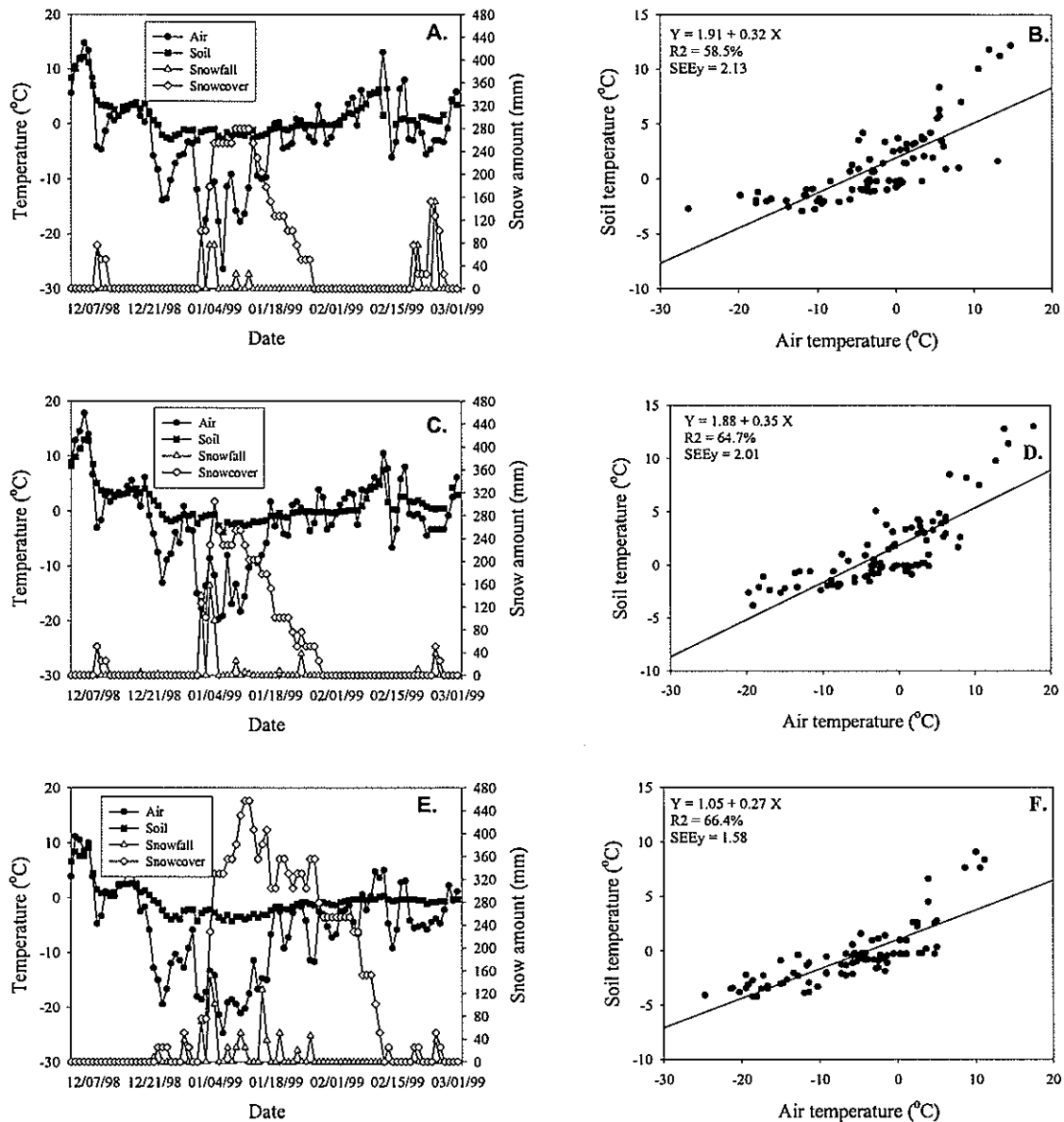Rapids during the same period.

Figure 3. Daily air temperature, soil temperature, snowfall, and snowcover information for (A) Chariton, (C) Crawfordsville, and (E) Kanawha, Iowa in 1998-1999 for the period 1 December through 28 February. Simple linear regression plots for soil temperature versus air temperature for (B) Chariton, (D) Crawfordsville, and (F) Kanawha during the same period.
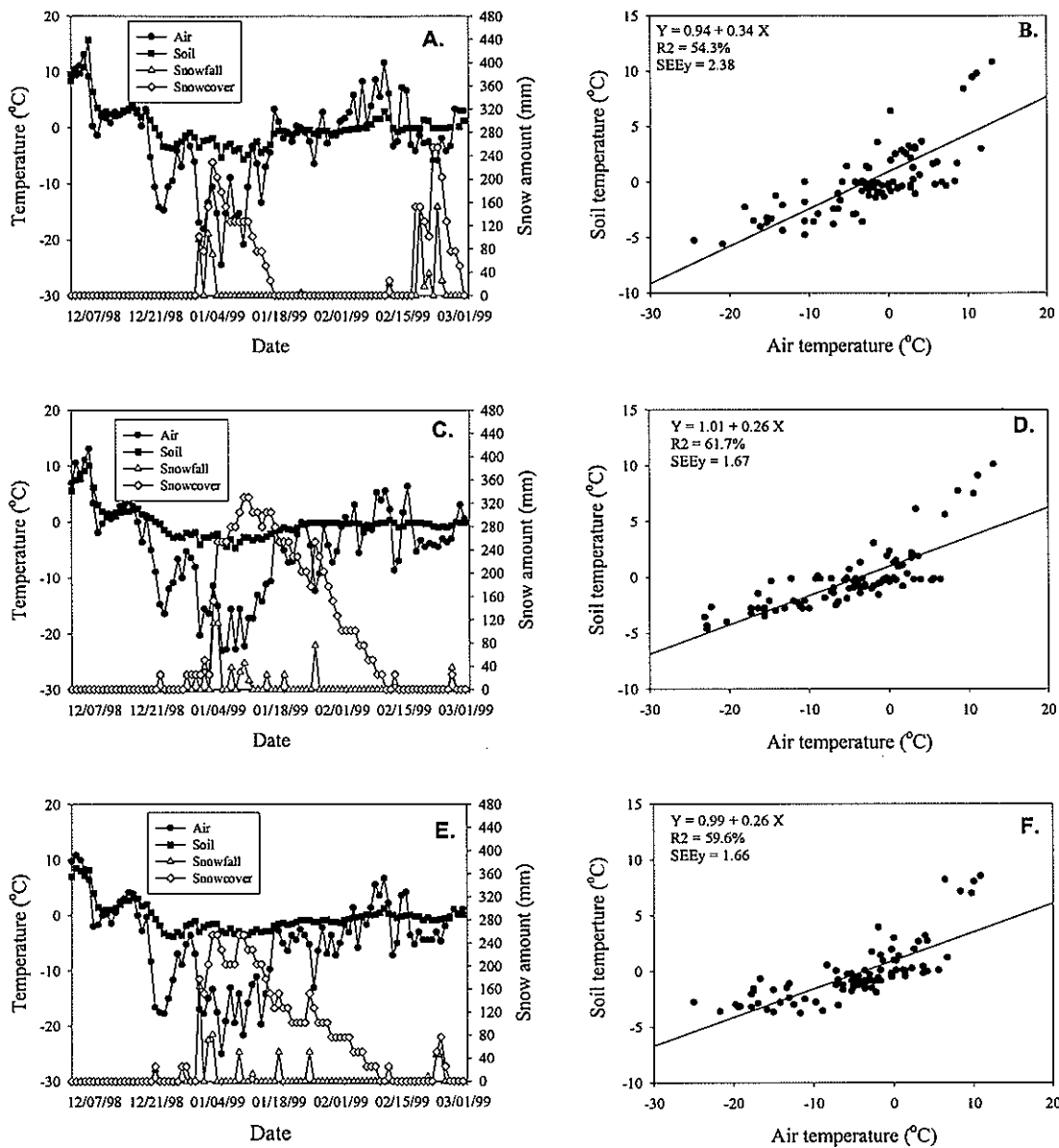
Figure 4. Daily air temperature, soil temperature, snowfall, and snowcover information for

(A) Lewis, (C) Nashua, and (E) Sutherland, Iowa in 1998-1999 for the period 1

December through 28 February. Simple linear regression plots for soil

temperature versus air temperature for (B) Lewis, (D) Nashua, and (F) Sutherland
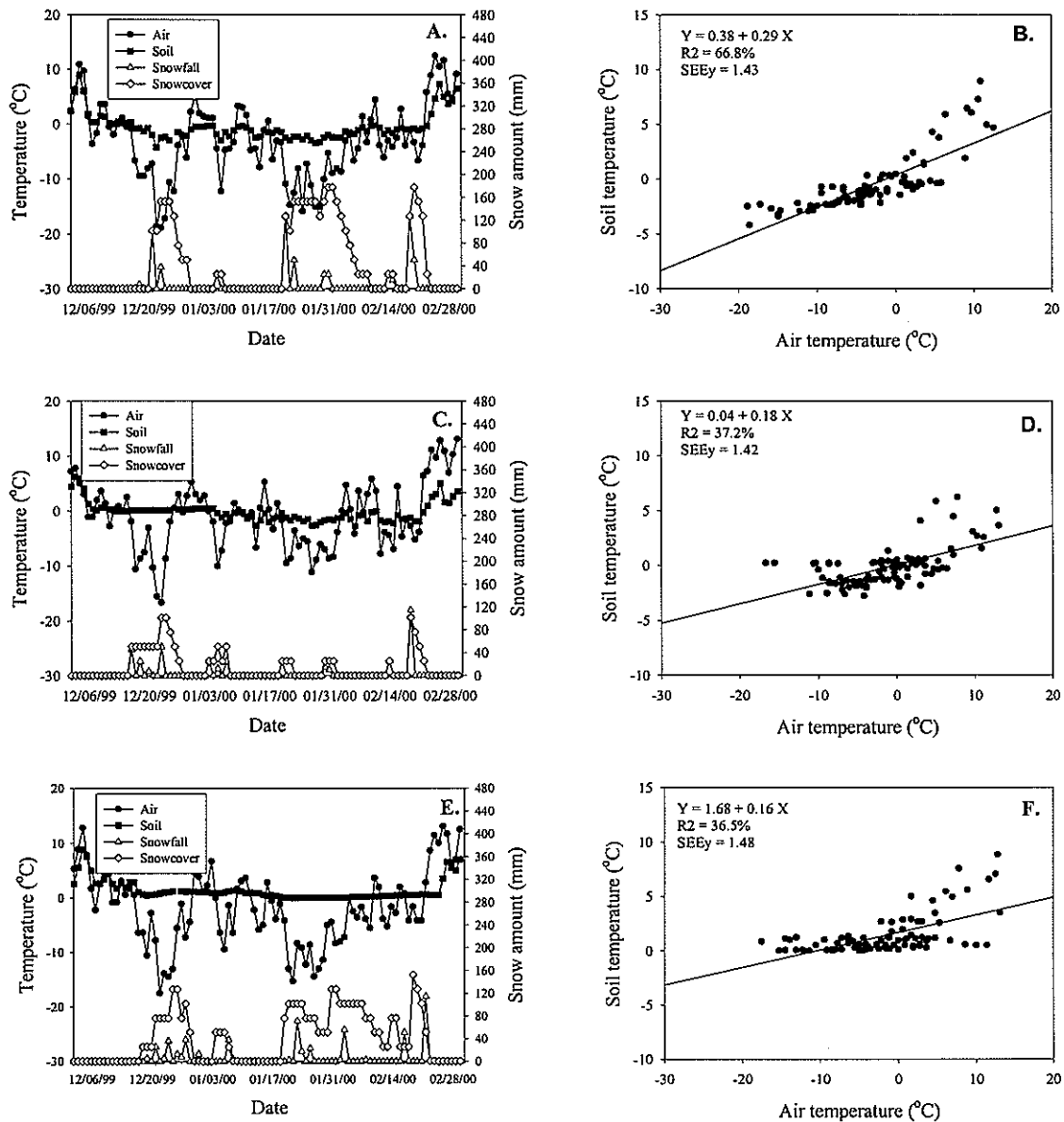
during the same period.

Figure 5. Daily air temperature, soil temperature, snowfall, and snowcover information for

(A) Ames, (C) Castana, and (E) Cedar Rapids, Iowa in 1999-2000 for the period 1

December through 29 February. Simple linear regression plots for soil

temperature versus air temperature for (B) Ames, (D) Castana, and (F) Cedar
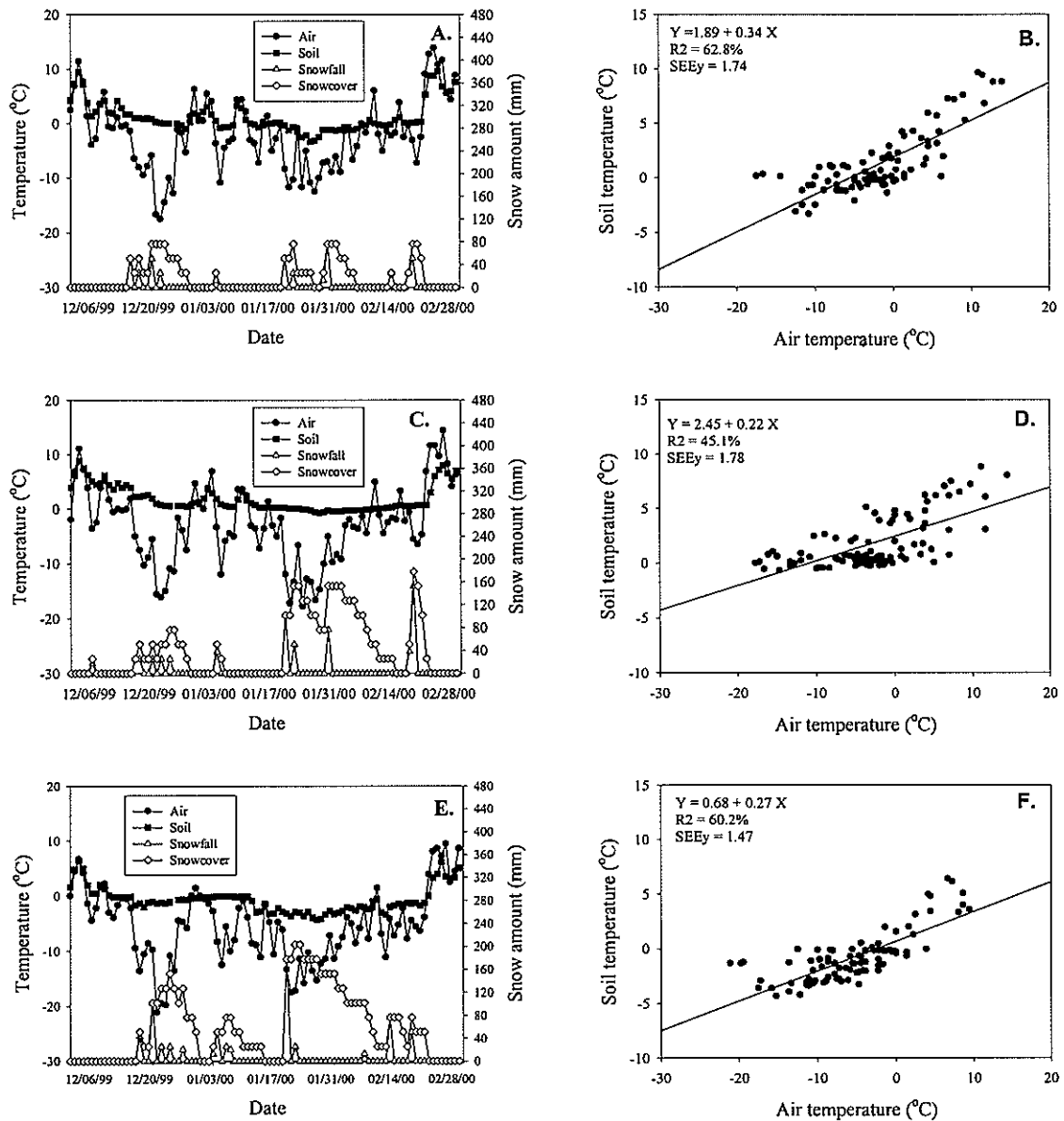
Rapids during the same period.

Figure 6. Daily air temperature, soil temperature, snowfall, and snowcover information for

(A) Chariton, (C) Crawfordsville, and (E) Kanawha, Iowa in 1999-2000 for the

period 1 December through 29 February. Simple linear regression plots for soil

temperature versus air temperature for (B) Chariton, (D) Crawfordsville, and (F)
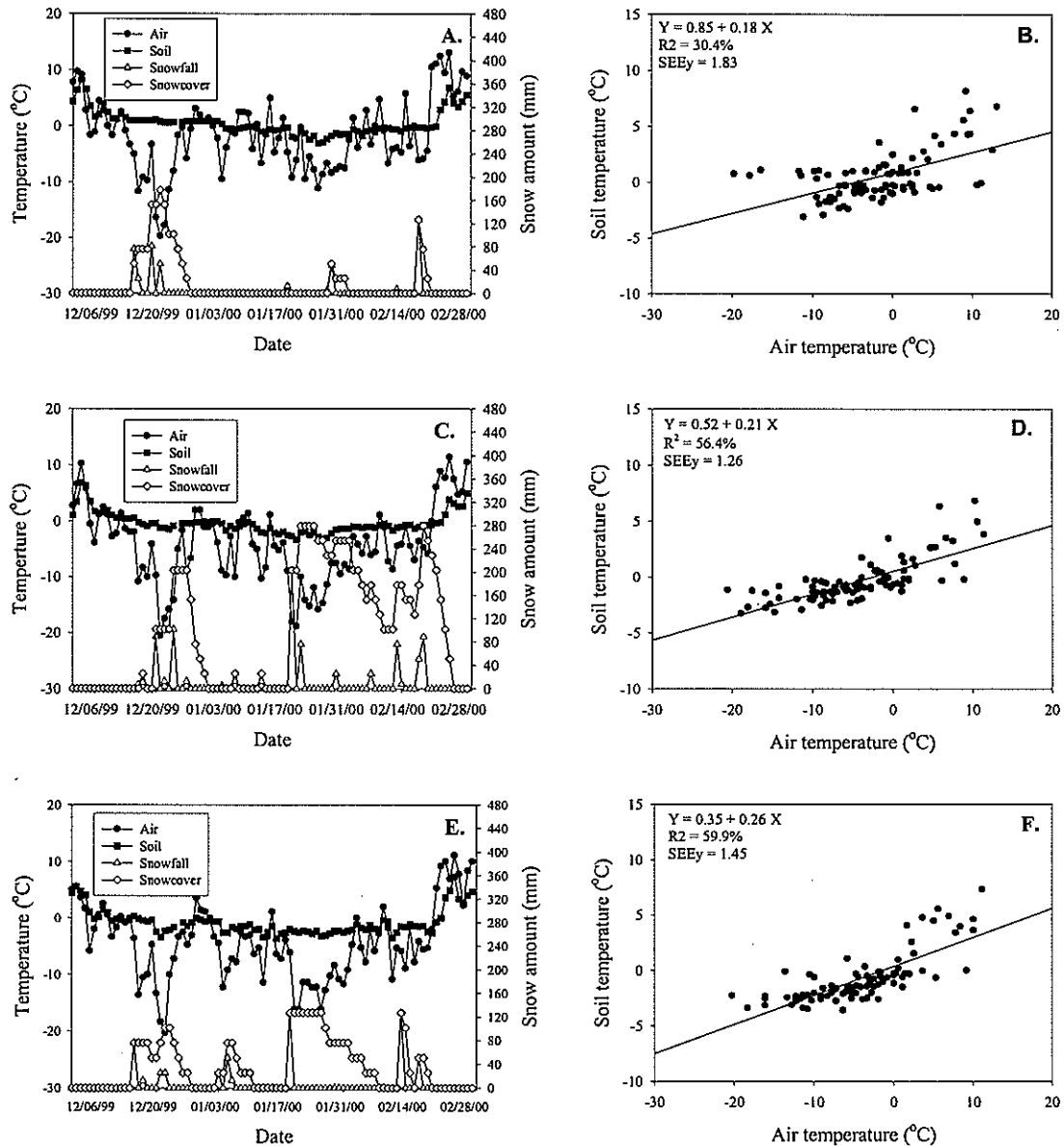
Kanawha during the same period.

Figure 7. Daily air temperature, soil temperature, snowfall, and snowcover information for

(A) Lewis, (C) Nashua, and (E) Sutherland, Iowa in 1999-2000 for the period 1

December through 29 February. Simple linear regression plots for soil

temperature versus air temperature for (B) Lewis, (D) Nashua, and (F) Sutherland

during the same period.

Appendix

Calculations made between °C and °F for Stewart's disease of corn need further explanation. It is apparent that 90°F does not equal -3.3°C when calculating the sum of the mean monthly temperatures for December, January, and February, but however, this is the proper calculation when summing these monthly temperatures. To provide a better understanding, an example will be presented.

The calculation of going from °F to °C is given here:

$$°C = \frac{°F - 32}{1.8}$$

The following table gives the sample calculation for determining predicted risk using the Stevens-Boewe system.

Month	Temperature (°F)	Temperature (°C)
December	32	0
January	27	-2.8
February	34	1.1
Sum	**93**	**-1.7**

CHAPTER 5

CONCLUSIONS/DISCUSSION

This thesis investigated the biology and importance of the corn flea beetle vector and its role in the Stewart's disease of corn pathosystem. This was accomplished by determining the number of corn flea beetle generations that occur in Iowa and by quantifying the proportions of those populations found to be infested with the causal agent of Stewart's disease, *Pantoea stewartii*. In addition, a preliminary study was conducted to determine how soil temperature was influenced by air temperature and how this may be applied to forecasting for Stewart's disease of corn. Research using yellow sticky cards and sweep netting demonstrated that there are overwintering, first, and second field generations of the corn flea beetle in Iowa. It was also observed that there was a period during June of both 1999 and 2000 when corn flea beetles were not found, which is important new management information. This research has also demonstrated that the incidence of *P. stewartii*-infested corn flea beetles can be monitored by ELISA testing and that the incidence fluctuates greatly throughout the corn growing season. The initial level of inoculum (*P. stewartii*-infested corn flea beetles in the adult overwintering generation) does not remain static during the spring as was previously hypothesized. This signals that additional research is needed concerning the mechanisms of fluctuation in the proportion of beetles infested with *P. stewartii*.

Finally, environmental variables other than just air temperature may be important in predicting the overwintering survival of corn flea beetle populations. For example, predicting the risk of Stewart's disease of corn might be improved by including other environmental variables such as soil temperature, snowfall, or snowcover duration in order to provide more accurate seasonal and site-specific risk predictions (forecasts). Making risk

information immediately available to seed corn producers via the internet will also help to facilitate the timely delivery of new risk information concerning Stewart's disease.

The development of positive and negative degree-day models would be extremely useful in predicting and managing within season corn flea beetle populations. The use of positive degree-days models would allow disease managers to predict when the overwintering generation is emerging from grassy areas adjacent to corn fields, when the initial corn flea beetles of the first and second generations are likely to emerge, and when peak population levels will occur. Negative degree-day models would allow researchers to predict when corn flea beetles will go into quiescence in the fall, and may also be used to model the effects of winter climate on the survival of corn flea beetle population. This information would be very useful for seed corn producers to make more timely disease management decisions, including when and where to plant corn seed (low risk sites and dates), whether or not insecticide seed treatments are needed, and when and where foliar insecticide sprays are likely to be needed to reduce corn flea beetle populations.

Another area of research that is needed concerns the lack of information regarding the influence of the microclimate on corn flea beetle population dynamics during the growing season. The 1999 and 2000 growing seasons, while being similar with respect to the number of corn flea beetle generations, had distinctly different within season population dynamics. The influence of microclimate during the growing season influences the corn flea beetle populations, but information is lacking concerning which environmental factors (i.e. air temperature, rainfall, etc.) can best account for changes in corn flea beetle population dynamics. This information will help us to devise improved sampling protocols which will

allow researchers to better predict changes in corn flea beetle populations within the corn growing season.

ELISA was used to test for the incidence of *P. stewartii*-infested corn flea beetles. This is just one method to determine the presence/absence of *P. stewartii* within individual members of the corn flea beetle population. Molecular-based techniques, such as polymerase chain reaction (PCR), could be applied with greater sensitivity to improve the detection of *P. stewartii*-infested corn flea beetles.

Also of interest is the possibility is that the corn flea beetle is not the only insect organism that can transmit *P. stewartii*. Some very preliminary work suggests that bean leaf beetles and cucumber beetles may also be sources of *P. stewartii* inoculum. Research from the 1920's and 1930's suggest that the spotted cucumber beetle is also a potential vector of the Stewart's disease bacterium. Experiments quantifying the relative importance of these potential vectors need to be addressed.

Another area of importance involves quantifying the transmission efficacy of corn flea beetles in relation to other insect vectors. For example, if 25% of the corn flea beetle population was found to be infested with *P. stewartii* by ELISA, would we expect that entire 25% to successfully transmit the bacterium? Acquisition and transmission studies conducted in the greenhouse and growth chamber (under controlled conditions) are needed.