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Quantifying the aggressiveness, temporal and spatial spread of *Pantoea stewartii* in sweet corn

by

Lu Liu

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Sustainable Agriculture

Program of Study Committee:
Forrest W. Nutter, Jr., Major Professor
Mark L. Gleason
Alison E. Robertson
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Iowa State University

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CHAPTER 1.

Introduction

ABSTRACT

Stewart's disease, caused by *Pantoea stewartii* (Smith), can cause severe economic damage to seed and sweet corn crops due to phytosanitary regulations that prevent the export of seed corn, as well as causing direct reductions in yield. To date, there are no quantitative data on the aggressiveness of *P. stewartii* isolates at the plant scale, nor on the temporal and spatial spread of this pathogen in the field. To quantify and compare the effect of temperature, leaf position (age) and isolate on the aggressiveness of *P. stewartii* isolates, growth chamber experiments were conducted using the sweet corn variety Jubilee. The aggressiveness of 13 *P. stewartii* isolates was quantified and compared by measuring incubation period, rate of lesion expansion/day, and time to leaf death. To study the effect of temperature, plants at the V7 growth stage were inoculated with the following isolates of *P. stewartii*: Rif 9A, a rifampicin-naldixic acid resistant isolate; and either ES 9211 or ES 9245 (both wild types). This was done by inoculating single leaves with 2 of the 3 isolates, one isolate on each side of the leaf midrib. There were 24 plants/ replication, and experiments were performed twice at each of 5 temperatures (21° C, 24° C, 27° C, 30° C, and 33° C). To study the effect of isolates, growth chamber experiments were conducted at what was found to be the optimal temperature (30˚ C). Sweet corn plants (variety "Jubilee") were inoculated at the V8 growth stage with 12 wild-type *P. stewartii* isolates and Rif 9A. Both sides of the mid-rib of 4 leaves per plant were inoculated with a single isolate of *P. stewartii*. There were

5 corn plants per isolate and 65 plants per replication. Experiments were performed twice. Lesion expansion (both acropetally and basipetally), were measured beginning on the day that lesions were first visible, and continued at 24-hour intervals until leaves were 100% senesced. The incubation period was shortest at 30° C, and rate of lesion expansion (averaged over isolates) was also fastest at this temperature. There were only small differences among isolates on components of aggressiveness. Of the 3 leaf positions (leaf ages) tested, the pathogen was least aggressive on the fifth true leaf. Isolate ES 0601 expanded significantly faster than other isolates. In these experiments, *P. stewartii* was most aggressive on the seventh and eighth leaf positions and least aggressive on the fifth leaf. To quantify the temporal and spatial spread of this pathogen, field experiments were conducted in 2007 in Ames, IA. Seeds (variety Jubilee) treated with insecticide (Cruiser) and seeds not treated with insecticide were planted in field plots (8 rows x 91 cm), with 25 plants per row planted at 30 cm spacing. The center six plants in each plot were inoculated with a double-marked rifampicin and nalidixic acid resistant isolate of *P. stewartii* to (Rif 9A). Feeding scars caused by the primary insect vector (corn flea beetle) were sampled from each field plot every 6 to 10 days, beginning 37 days after inoculation and continuing until 16 August. The double-marked isolate of *P. stewartii* was first detected in field samples on 13 July. Incidence of *P. stewartii*-infected plants was 4% in seed-treated plots compared to 4% to 6.7% in non-treated plots. By the end of the season, mean incidence for the insecticide-treated plots was 14.2 ± 3.15%, and mean incidence within non-treated plots was 18.5 ± 1.92%. The exponential model best fit the *P. stewartii* progress curves, but there was no statistical difference between the mean rate of plant-to-plant spread over time in nontreated versus insecticide

seed-treated plots (0.0105± 0.0010 log units per day versus 0.0089 ± 0.0019 log units per day, respectively). The spatial pattern of *P. stewartii*-infected plants (based on ordinary runs analysis) was random for all plots and all sampling times.

LITERATURE REVIEW

History and occurrence of *Pantoea stewartii* subsp. *stewartii* (syn. *Erwinia stewartii*)

In the late 1880s, T. J. Burrill described a new bacterial disease of corn (Burrill, 1887). F. C. Stewart was the first to isolate the causal bacterium in Long Island, New York in 1895, however, he did not assign a Latin binomial to the causal agent (Stewart et al., 1897). E. F. Smith later described the bacterium and gave it the name *Pseudomonas stewarti* (Smith) (Smith, 1898). Later, the name was changed to *Erwinia stewartii* (Smith) (Dye, 1963). Most recently, the name *Pantoea stewartii* (Smith) has been proposed, based upon the results of DNA-DNA hybridization studies (Mergaert et al., 1993).

Pantoea stewartii, which has been reported to occur in many U.S. states (Connecticut, Illinois, Indiana, Iowa, Kentucky, Louisiana, Missouri, Nebraska, New York, North Dakota, Ohio, Pennsylvania, Wisconsin), is also present in other parts of the world, including Austria, Brazil, Canada, China, Costa Rica, Greece, Guyana, Malaysia, Mexico, Peru, Poland, Puerto Rico, Romania, Russia, Thailand, and Viet Nam (EPPO, 2008).

Importance of *Pantoea stewartii* subsp. *stewartii*

Pantoea stewartii can have severe negative impacts on corn production (Stewart, 1897; Pepper, 1967; Suparyono and Pataky, 1989; Pataky, 2003). In the 1930s, Stewart's disease (also known as Stewart's wilt) was reported to cause substantial economic losses to sweet corn production in Connecticut (Stevens, 1934; Elliot, 1935), and occasionally to field corn as well. However, since resistant hybrids have been introduced, Stewart's disease has had little impact on the grain yield of resistant field corn hybrids (Pataky et al., 1990). It is important to note, however, that Stewart's disease continues to have significant negative impacts on susceptible sweet corn hybrids and seed corn inbreds. According to Suparyono and Pataky (1989), Stewart's disease was responsible for 40% to 100% yield loss in susceptible sweet corn hybrids, when infection occurred at or before the fifth leaf growth stage (V5).

In Iowa, the greatest economic damage from Stewart's disease is caused by the enforcement of zero-tolerance phytosanitary regulations that are imposed by many international trading partners (Pepper, 1967; McGee, 1995). When regulations are enforced, all seed produced from *P. stewartii*-infected seed corn fields may: (i) be sold as feed corn, at a greatly reduced price per bushel, rather than as seed corn, (ii) be redirected to the domestic seed corn market, or (iii) require seed companies to incur expensive laboratory fees to test seed corn lots for *P. stewartii* (Pepper, 1967; McGee, 1995).

Since seed corn field inspections began in Iowa in 1972, Stewart's disease was detected in Iowa in 21 of 32 years (through 2003). Peak prevalence of this disease was as high as 58% in both 1999 and 2000 (Figure 1) (Nutter et al., 1998; Esker and Nutter, 2001;

Nutter et al., 2002).

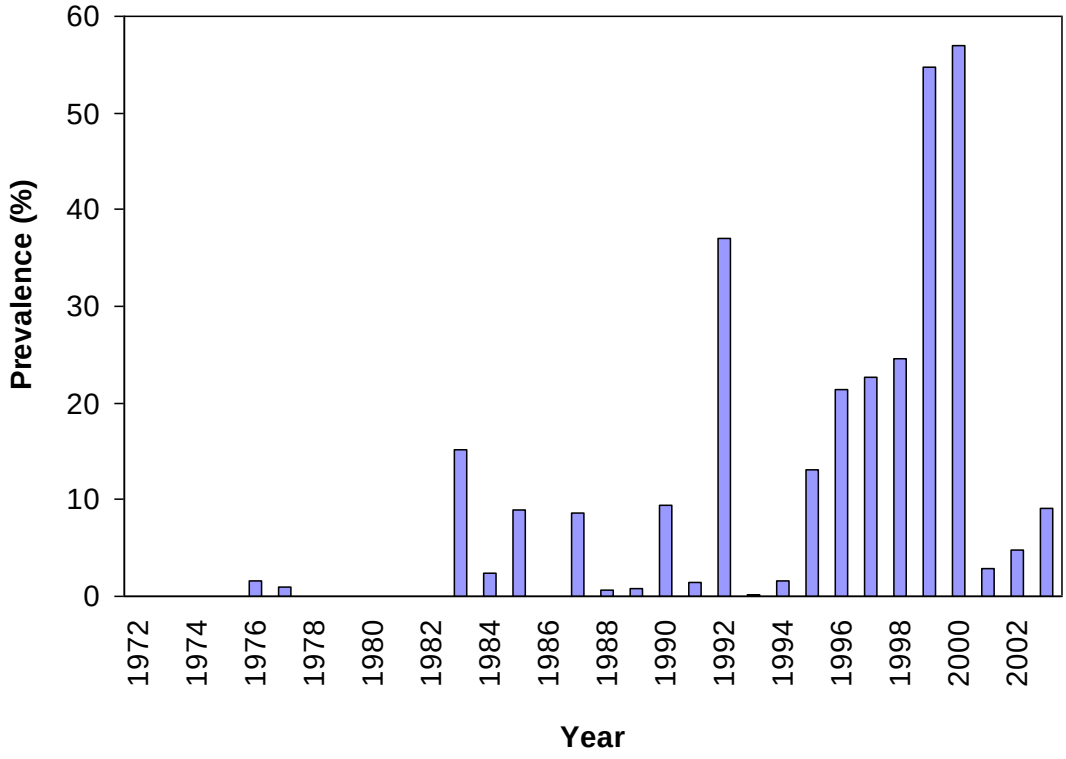

Figure 1. Prevalence of Stewart's disease in Iowa seed corn fields from 1972 to 2003.

Morphological and Genomic characteristics of *Pantoea stewartii*

Pantoea stewartii is described as a facultative, anaerobic, gram-negative, nonflagellate, nonspore-forming, nonmotile rod-shaped bacterium (Bradbury, 1967). Rods measure approximately 0.4 to 0.8 × 0.9 to 2.2 µm (Pepper, 1967; Pataky, 2003). In 2008, however, Herrera et al. p showed that *Pantoea stewartii* has flagella and EPS-dependent surface motility on semisolid agar. Transmission electron microscopy imagery confirmed the presence of thin flagella emerging laterally from each cell (Herrera et al.,

2008). Culture media can affect both colony color and growth. For example, colonies on yeast extract-dextrose-calcium carbonate agar are yellow and convex in appearance, whereas colonies on nutrient-glucose agar are cream-yellow to orange-yellow (Pataky, 2003).

The genome of *P. stewartii* is approximately 5 Mbp, which includes an array of plasmids (Perna et al., 2003). The GC content ranges from 50 to 80% (Coplin et al., 2002). Uniformity in pulsed field gel electrophoresis (PFGE) patterns of different *P. stewartii* isolates signifies the clonal nature of this species (Coplin et al., 2002).

Stewart's Disease Symptoms and Disease Development

Stewart's disease causes both a seedling wilt phase and a leaf blight phase (Stewart, 1897; Smith, 1914; Pepper, 1967; Mergaert et al., 1993). When corn plants are infected on or before the V5 stage, seedlings often become stunted, and die. The leaf blight phase occurs when leaves of older corn plants become infected, resulting in long, yellow-chlorotic streaks with irregular margins that expand parallel to the leaf veins. As lesions coalesce, entire leaves may become blighted (White, 1999). Both phases of Stewart's disease can reduce seed corn yields (Pataky et al., 1985; Pepper, 1967; Suparyono and Pataky, 1989).

Pantoea stewartii grows within the intercellular spaces of corn leaves, invades the xylem vessels, and adheres to cell walls. The pathogen then produces biofilms that accumulate in vessels, thereby obstructing water movement (Pataky 2003). Plants

subsequently become wilted, with chlorotic streaks (foliar lesions). Wilting is followed by plant death. The pathogen grows primarily basipetally within corn leaves (Koutsoudis et al., 2006). In older plants, the leaf blight phase lesions usually originate from the site of corn flea beetle feeding scars. The chlorotic water-soaked lesions are due to the loss of cell membrane function and accumulation of fluids in the leaf tissue due to vascular blockage (Pataky et al., 2003). Wilt symptoms are also the result of effector proteins, which are delivered into the corn cells by a Hrp type III secretion system (Frederick et al., 2001). An extracellular polysaccharide (EPS), known as stewartan, occludes xylem vessels during the leaf blight phase of the pathogenesis (Braun, 1982; 1990).

Seed-to-Seedling and Insect Transmission of *Pantoea stewartii*

Seed-to-seedling transmission. Although *Pantoea stewartii* was reported to be seedborne in 1933 (Rand and Cash, 1993), more recent research has shown that seed-to-seedling transmission is not an epidemiologically-important source of initial inoculum (Block et al., 1998). Seed-to-seedling transmission rates for *P. stewartii* are reported to be about 1 in 50,000 for seed obtained from systemically-infected, susceptible seed corn parent plants, and 1 in 20,000,000 from seed produced from resistant corn plants expressing symptoms of the leaf blight phase of Stewart's disease (Pataky, 2003). Although seed-to-seedling transmission is negligible, phytosanitary regulations continue to be strictly enforced to ensure that seed lots from Stewart's disease-infected seed corn fields are not exported to countries where Stewart's disease has not been reported. Seed lots arising from *P. stewartii*-infected fields, however, may be exported if seed lots are

sampled, tested by ELISA, and yield negative test results (Block et al., 1999).

Transmission by corn flea beetles. Rand and Cash (1933) reported that the corn flea beetle, *Chaetocnema pulicaria* (Melsheimer), was the primary vector of *P. stewartii*. In 1934, Elliott and Poos reported that *P. stewartii* can overwinter in the gut of corn flea beetles, and that overwintering *P. stewartii*-infested corn flea beetles were the most important source of initial inoculum for the seedling wilt phase of this disease. Based on their research, the corn flea beetle is generally recognized as the primary vector for *P. stewartii* dissemination. Several other insects such as the toothed flea beetle *(Chaetocnema denticulata* (Illiger)), corn rootworm larvae (*Diabrotica barberi* (Smith and Lawrence)), and the seed corn maggot (*Delia platura* (Meigen)), can acquire and transmit the bacterium. The latter three species, however, are of minor importance compared to the corn flea beetle.

Corn Flea Beetle

The corn flea beetle (*Chaetocnema pulicaria*) has several life cycles per year in Iowa: the overwintering generation and two generations per growth season (Esker et al. 2002). In Iowa, there is a beetle-free period, lasting 2 to 4 weeks in June, which begins with the death of the adult overwintering generation and ends with the emergence of the first summer (adult) generation. Generally, there are two growth peaks during summer that coincide with the two summer generations. Time between summer generations is approximately 30 days (Esker et al. 2002). The second summer generation becomes the overwintering generation. After corn fields senesce, corn flea beetles migrate to grass

borders that are adjacent to corn fields to overwinter. The mean acquisition feeding period for 50% of the corn flea beetle population to acquire *P. stewartii* is 36.5 ± 11.6 h and the mean transmission period for 50% of the corn flea beetle population to transmit *P. stewartii* is 7.6 ± 0.87 h (Menelas et al., 2006).

According to Esker et al. (2004), vertical yellow sticky cards placed 0.3 m above the ground had the highest *C. pulicaria* beetle capture efficiency, suggesting that this may be the most effective trap location to monitor corn flea beetle populations. The relationship between corn flea beetle population density and incidence of Stewart's disease was described by the equation y = 4.33 + 0.89 (x), where y is the maximum incidence (%) of the disease in each planting and x is the mean number of corn flea beetles per trap per day beginning 1 week after planting (Cook et al., 2005).

Management of Stewart's Disease

Successful management of Stewart's disease involves five integrated disease management principles: (i) exclusion, (ii) avoidance, (iii) eradication, (iv) protection, and (v) host resistance (Nutter, 2007).

Exclusion is defined as the use of management practices designed to keep a pathogen out of a crop production area, to keep initial inoculum (y_o) at zero (Nutter, 2007). For Stewart's disease, this means keeping *P. stewartii*-infected seed out of areas where the disease has not yet been reported to occur. Within the U.S., there are no exclusion regulations for Stewart's disease, but for most European and Asian countries, this

management principle is very important. Each year, substantial labor and money is spent to conduct field inspections and perform seed tests in order to meet phytosanitary regulations for Stewart's disease. These regulations stipulate that seed harvested from *P. stewartii*-infected fields and seed lots that test positive for Stewart's disease, should not be exported to the countries where Stewart's disease has not yet been reported.

Avoidance is defined as the use of management practices that reduce disease risk by avoiding pathogen inoculum or environmental conditions that minimize crop infection efficiency of y_o (Nutter, 2007). Specifically, choices concerning planting date and planting sites are made with regards to the risk for Stewart's disease, i.e., to avoid the overwintering generation of adult corn flea beetles, thereby reducing early season infection and spread of the pathogen (Menelas, 2003). Although delayed planting reduces the risk for *P. stewartii* infection, delayed planting may also adversely affect corn yields, because late planting can reduce yield potential (Pataky, 2003).

Eradication is defined as the use of management practices that reduce y_o at the source (Nutter, 2007). For this pathosystem, primary initial inoculum (in the form of *P. stewartii*-infested corn flea beetles) may be reduced by deploying seed and/or foliar insecticides to reduce early season disease incidence. However, a cost/benefit analysis should be done before these tactics are adopted at a regional or local scale. To date, quantitative data on the impact of seed and foliar insecticides on the temporal and spatial spread of *P. stewartii* is lacking.

Protection is defined as any tactic that is used to shield the crop (Nutter, 2007). Here it refers to insecticide seed treatment. Insecticide seed treatments have been reported to be

effective up to the fifth leaf growth stage (V5) of corn (Munkvold et al., 1996). This tactic would protect corn seedlings during the early wilt phase of the disease. After the V5 growth stage, foliar insecticides could be supplemented to further protect the crop for the rest of the growing season.

Another protective measure, host resistance, can reduce initial inoculum (y_o) or the rate of an epidemic (r) (Nutter, 2007). Host resistance involves finding resistance genes (or DNA) and incorporating these genes into corn inbreds and hybrids. There are several different levels of resistance to Stewart's disease in hybrids, ranging from highly resistant to highly susceptible (Pataky, 2003). Generally, corn inbreds are moderately to highly susceptible (Kuhar et al., 2002).

When integrated, the above disease management tactics can help to reduce the risk of epidemics of Stewart's disease in the field. However, another risk factor that also needs to be taken into account is the direct feeding injury caused by corn flea beetles. This insect can also cause serious injury to corn seedlings and leaves when population densities are high (Suparyono and Pataky, 1989).

Disease Forecasting

Accurately predicting the risk of Stewart's disease, prior to spring planting, can be of tremendous benefit to corn growers (Nutter et al., 2002; Esker et al., 2003). The theory behind Stewart's disease forecasting is that overwintering corn flea beetle populations are more likely to survive mild winters than severe winters (Stevens, 1934). The size of the

surviving corn flea beetle population is directly related to level of initial inoculum, because corn flea beetles provide an overwintering refuge for the bacterium, as well as a means of dissemination. It has been demonstrated that winter temperatures provide a good indicator of the potential for corn flea beetle populations to survive the intercrop period (winter) and can be used to measure the seasonal risk for this disease prior to planting (Stevens, 1934). Stevens developed the first Stewart's disease forecasting system, which was based on the sum of the mean monthly temperatures for December, January, and February. An index value (the mean sum of the three monthly means) greater than 90˚ F indicates there is a high risk of Stewart's disease in the ensuing season. If the index is less than 90˚ F, then the risk for Stewart's disease is nil or low.

Boewe (1949) modified Stevens' model and developed what is known as the "Stevens-Boewe" Model. This model predicts that when the sum of the same three mean monthly temperatures is lower than 80˚ F, the risk for the leaf blight phase of Stewart's disease is negligible. However, if the sum is higher than 90˚ F, the risk is severe. An index between 80˚ F and 85˚ F indicate low risk, and temperature sums between 85˚ F and 90˚ F indicate moderate disease risk.

Nutter et al. (1998) later improved the accuracy of forecasting Stewart's disease of corn in Iowa by developing a new model. The "Iowa State Model" is based on the mean monthly temperatures for December, January, and February separately; i.e., if the mean monthly temperature of only one of the three month is above 24˚ F, then there is a low risk for Stewart's disease. If two of the three months are above 24˚ F, then there is a moderate risk, and if all three months are above 24˚ F, then there is a high risk for Stewart's disease.

JUSTIFICATION

To date, there is no quantitative data concerning the effect of temperature on components of pathogen aggressiveness for different isolates of *Pantoea stewartii*. These components include infection efficiency, length of the incubation period, rate of lesion expansion, and time to leaf death. Baseline information is needed to detect the occurrence of future changes in pathogen aggressiveness or loss of host resistance. This information may also be used to assess the need for management tactics to reduce disease risk. Moreover, this information may also help corn growers and seed companies to understand the epidemiology of this pathosystem and predict the seasonal and site-specific risks for Stewart's disease.

Quantitative data concerning the temporal and spatial spread of *P. stewartii* in sweet corn is currently lacking. For field inspectors, knowledge concerning the spatial pattern of this disease would likely help to develop more efficient sampling protocols for field inspectors (Campbell and Madden, 1990). Finally, the effect of insecticide seed treatment on the temporal and spatial spread of *P. stewartii* is largely unknown. Therefore, the objectives of this study were to: i) quantify and compare the effect of temperature on the aggressiveness of *P. stewartii* isolates, ii) quantify and compare aggressiveness of different *P. stewartii* isolates collected in Iowa, and iii) quantify the temporal and spatial spread of *P. stewartii* within sweet corn fields.

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CHAPTER 2.

Quantifying and Comparing Disease and Pathogen Aggressiveness of *Pantoea stewartii* in Sweet Corn

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ABSTRACT

Stewart's disease, caused by *Pantoea stewartii*, can cause severe economic damage to seed and sweet corn crops. Economic damage results from direct losses caused by *P. stewartii* and from costs and losses associated with the enforcement of phytosanitary regulations. To date, quantitative data concerning the aggressiveness of *P. stewartii* isolates on corn is not available. To quantify and compare the effects of temperature, leaf position (age), and pathogen isolate on the aggressiveness of *P. stewartii*, growth chamber experiments were conducted using the sweet corn variety Jubilee. Isolate aggressiveness was determined for 13 *P. stewartii* isolates by quantifying several components of aggressiveness. The components evaluated were: infection efficiency, incubation period (day), rate of lesion expansion, and time to leaf death. To determine the effect of temperature on isolate aggressiveness, plants at the seventh leaf stage were inoculated with three different *P. stewartii* isolates: Rif 9A, a rifampicin-naldixic acid resistant isolate and two wild types, ES9211 and ES 9245, and incubated in growth chambers at constant temperatures of 21° C, 24° C, 27° C, 30° C, and 33° C. Infection efficiency was highest, incubation period was shortest, and rate of lesion expansion was fastest at 30° C compared to the other temperatures. To quantify pathogen

aggressiveness among isolates, growth chamber experiments were conducted at 30° C (the optimum temperature for the rate of lesion expansion). Sweet corn plants were inoculated at the eighth leaf stage with 12 wild-type *P. stewartii* isolates, plus a double-marked isolate (rifampicin-nalidixic acid resistant isolate, Rif 9A. Lesion lengths (both acropetally and basipetally) was measured daily, beginning when lesions were first visible, and lesion expansion measurements were discontinued when no further lesion expansion was possible (leaves were 100% senesced). Rates of lesion expansion were calculated based on the change in lesion lengths over time. Isolate ES 0601 had the fastest rate of lesion expansion. Of the 4 leaf positions tested in this experiment, *Pantoea stewartii* was most aggressive on the seventh and eighth leaf positions (the youngest leaves tested), and less aggressive on the fifth (oldest) leaf.

INTRODUCTION

In the late 1880s, T. J. Burrill described a new bacterial disease of corn (Burrill et al., 1887). The bacterium causing this new disease was first isolated by F. C. Stewart in Long Island, New York in 1895, however, the causal agent remained unnamed (Stewart et al., 1897) until E. F. Smith later described and named the bacterium (*Pseudomonas stewarti*) in 1898 (Smith, 1898). The name was subsequently changed to *Erwinia stewartii* (Smith) by Dye in 1963 (Dye, 1963). Recently, the name *Pantoea stewartii* (Smith) was proposed, based upon the result of DNA-DNA hybridization studies (Mergaert et al., 1993).

The causal agent of Stewart's disease, *Pantoea stewartii*, has flagella and EPS-dependent surface motility on semi-solid agar. Transmission electron microscopy imagery has confirmed the presence of thin flagella emerging laterally from the bacterium cells (Herrera et al., 2008). *P. stewartii* grows within the intercellular spaces of leaves, invades the xylem vessels, and produces biofilms that adhere to and accumulate in xylem vessels, resulting in the blockage of water movement within plants (Braun, 1982). Reproduction and movement of the pathogen within corn leaves occurs primarily in the basipetal direction (Koutsoudis et al., 2006).

Stewart's disease (also known as Stewart's wilt) has two distinct phases of pathogenesis: the seedling wilt phase and the leaf blight phase (White, 1999). The seedling wilt phase occurs when corn seedlings are infected on or before the fifth leaf (V5) growth stage, and results in seedling wilt, stunting, and seedling death (Dill, 1979; Pepper, 1967). The leaf blight phase generally occurs later in the growing season, when leaves of older plants become infected. Foliar symptoms begin as long, chlorotic streaks with wavy margins. The lesions expand longitudinally and often coalesce, resulting in leaf death (White, 1999). Both phases of Stewart's disease can reduce yield. The seedling phase primarily reduces plant stand (plants/m^2), whereas the leaf blight phase primarily reduces ear size and kernel weight (Pepper, 1967; Suparyono and Pataky, 1989; Pataky et al., 1995).

Stewart's disease was first reported to cause substantial economic losses in the 1930's in Connecticut (Stevens, 1934; Elliot, 1935). However, after resistant corn hybrids were introduced, Stewart's disease has had less impact on the grain yields of resistant hybrids (Pataky et al., 1990). Stewart's disease, however, continues to exert a large negative

impact on susceptible sweet corn hybrids and seed corn inbreds (Suparyono and Pataky, 1989; Esker et al., 2006). According to Suparyono and Pataky (1989), Stewart's disease can cause 40% to 100% yield loss in susceptible sweet corn hybrids. This disease can be particularly devastating when sweet corn fields are infected at or before the V5 growth stage.

In Iowa, the greatest negative economic impact of Stewart's disease is due to zero tolerance phytosanitary regulations that are imposed by many international trade partners, and result in added production costs (Pepper, 1967; McGee, 1995). Producers pay expensive fees for field inspections for seed certification, as required by the Iowa Department of Agriculture and Land Stewardship. Additional fees may be required to pay for expensive phytosanitary seed tests to ensure that *P. stewartii* is below detectable levels in seed corn lots destined for the export market (Pepper, 1967; McGee, 1995). Additional production costs are also incurred by hybrid field corn producers, who must pay more to purchase resistant hybrids.

The primary insect vector for winter survival and pathogen transmission is the corn flea beetle (*Chaetocnema pulicaria* (Melsheimer)) (Rand and Cash, 1933). If corn flea beetle population densities are high in the spring, then the risk for Stewart's disease is also high. In high risk years, seed and foliar insecticides may be required to reduce disease risk (Stevens, 1934; Esker et al., 2002). Data obtained from seed corn field inspections performed from 1972 through 2003 (under the auspices of the Iowa Department of Agriculture and Land Stewardship) indicate that Stewart’s disease has been detected in Iowa seed corn fields in 21 out of 32 inspection years (65.6%). Peak prevalence of this disease has been reported to be as high as 58% (in both 1999 and 2000)

(Esker et al., 2006) (Figure 1).

In 1934, Elliott and Poos (1934) reported that *P. stewartii* overwinters within the gut of the corn flea beetle (*Chaetocnema pulicaria*), and that the overwintering population of *P. stewartii*-infested corn flea beetle is (epidemiologically) the most important source of initial inoculum to initiate the early-season wilt phase of this disease (Elliott and Poos, 1934). Other insect species have also been reported to be potential vectors of *P. stewartii*, albeit with reduced transmission efficiencies. These include the toothed flea beetle (*Chaetocnema denticulata* (Illiger)), the corn rootworm larva (*Diabrotica barberi* (Smith and Lawrence)), and the seed corn maggot (*Delia platura* (Meigen)). However, these vectors are considered to be of minor importance compared to the corn flea beetle (Rand and Cash, 1933).

The relative aggressiveness of pathogen isolates can be assessed by quantifying one or more components of pathogen aggressiveness (Webb and Nutter, 1997). These components are: infection efficiency, incubation period, rate of lesion/pathogen expansion, rate of reproduction, and time-to-death. Infection efficiency is defined as the number of successful infections per unit of pathogen inoculum, and is expressed as a percentage. Incubation period is the time (h) from inoculation until lesions (symptoms) are apparent. Rate of lesion expansion is the change in lesion size or extent in the host per unit of time. Rate of reproduction is the rate of production of new pathogen dispersal units. Time-to-death is the period of time from inoculation of the host to host death.

Quantitative data concerning the impact of temperature, leaf position (leaf age) and pathogen isolate on components of pathogen aggressiveness are needed to assess the

seasonal and biological risk for Stewart's disease epidemics. Little is known about the variation in pathogen aggressiveness that may exist among *P. stewartii* isolates, as affected by temperature and leaf age. To date, there is no quantitative data concerning components of aggressiveness for *P. stewartii*, which would serve as a reference (or baseline) to detect future shifts in pathogen aggressiveness and/or the emergence of pathogen isolates resistant to insecticides and/or host resistance. Therefore, the objectives of this study were to: (i) quantify the effects of temperature and leaf age on the components of aggressiveness for *Pantoea stewartii* isolates in sweet corn, and (ii) quantify and compare the relative differences in components of pathogen aggressiveness for thirteen isolates of *P. stewartii* from Iowa.

MATERIALS AND METHODS

Plant preparation. Sweet corn variety Jubilee (Syngenta Seeds Inc.) was planted in the greenhouse in 12.5-cm-diameter clay pots containing a pasteurized 1:2:1 mixture of peat, perlite, and soil. One seed was planted per pot and plants were fertilized weekly with a solution of 21-5-20 (N-P-K) fertilizer (200 ppm N; Miracle-Gro, Marysville, OH), beginning at the V5 growth stage. For temperature studies, 25-day-old corn seedlings (V7 growth stage) were transferred to growth chambers 3 days prior to inoculation at constant temperatures of 21° C, 24° C, 27° C, 30° C, and 33° C. To quantify isolate aggressiveness, 30-day-old corn seedlings (V8 growth stage) were transferred and randomly placed (3 days prior to inoculation) within each of two 30° C growth chambers.

Inoculum preparation.

Temperature study. Three *P. stewartii* isolates (Rif 9A (Menelas, 2006), ES 9211, and ES 9245) were obtained from Dr. Charles Block, United States Department of Agriculture (USDA) Plant Introduction Station, Iowa State University, Ames, Iowa. Isolate Rif 9A is a rifampicin- and nalidixic acid-resistant isolate derived from a wild type isolate (SS104). Prior to experiments, stock cultures of all isolates were stored at -75º C in 15% glycerol. Before experiments began, the cultures were incubated for 48 h at 25º C on nutrient broth yeast extract agar (NBY). Prior to inoculation, inoculum concentrations were adjusted to approximately 1×10^8 CFU/ml in sterile distilled water.

Isolate aggressiveness study. Thirteen *P. stewartii* isolates (Table 1) originally collected from Iowa corn fields were obtained from Dr. Charles Block. Before experiments began, cultures were incubated for 48 h at 25º C on nutrient broth yeast extract agar (NBY), and inoculum concentrations were adjusted to approximately 1×10^8 CFU/ml as described above.

Inoculation of corn leaves.

Inoculation site study. Before experiments began, a preliminary study was performed to determine if inoculation site on corn leaves had an impact on components of aggressiveness, (i.e., did inoculations between leaf veins as opposed to directly into the leaf veins have an effect on components of aggressiveness). Single leaves (growth stage V6 or V7) from each of 70 corn seedlings were inoculated with the rifampicin- and nalidixic acid-resistant *P. stewartii* isolate (Rif 9A) and then placed in a growth chamber at 30º C. Thirty-five seedlings were inoculated on each side of the leaf midrib, between the leaf veins, and 35 seedlings received inoculum into the leaf veins. After a 15-µl

aliquot of inoculum was placed at each inoculation site, and 6 small punctures were made using a 0.45-mm syringe, either (i) between or (ii) into the leaf veins. Every twenty-four hours after inoculation until the pathogen reached the end of the leaf, 10 leaves were randomly selected from seedlings receiving inoculum between and 10 leaves from seedlings receiving inoculum from into the leaf veins. Then 1-cm-long leaf sections were cut from above and below each inoculation site until the end of the leaf for each leaf selected. The leaf sections were placed onto a selective medium, and incubated at 25° C. After 2-3 days, plates were examined for the presence of *P. stewartii* colonies (Rif 9A). The pathogen was considered to have reached the leaf section if the marked Rif 9A isolate was recovered from the edge of a leaf section placed on NBY-CRN media. To quantify the rate of lesion expansion, the length of lesion on each of the leaves before cutting were measured from the point of inoculation, both acropetally and basipetally. The experiment was performed twice.

Temperature study. Three leaf positions (V5, V6 and V7) of each corn seedling within each growth chamber (21° C, 24° C, 27° C, 30° C, or 33° C) were inoculated with two of three isolates of *P. stewartii*, one on each side of the leaf midrib. There were 24 plants per replication for each pairwise comparison of isolates. Three isolates were used: Rif 9A (a rifampicin-naldixic acid resistant isolate), ES9211, and ES 9245 (both wild types). Each leaf was inoculated with two of the three isolates, with one isolate on each side of the leaf midrib. At each inoculation site, a 15-µl aliquot of inoculum was placed between two main leaf veins on each side of the leaf midrib, and a 0.45-mm syringe was used to puncture 6 small holes through the inoculum droplet into the leaf tissue. Experiments were performed twice.

Isolate aggressiveness study. Five corn plants were randomly chosen from the 65 plants in two 30° C growth chambers, and both sides of the mid-rib of four leaves (V5, V6, V7 and V8) per plant were inoculated with a single isolate out of 13 *P. stewartii* isolate (12 wild types plus Rif 9A). A 15-µl aliquot of inoculum was placed between main leaf veins, and introduced by puncturing leaf tissue with a 0.45-mm syringe for each inoculation site as described above. Experiments were performed twice.

Components of aggressiveness. Four components of aggressiveness were quantified: infection efficiency, incubation period, rates of lesion/pathogen expansion and time-to-death:

Infection efficiency. Infection efficiency was operationally defined as the number of *P. stewartii* inoculations that resulted in Stewart's disease lesions divided by the total number of inoculations performed.

Incubation period. Incubation period was operationally defined as the time from inoculation to the time (h) that visible lesions were observed.

Rate of lesion expansion. As soon as lesions appeared on inoculated corn leaves, and every 24 h thereafter, the length of each developing lesion was measured in both acropetal and basipetal directions, from the point of inoculation to the outer edge of the lesions. Lesion length measurements continued until lesions reached the acropetal or basipetal edge of the corn leaf, or until leaf death occurred (whichever occurred first). Rate of lesion expansion was defined as the slope of the regression equation relating lesion size (y) to time after inoculation (x). Slopes were obtained from the growth model that best fit the data (Nutter, 1997).

Time-to-death. Time-to-death was operationally defined as the time from inoculation until leaf death.

Data analyses. The experimental design was a randomized split-split-split block to compare: (i) infection efficiency, (ii) incubation period, (iii) rates of lesion expansion, and (iv) time-to-death among isolates (factor A), leaf positions (factor B), and temperature (factor C). The optimum temperature for the rate of lesion expansion was determined in prior experiments, and this temperature was then used for all experiments to quantify differences in components of aggressiveness among isolates.

To select the most appropriate population growth model to linearize the change in lesion length with the change in time, pathogen progress curves were plotted for each factor. To accomplish this, lesion lengths were first divided by the final lesion length and then plotted against hours after inoculation for each factor. The change in the proportion of lesion length versus time (dy/dt) was plotted with respect to hours after inoculation for each factor (Padgett et al., 1990; Steinlage et al., 2002). Based upon the shapes of pathogen/disease progress and rate curves, one or more population growth models (e.g., linear, monomolecular, exponential, logistic, and Gompertz) were selected and further evaluated for goodness of fit (Madden et al., 2007). The criteria used to select the best population growth model were: (i) the F-statistics for each overall model, (ii) the coefficient of determination (R^2), and (iii) the standard error of the estimate for y (SEy) (Nutter, 1997). After selecting the most appropriate population growth model, the slope parameter and statistics for each model, as affected the temperature, leaf position, or *P. stewartii* isolate were obtained and subjected to ANOVA and mean separations using Tukey's test ($P \leq 0.05$) (SAS 9.1.3, SAS Institute, Inc, Cary, NC).

RESULTS

Effect of inoculation method on components of aggressiveness components. There were no statistical differences between acropetal and basipetal rates of pathogen invasion ($F = 0.01$, $P = 0.9069$) and lesion expansion ($F = 0.01$, $P = 0.9131$). Therefore, the rates of pathogen invasion and lesion expansion were averaged for the two directions to determine the effect of inoculation method on components of aggressiveness.

Effect of inoculation method on incubation period. Inoculation method significantly affected incubation period. The inoculation period for inoculations performed between the leaf veins was 6.0 days, compared to 3.5 days for within-vein inoculations (Table 2). Similar results were obtained in the repeated experiment (4 days and 3 days, respectively).

Effect of inoculation method on the rate of lesion and pathogen expansion. The exponential model best explained the rate of lesion expansion, based on *F*-statistics, R^2 values, and SE*y* values (Table 3). Therefore the exponential model was used to calculate and compare rates of lesion and pathogen expansion.

There were significant differences between the rates of pathogen expansion and rates of visible lesion expansion, with rates of pathogen expansion (0.548 ± 0.264 ln units per day) being approximately three times faster than rates of lesion expansion (0.170 ± 0.029 ln units per day) (Figs 2 and 3). The rate of lesion expansion was 0.320 ln units per day for inoculations between the veins, whereas the rate of lesion expansion when inoculated directly into leaf veins was more than two times faster (0.777 ln units per day). The rate of pathogen expansion for inoculations between the veins was significantly slower (0.149

ln units per day) compared to inoculations directly into leaf veins (0.190 ln units per day) (Table 2; Figs 2 and 3).

In the repeated experiment, the rate of pathogen expansion was 0.333 ± 0.032 ln units per day, which again more than four times faster than the rate of lesion expansion (0.078 ± 0.013 ln units per day) (Figs 2 and 3).

Temperature, leaf position and pathogen isolate studies. The exponential model best explained the rate of lesion expansion, based on *F*-statistics, R^2 values, and SE*y* values (Table 3). Therefore the exponential model was used to calculate and compare rates of lesion and pathogen expansion.

Effect of temperature, leaf position and pathogen isolates on infection efficiency. Based on ANOVA table results for infection efficiency (Table 4), leaf position ($P < 0.0001$) and isolate ($P = 0.0007$) significantly affected infection efficiency, but temperature ($P = 0.1289$) and direction ($P = 0.5593$) did not. Temperature X leaf position ($P = 0.0197$), temperature X isolate ($P = 0.0004$), and leaf position X isolate ($P = 0.0028$). 2-way interactions were significant, indicating that infection efficiency data could not be averaged over these variables (Kirk, 1982). All other 2-way and 3-way interactions were not significant. For the temperature X leaf position interaction, at 21° C, 27° C and 33° C, infection efficiency was significantly lower on the fifth leaf (ranging from 37.0 % to 60.0 %) compare to the seventh leaf and eighth leaf positions (ranging from 82.3 % to 99.0 %). The fifth leaf position at 21° C had the lowest infection efficiency (37.0%) among all the temperatures and leaf position combinations (Figure 4). On the sixth and seventh leaf positions, infection efficiency was not significantly affected

by temperature, ranging from 80.7 % to 99.0 %. On the fifth leaf position, infection efficiency was lowest at 21° C, and higher at other temperatures (ranging from 58.3 % to 77.6 %) (Figure 4). For temperature X isolate interactions, isolate ES 9245 had the lowest infection efficiency at 21° C (60.0 %), and highest infection efficiency at 24° C (95.4 %) (Figure 5). For leaf position and isolate interaction, for the same isolate, infection efficiency was lower on the fifth leaf (from 53.6 % to 65.9 %) and higher on the sixth and seventh leaf (from 84.1 % to 94.1%), and isolate Rif 9A had the lowest infection efficiency on the fifth leaf (53.6 %) (Figure 6).

Effect of temperature, leaf position and pathogen isolates interactions on incubation period. At 21° C, lesion development was totally inhibited, and therefore, incubation period could not be measured. For all other temperatures, leaf positions ($P < 0.0001$) and pathogen isolates ($P < 0.0001$) significantly affected the incubation period of *P. stewartii* (Table 5). Temperature ($P = 0.5221$) and direction of lesion expansion, however, did not significantly affect incubation period ($P = 0.2262$). Temperature X leaf position 2-way interaction were significant ($P < 0.0001$), and all other 2-way and 3-way interactions were not statistically significant, indicating that only isolate main effects can be averaged over other variables. The incubation period for isolate Rif 9A (196.8 hours) was significantly longer than the incubation period for isolate ES 9211 (184.6 hours). Isolate ES 9245 (177.2 hours) had the shortest incubation period (Tables 6). For the temperature and leaf position interaction, the fifth leaf had the longest incubation period at 33° C (259.2 hours) and the seventh leaf had the shortest incubation period at 30° C (131.8 hours) among all the temperature and leaf position combinations (Figure 7).

Effect of temperature, leaf position and pathogen isolates on rate of lesion expansion. Based on the ANOVA table results for rate of lesion expansion (Table 7), temperature ($P = 0.0006$), leaf position ($P = 0.0237$) and direction of lesion expansion ($P < 0.0001$) significantly affected the rate of lesion expansion, with the exception of isolate ($P = 0.9339$). The 2-way interactions leaf position X isolate ($P < 0.0001$), temperature X direction of lesion expansion ($P < 0.0001$) and leaf position X direction of lesion expansion ($P = 0.0204$) were significant, indicating that rate data could not be averaged over these variables (Kirk, 1982). All other 2-way and 3-way interactions were not significant ($P > 0.05$). For the leaf position X isolate interaction, lesion expansion was fastest for isolate ES 9245 on the fifth leaf position (0.01724 ln units per hour) and slowest (0.01371 ln units per hour) on the seventh leaf (Figure 8). Lesion expansion did not occur at 21° C. For the other temperatures tested, lesion expansion was slowest at 24° C (0.01256 ln units per hour acropetally and 0.01234 ln units per hour basipetally), and fastest at 27° C (0.02228 ln units per hour acropetally and 0.02350 ln units per hour basipetally). At 30° C, the rates were 0.02056 ln units per hour acropetally and 0.02489 ln units per hour basipetally, and at 33° C, the rates were 0.01779 ln units per hour acropetally and 0.01929 ln units per hour basipetally (Figure 9). For the leaf position X direction of lesion expansion interaction, acropetal lesion expansion (0.01464 ln units per hour) was significantly slower than basipetal lesion expansion (0.01600 ln units per hour) at all leaf positions. Rate of lesion expansion was slowest on the seventh leaf acropetally (0.01355 ln units per hour) (Figure 10).

Leaf position and pathogen isolates study. *Effect of pathogen isolates and leaf position on infection efficiency.* Based on the ANOVA table results for infection

efficiency (Table 8), leaf position significantly affected infection efficiency ($P < 0.0001$), but isolate did not ($P = 0.7471$). All 2-way and 3-way interactions were not significant. Infection efficiency varied among isolates, ranging from 86.0 % (Rif 9A) to 96.3 % (ES 9244 and ES 9208) (Table 9). Infection efficiency was lowest at the fifth leaf (71.6%), and was significantly higher at the sixth (96.2%), seventh (99.3%) and eighth leaf position (97.9%) (Table 9).

Effect of pathogen isolate and leaf position on incubation period. Based on the ANOVA table results for incubation period (Table 10), leaf position significantly affected incubation period ($P < 0.0001$) for the 13 isolates. Only isolate ($P = 0.7497$) did not significantly affect incubation period. All 2-way and 3-way interactions were not significant. Incubation period among isolates ranged from 198.9 hours (ES9201) to 272.0 hours (ES 9207) after inoculation. The average incubation period was longest for the fifth leaf (273.6 hours), and shortest for the seventh leaf (185.1 hours) and eighth leaf (177.5 hours) (Table 11).

Effect of pathogen isolate and leaf position on rate of lesion expansion. Based on ANOVA table results for lesion expansion (Table 12), isolate ($P = 0.0103$), and leaf position ($P < 0.0001$) all significantly affected the rate of lesion expansion. The leaf X direction ($P = 0.0235$) 2-way interaction was significant, indicating that rate data could not be averaged over these variables (Kirk, 1982). All other 2-way and 3-way interactions were not significant. The rate of lesion expansion was fastest for isolate ES 0601 (0.0231 ln units per hour), and slowest for isolate ES 9253 (0.01505 ln units per hour) (Table 13). For the leaf X direction interaction, rate of lesion expansion was fastest at the sixth leaf position, both acropetally (0.02254 ln units per hour) and basipetally

(0.02281 ln units per hour). The rate of lesion expansion was slowest on the fifth leaf position acropetally (0.01571 ln units per hour) (Figure 11).

Effect of pathogen isolates and leaf position on time-to-death. Based on ANOVA table results for time-to-death (Table 14), leaf position ($P < 0.0001$) significantly affected time-to-death, but main effects for isolate were not significant ($P = 0.7596$). All 2-way and 3-way interactions were not significant. Time-to-death varied among isolates from 458.0 hours (ES 9257) to 567.2 hours (ES 9203) after inoculation. Time-to-death was shortest at the fifth leaf position (oldest leaf) (435.1 hours), and longest at the eighth leaf position (youngest leaf) (618.5 hours) (Table 11).

DISCUSSION

This is the first study to quantify the aggressiveness of *Pantoea stewartii* isolates on corn leaves at different temperatures and for different leaf positions, by measuring the rate of lesion expansion over time, incubation period, and time to death. In previous studies, Koutsoudis, et al. 2006 indicated lesions expand mostly basipetally, based on the nature of the bacterium movement. The three-isolate comparison using the exponential model confirmed their observation, however, for the thirteen-isolate study, difference of lesion expansion rates for different directions were not detected.

Koutsoudis et al's study was based on the movement of the pathogen inside the xylem of the corn leaves. However, in this study, the inoculation site was between the leaf veins, which means the inoculations was avoiding the xylem. This may be able to

explain the differences. The reason for inoculating between the veins was used in this study was based on field and greenhouse observation of the corn flea beetle feeding behavior, which shows that corn flea beetles tend to feed between the corn leaf vein rather than on the leaf veins. A preliminary study showed a large difference for the lesion expansion between different inoculation points (Figure 2). In nature, corn flea beetles might also hurt the leaf veins during feeding, the lesion expansion pattern in the real world would be a combination of inoculation between and in the leaf vein. Since lesion expansion tends to be slower between the veins than in the veins, this study can serve as a lower band of the lesion expansion in real world.

For the *Pantoea stewartii* isolates tested, ES 0601 was more aggressive than other isolates for the rate of lesion expansion. This may be explained by the origin of the isolates. The isolate ES 0601 was isolated from *Coix lachryma-jobi* rather than corn. Maize is a more common host of this bacterium, and since ES 0601 was virulent on a less susceptible host, when inoculated on sweet corn, this isolate can be more aggressive than the isolates isolated from Maize.

At 21° C, lesion expansion was totally prohibited, however, when the temperature was increased to 30° C from 21° C, lesions became visible after incubating for one day. This indicates that the pathogen is still moving in the leaf though the lesion is not visible. However, corn flea beetles can still acquire the pathogen and spread it in the field. So, corn inspection after some consecutive cool days may not be able to accurately estimate the incidence and prevalence of Stewart's disease in the field. Also, corn field inspectors need to watch the weather (mainly temperature) to conduct appropriate inspection date.

Days with temperature between 27° C and 30° C would likely be the best for discovering Stewart's disease in the field.

Of the four leaves tested, *Pantoea stewartii* was more aggressive on the seventh and eighth leaf and less aggressive on the fifth leaf of corn plant. This may be due to the fact that water and nutrient transports is faster in the newly developed corn leaves than older leaves, but also because newer leaves are provided with more water and nutrients than older leaves. Water and nutrients will influence the movement of the bacterium, while they also influence the growth and multiplication of the bacterium. Another reason may be the senescence of the corn plants, as the older leaves, especially the fifth true leaf, will die faster than the newer leaves, which would influence the time-to-death. Finally, from observation, there is more wax on the leaf surfaces of the fifth leaf than on newer leaves, which would also influence the infection efficiency.

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TABLES AND FIGURES

Table 1. *Pantoea stewartii* isolates and their origins used in this thesis.

Isolate Number	Genus	species	subsp. or pathovar	Host	Origin
Rif 9A	*Pantoea*	*stewartii*	*stewartii*	corn	Rifampicin-nalidixic acid resistant isolate from DC283 by G. Lamka (ISU)
ES 0601	*Pantoea*	*stewartii*	*stewartii*	Coix	Ames, IA, 2006
ES 9201	*Pantoea*	*stewartii*	*stewartii*	corn	Washington, IA, 1992
ES 9203	*Pantoea*	*stewartii*	*stewartii*	corn	Lone Tree, IA, 1992
ES 9204	*Pantoea*	*stewartii*	*stewartii*	corn	Washington, IA, 1992
ES 9207	*Pantoea*	*stewartii*	*stewartii*	corn	Story City, IA, 1992
ES 9208	*Pantoea*	*stewartii*	*stewartii*	corn	Boone, IA, 1992
ES 9211	*Pantoea*	*stewartii*	*stewartii*	corn	Slater, IA, 1992
ES 9230	*Pantoea*	*stewartii*	*stewartii*	corn	Maxwell, IA, 1992
ES 9243	*Pantoea*	*stewartii*	*stewartii*	corn	Madrid, IA, 1992
ES 9244	*Pantoea*	*stewartii*	*stewartii*	corn	Nevada, IA, 1992
ES 9245	*Pantoea*	*stewartii*	*stewartii*	corn	Nevada, IA, 1992
ES 9246	*Pantoea*	*stewartii*	*stewartii*	corn	Coon Rapids, IA, 1992
ES 9253	*Pantoea*	*stewartii*	*stewartii*	corn	Schaller, IA, 1992
ES 9257	*Pantoea*	*stewartii*	*stewartii*	corn	Wellsburg, IA, 1992

Table 2. Effect of inoculation methods on the rate of lesion and pathogen expansion on sweet corn variety "Jubilee" inoculated with a rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* (Rif 9A) at 30 ˚C.

Experiment	Inoculation method	Test	Incubation period (days)	Rate of expansion (ln units of y/day)
1	Between the veins	Lesion expansion	1	0.149 e*
1	Into the veins	Lesion expansion	1	0.190 d
1	Between the veins	Pathogen expansion	6	0.320 bc
1	Into the veins	Pathogen expansion	3.5	0.777 a
2	Between the veins	Lesion expansion	1	0.076 f
2	Into the veins	Lesion expansion	1	0.080 f
2	Between the veins	Pathogen expansion	4	0.313 c
2	Into the veins	Pathogen expansion	3	0.354 b

* Means with the same letters within columns are not significantly different ($P \leq 0.05$) based on a Tukey means separation test.

Table 3. Percentage of lesion and pathogen growth curves for isolates of *Pantoea stewartii* on sweet corn variety "Jubilee" that best fit one of five population growth models (based on *F*-statistics, R^2 values, and SE*y* values).

Growth model	Linearized form	R^2 (%) [a]	
		3 isolates	13 isolates
Linear	$y = y_0 + rt$	70.26	78.75
Exponential	$\ln y = \ln y_0 + rt$	70.76	84.81
Monomolecular	$\ln \{1/(1-y)\} = \ln \{1/(1- y_0)\} + rt$	47.55	36.83
Logistic	$\ln (y/(1-y) = \ln \{y_0/(1- y_0) + rt\}$	52.06	66.49
Gompertz	$-\ln (-\ln y) = -\ln (-\ln y_0) + rt$	55.30	72.19

[a] Number of data curves that best fit a model data (p-value ≤ 0.05), divided by total number of data curves analyzed.

Table 4. ANOVA table for the effects of temperature, leaf position, direction of lesion growth (acropetally versus basipetally), pathogen isolate, and their interactions on infection efficiency of *Pantoea stewartii* on sweet corn variety "Jubilee".

Variable(s)	DF	F Value	Pr > F
Temperature	4	2.89	0.1289
Leaf	2	70.81	<.0001 ***[z]
Temperature*Leaf	8	2.3	0.0197 *
Isolate	2	7.42	0.0007 **
Temperature*Isolate	8	3.64	0.0004 **
Leaf*Isolate	4	4.12	0.0028 *
Temperature*Leaf*Isolate	16	1.06	0.3902
Direction	1	0.34	0.5593
Temperature*Direction	4	0.65	0.6277
Leaf*Direction	2	0.09	0.9183
Temperature*Leaf*Direction	8	0.54	0.8307
Isolate*Direction	2	0.88	0.4146
Temperature*Isolate*Direction	8	0.12	0.9983
Leaf*Isolate*Direction	4	0.45	0.769
Temperature*Leaf*Isolate*Direction	16	0.27	0.9984

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 5. ANOVA table for the effects of temperature, leaf position, direction of lesion growth (acropetally versus basipetally), pathogen isolate, and their interactions on incubation period of *Pantoea stewartii* on sweet corn variety "Jubilee".

Variable(s)	DF	F Value	Pr > F
Temperature	3	0.88	0.5221
Leaf	2	65.13	<.0001 ***[z]
Temperature*Leaf	6	10.21	<.0001 ***
Isolate	2	24.56	<.0001 ***
Temperature*Isolate	6	1.44	0.2027
Leaf*Isolate	4	1.25	0.293
Temperature*Leaf*Isolate	12	1.06	0.3929
Direction	1	1.47	0.2262
Temperature*Direction	3	0.67	0.5698
Leaf*Direction	2	0.3	0.741
Temperature*Leaf*Direction	6	0.1	0.9965
Isolate*Direction	2	0.65	0.5223
Temperature*Isolate*Direction	6	0.25	0.96
Leaf*Isolate*Direction	4	0.4	0.8078
Temperature*Leaf*Isolate*Direction	12	0.16	0.9994

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 6. The effect of isolates of *Pantoea stewartii* on the incubation period of *P. stewartii* on sweet corn variety "Jubilee", averaged over direction of lesion expansion (acropetal and basipetal), leaf positions, and temperature.

Isolate	Incubation period (hours)
Rif 9A	196.8 a
ES 9211	184.6 b
ES 9245	177.2 c

* Means with the same letters within columns are not significantly different ($P \leq 0.05$) based on a Tukey means separation test.

Table 7. ANOVA table for the effects of temperature, leaf position, direction of lesion growth (acropetally versus basipetally), pathogen isolate, and their interactions on rate of lesion expansion of *Pantoea stewartii* on sweet corn variety "Jubilee".

Variable(s)	DF	F Value	Pr > F
Temperature	4	33.97	0.0006***[z]
Leaf	2	3.77	0.0237*
Temperature*Leaf	8	1.43	0.1807
Isolate	2	0.07	0.9339
Temperature*Isolate	8	1.12	0.3513
Leaf*Isolate	4	6.29	<.0001***
Temperature*Leaf*Isolate	16	1.16	0.3019
Direction	1	33.96	<.0001***
Temperature*Direction	4	12.89	<.0001***
Leaf*Direction	2	3.9	0.0204*
Temperature*Leaf*Direction	8	1.45	0.1706
Isolate*Direction	2	0.28	0.7591
Temperature*Isolate*Direction	8	0.76	0.6371
Leaf*Isolate*Direction	4	0.58	0.6795
Temperature*Leaf*Isolate*Direction	16	0.57	0.9100

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 8. ANOVA table for the effects of *Pantoea stewartii* isolate, leaf position, direction of lesion growth (acropetally versus basipetally), and their interactions on infection efficiency of *P. stewartii* on sweet corn variety "Jubilee" at 30° C.

Variable(s)	DF	F Value	Pr > F
Isolate	12	0.67	0.7471
Leaf	3	39.91	<.0001 ***[z]
Isolate*Leaf	36	0.64	0.9515
Direction	1	1.59	0.2073
Isolate*Direction	12	0.68	0.7754
Leaf*Direction	3	0.38	0.7683
Isolate*Leaf*Direction	36	0.5	0.9948

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 9. The effect of isolate and leaf position on infection efficiency of thirteen isolates of *Pantoea stewartii* on the sweet corn variety "Jubilee" at 30° C.

Effect		Infection efficiency (%) [a]	
Isolate	ES 9201	95.6	a
	ES 9243	93.8	a
	ES 9244	96.3	a
	ES 9208	96.3	a
	ES 9253	88.8	a
	ES 9257	90.0	a
	ES 9204	95.0	a
	ES 0601	93.1	a
	ES 9203	88.8	a
	ES 9207	82.5	a
	ES 9230	90.0	a
	ES 9246	90.0	a
	Rif 9A	86.0	a
Leaf position	V 5	71.6	b
	V 6	96.2	a
	V 7	99.3	a
	V 8	97.9	a

[a] Infection efficiency (%) was defined as the number of leaves showing visible lesion divided by the total number of leaves inoculated.

Table 10. ANOVA table for the effects of *Pantoea stewartii* isolate, leaf position, direction of lesion growth (acropetally versus basipetally), and their interactions on incubation period of *P. stewartii* on sweet corn variety "Jubilee" at 30° C.

Effect	DF	F Value	Pr > F
Isolate	12	0.68	0.7497
Leaf	3	123.06	<.0001 ***[z]
Isolate*Leaf	36	0.58	0.9757
Direction	1	1.67	0.1963
Isolate*Direction	12	0.32	0.9868
Leaf*Direction	3	0.67	0.5683
Isolate*Leaf*Direction	36	0.12	1

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 11. The effect of leaf position on the incubation period and time-to-death of thirteen isolates of *Pantoea stewartii* on the sweet corn variety "Jubilee" at 30 ˚C averaged over isolates.

Leaf	Incubation period (hours)	Time-to-death (hours)
V5	273.6 a*	435.1 d
V6	235.4 b	513.7 c
V7	185.1 c	593.0 b
V8	177.5 c	618.5 a

* Means with the same letters within columns are not significantly different ($P \leq 0.05$) based on the Tukey method.

Table 12. ANOVA table for the effects of *Pantoea stewartii* isolate, leaf position, direction of lesion growth (acropetally versus basipetally), and their interactions on rate of lesion expansion of *P. stewartii* on sweet corn variety "Jubilee" at 30° C.

Effect	DF	F Value	Pr > F
Isolate	12	3.35	0.0105 *[z]
Leaf	3	10.99	<.0001 ***
Isolate*Leaf	36	0.85	0.7136
Direction	1	0.01	0.9391
Isolate*Direction	12	1.66	0.0703
Leaf*Direction	3	3.17	0.0235*
Isolate*Leaf*Direction	36	0.9	0.6477

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

Table 13. The effect of isolate on the rate of lesion expansion of thirteen isolates of *Pantoea stewartii* on the sweet corn variety "Jubilee" at 30° C , averaged over directions of lesion expansion (acropetal and basipetal) and leaf positions.

Isolate	Rate of lesion expansion (ln units per hour)	
ES 9201	0.01767	ab
ES 9243	0.02089	ab
ES 9244	0.01791	ab
ES 9208	0.02011	ab
ES 9253	0.01505	b
ES 9257	0.01972	ab
ES 9204	0.02078	ab
ES 0601	0.02313	a
ES 9203	0.01931	ab
ES 9207	0.01659	ab
ES 9230	0.01687	ab
ES 9246	0.01616	ab
Rif 9A	0.02034	ab

* Means with the same letters within columns are not significantly different ($P \leq 0.05$) based on the Tukey method

Table 14. ANOVA table for the effects of *Pantoea stewartii* isolate, leaf position, direction of lesion growth (acropetally versus basipetally), and their interactions on time-to-death of *P. stewartii* on sweet corn variety "Jubilee" at 30° C.

Effect	DF	F Value	Pr > F
Isolate	12	0.66	0.7596
Leaf	3	151.49	<.0001 ***[z]
Isolate*Leaf	36	0.97	0.5255
Direction	1	0.33	0.5637
Isolate*Direction	12	0.35	0.9805
Leaf*Direction	3	0.36	0.7803
Isolate*Leaf*Direction	36	0.37	0.9998

[z] Indicates significance at $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***).

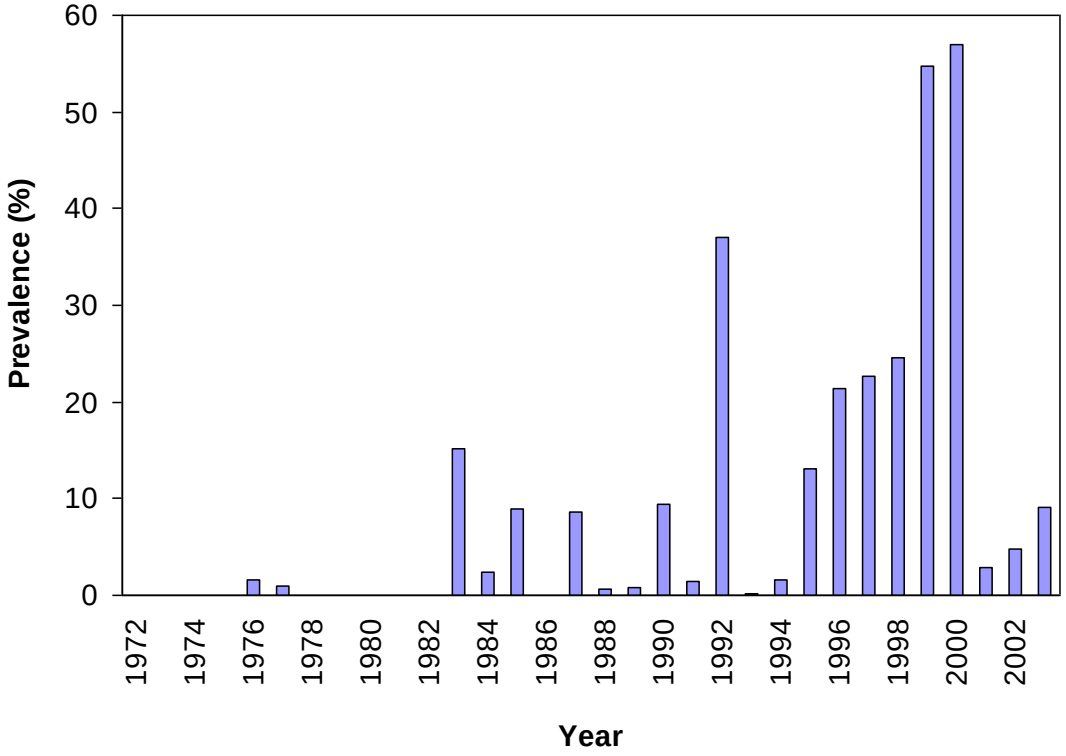

Figure 1. Prevalence of Stewart's disease of corn in Iowa seed corn fields from 1972 to 2003.The number of seed corn fields inspected ranged from approximately 500 seed corn fields in the early 1970s to > 1,300 fields in the latter two decades.

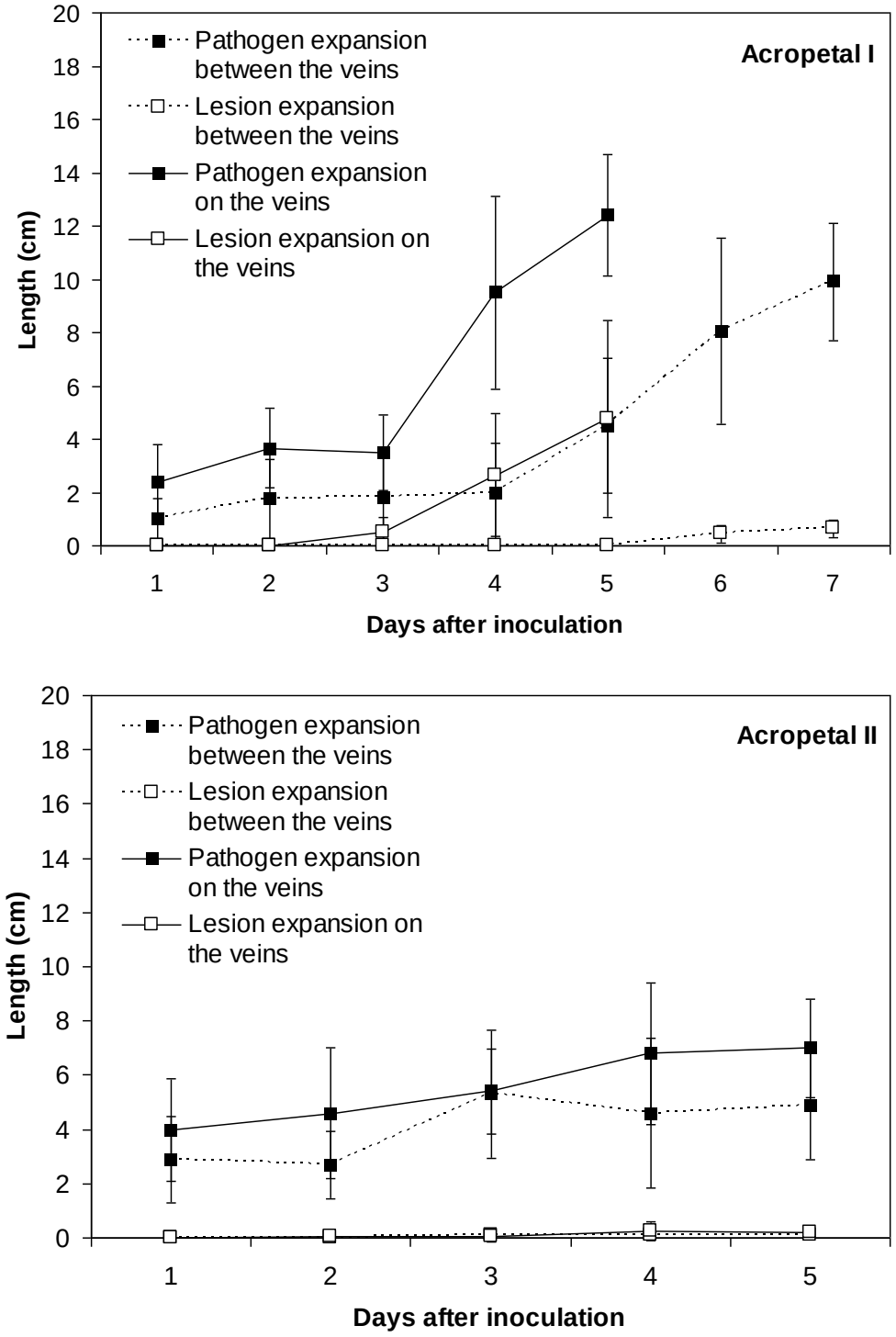

Figure 2. Acropetal disease and pathogen expansion of *Pantoea stewartii* isolate (Rif 9A) over time for two different inoculation procedures (between leaf veins versus directly into leaf veins) at 30˚ C on sweet corn variety "Jubilee" for (I) the initial experiment, and (II) the repeated experiment.

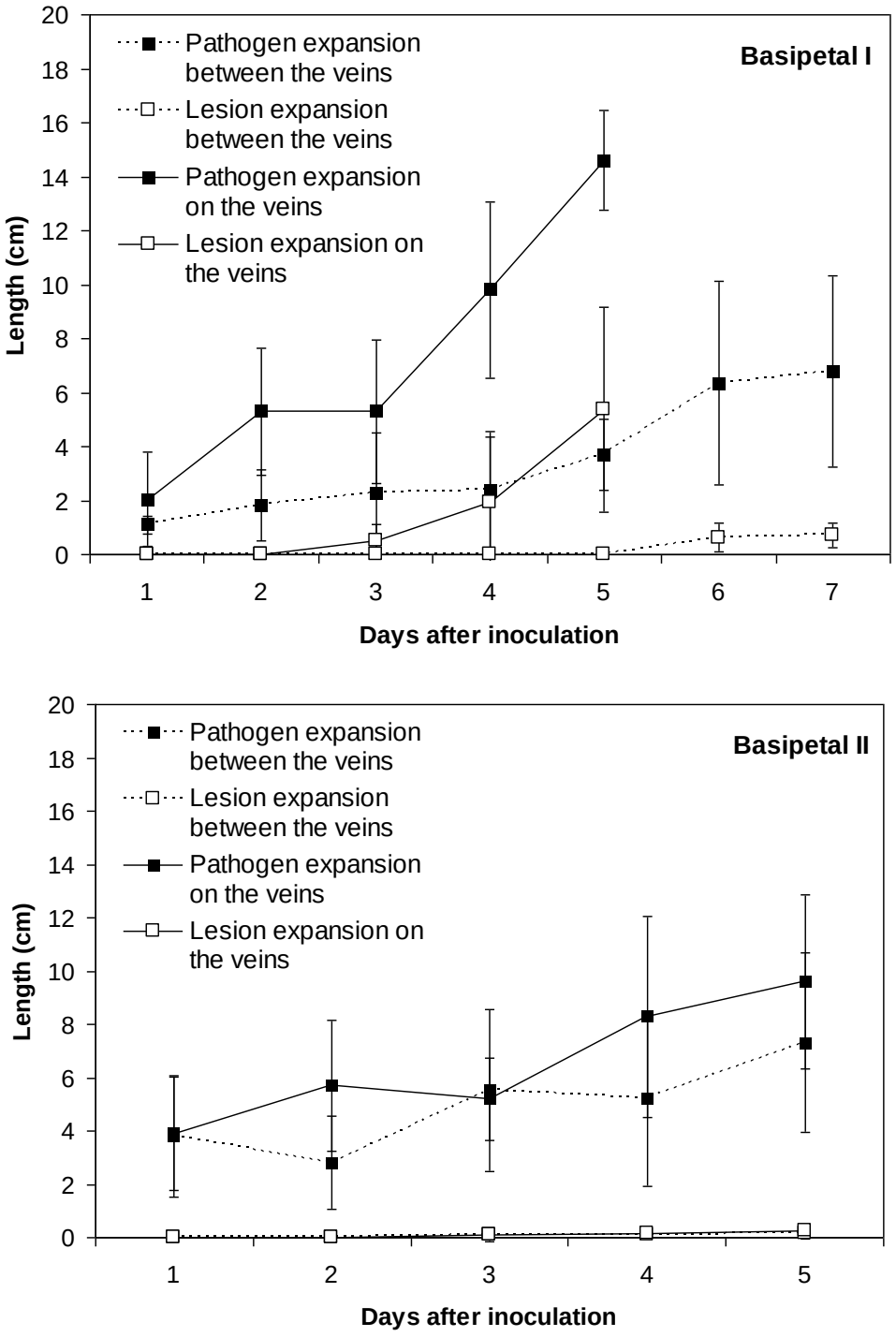

Figure 3. Basipetal disease and pathogen expansion of *Pantoea stewartii* isolate (Rif 9A) over time for two different inoculation procedures (between leaf veins versus directly into leaf veins) at 30˚ C on sweet corn variety "Jubilee" for (I) the initial experiment, and (II) the repeated experiment.

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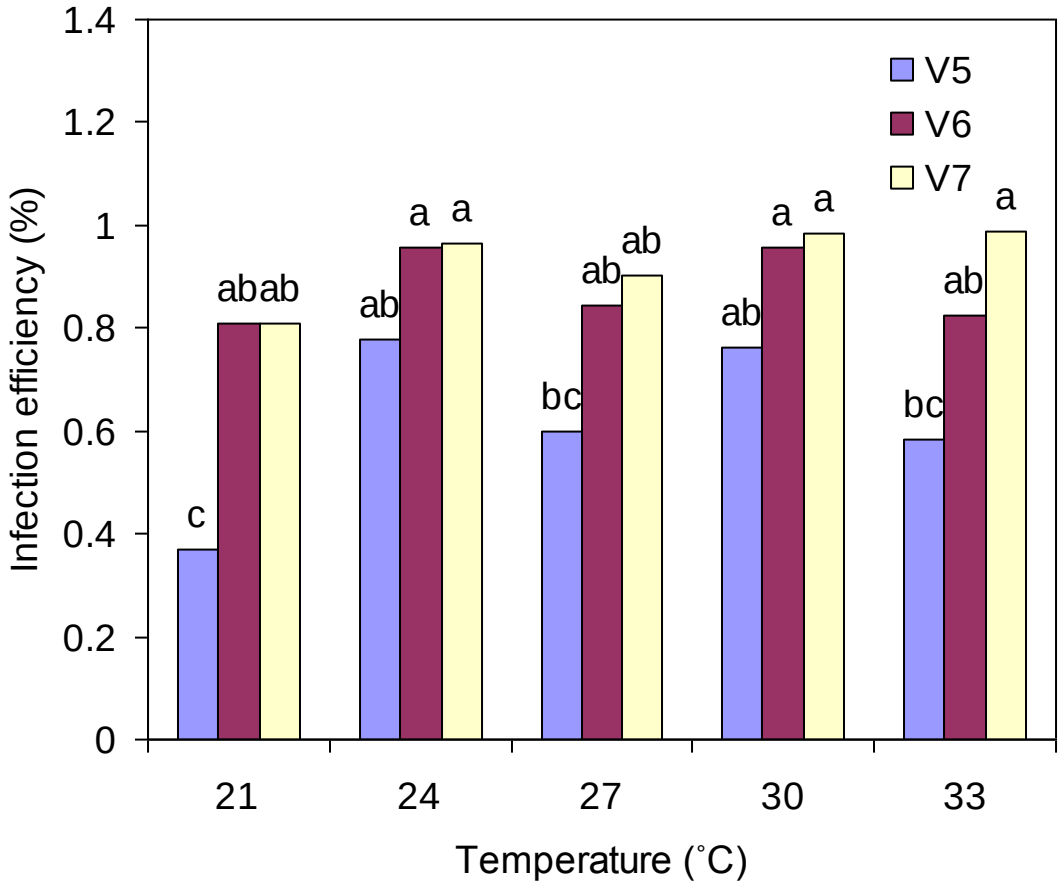

Figure 4. Effect of temperature and leaf position (leaf age) on the infection efficiency of isolates of *Pantoea stewartii*, averaged over 3 isolates of *P. stewartii*, on the sweet corn variety "Jubilee".

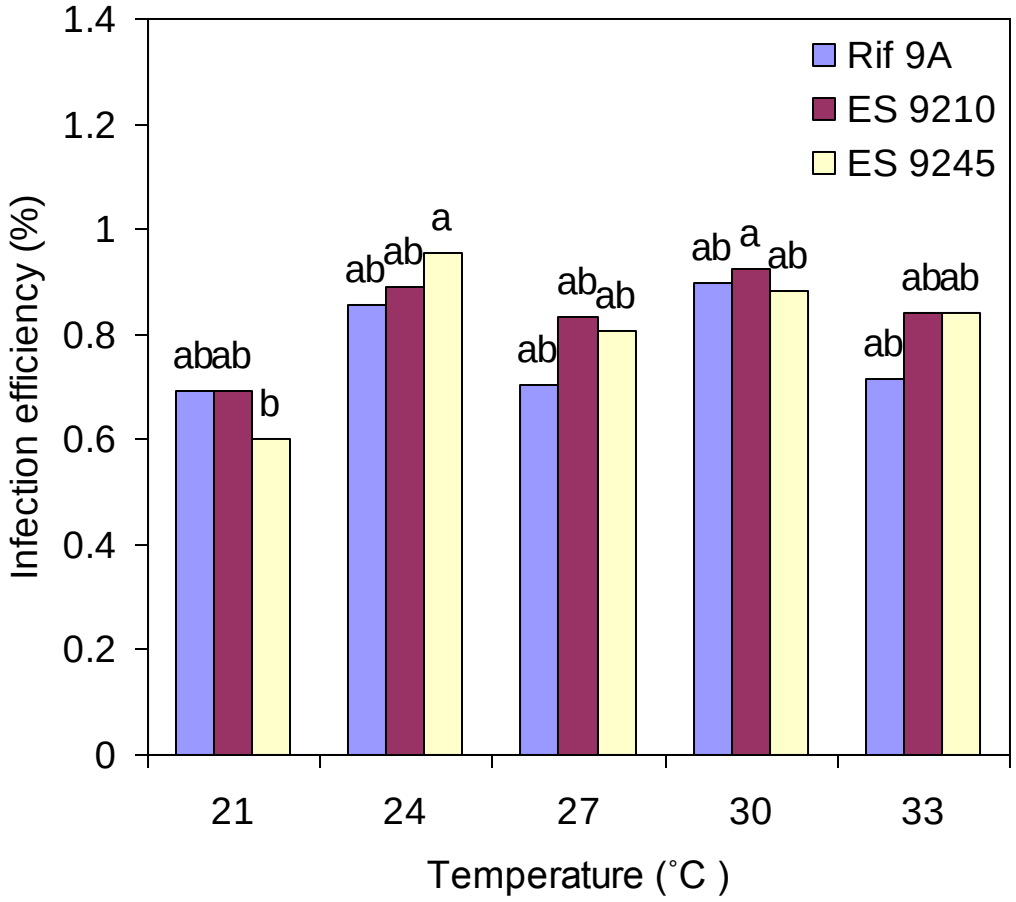

Figure 5. Effect of temperature on the infection efficiency of 3 isolates of *Pantoea stewartii*, averaged over three leaf positions, on the sweet corn variety "Jubilee".

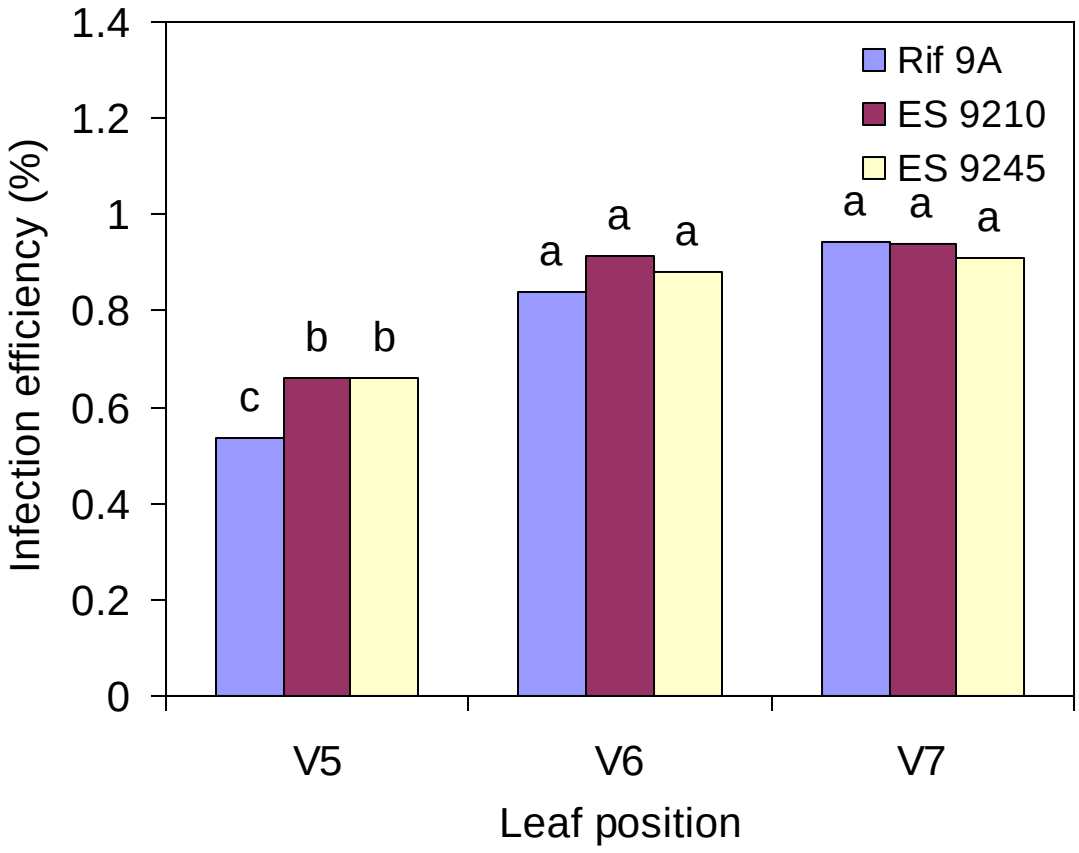

Figure 6. Effect of leaf position on infection efficiency for 3 isolates of *Pantoea stewartii*, averaged over the direction of lesion expansion (acropetally and basipetally) and temperature, on the sweet corn variety "Jubilee".

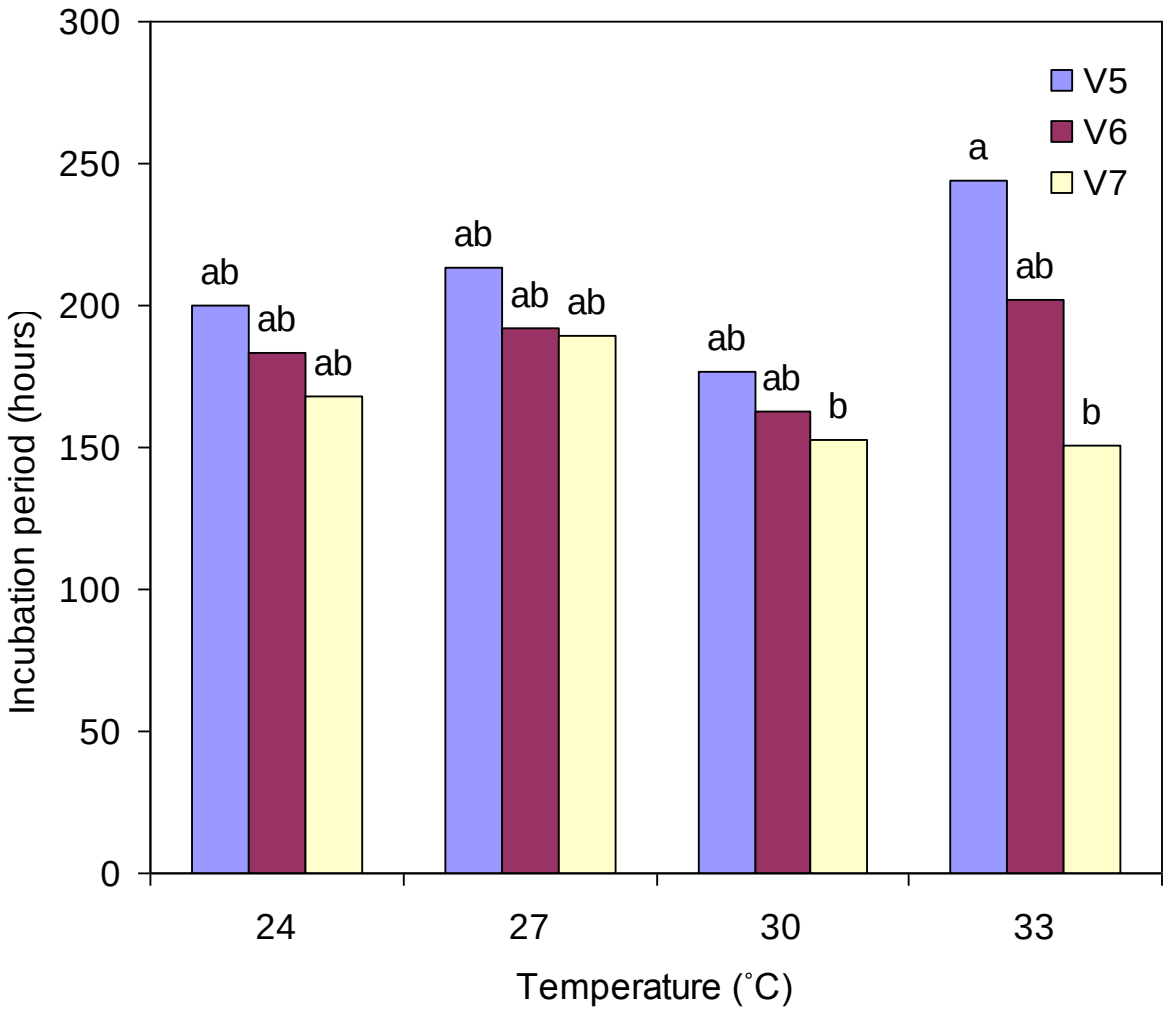

Figure 7. Effect of temperature on mean incubation period for three isolates of *Pantoea stewartii*, temperature, and leaf position (V5, V6 and V7) averaged over the direction of lesion expansion (acropetally and basipetally), on the sweet corn variety "Jubilee".

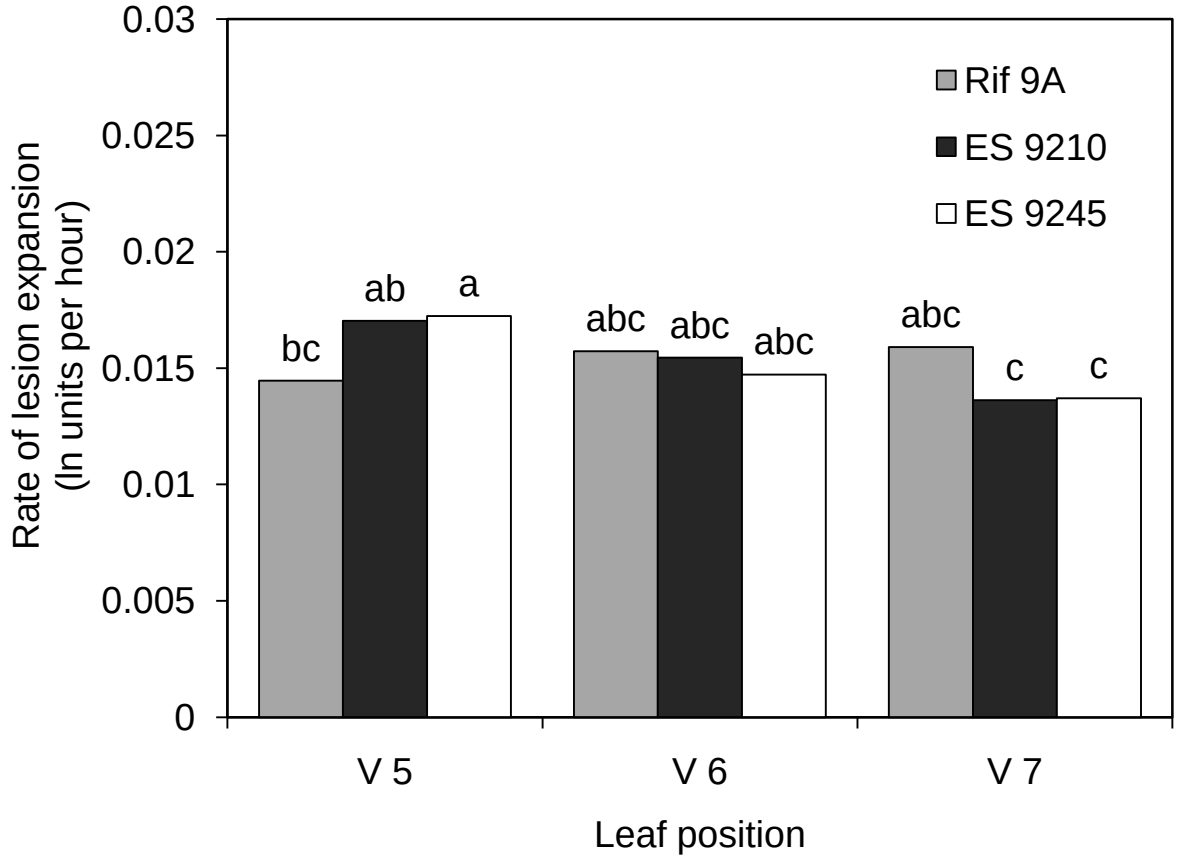

Figure 8. Effect of leaf position on the rate of lesion expansion for three isolate of *Pantoea stewartii* averaged over the direction of lesion expansion (acropetally and basipetally) and temperature, on the sweet corn variety "Jubilee".

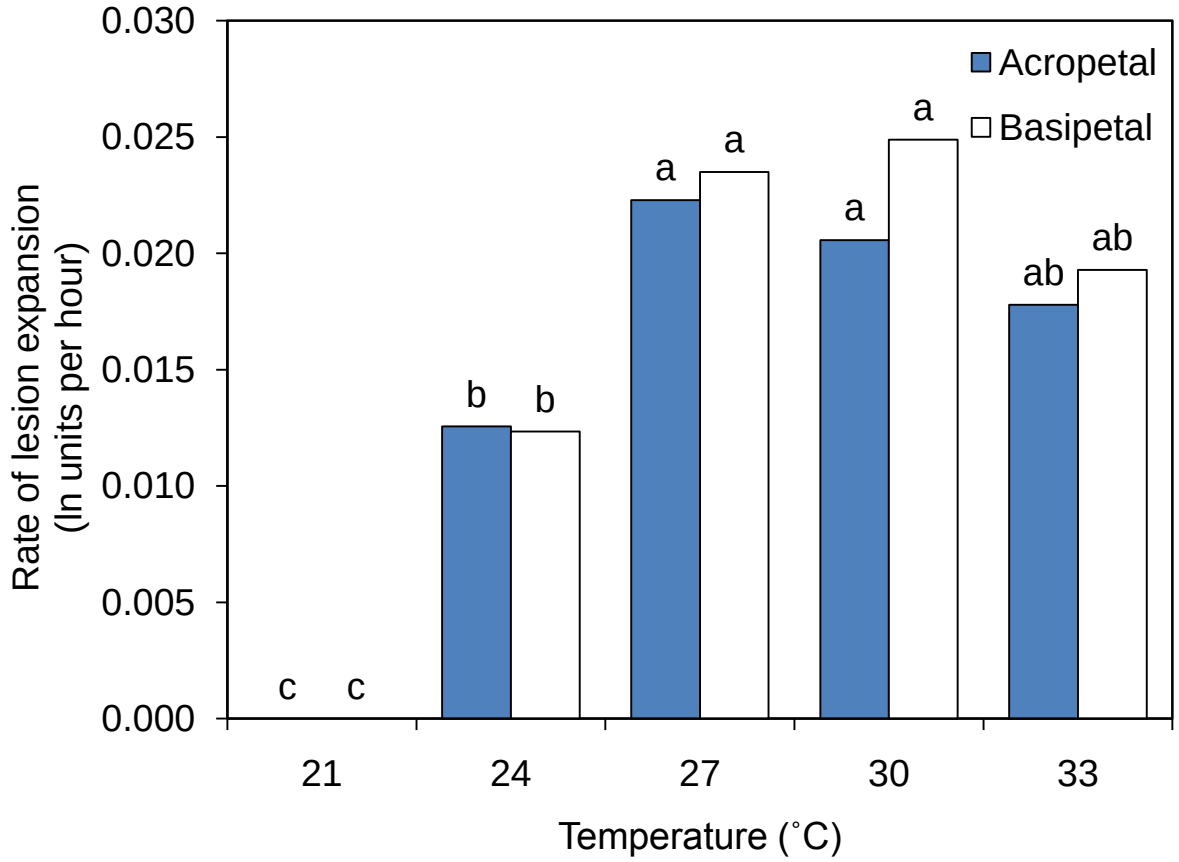

Figure 9. Effect of temperature on the mean rate of lesion expansion of *Pantoea stewartii* isolates Rif 9A, ES 9211, and ES 9245 averaged over leaf position, on the sweet corn variety "Jubilee",

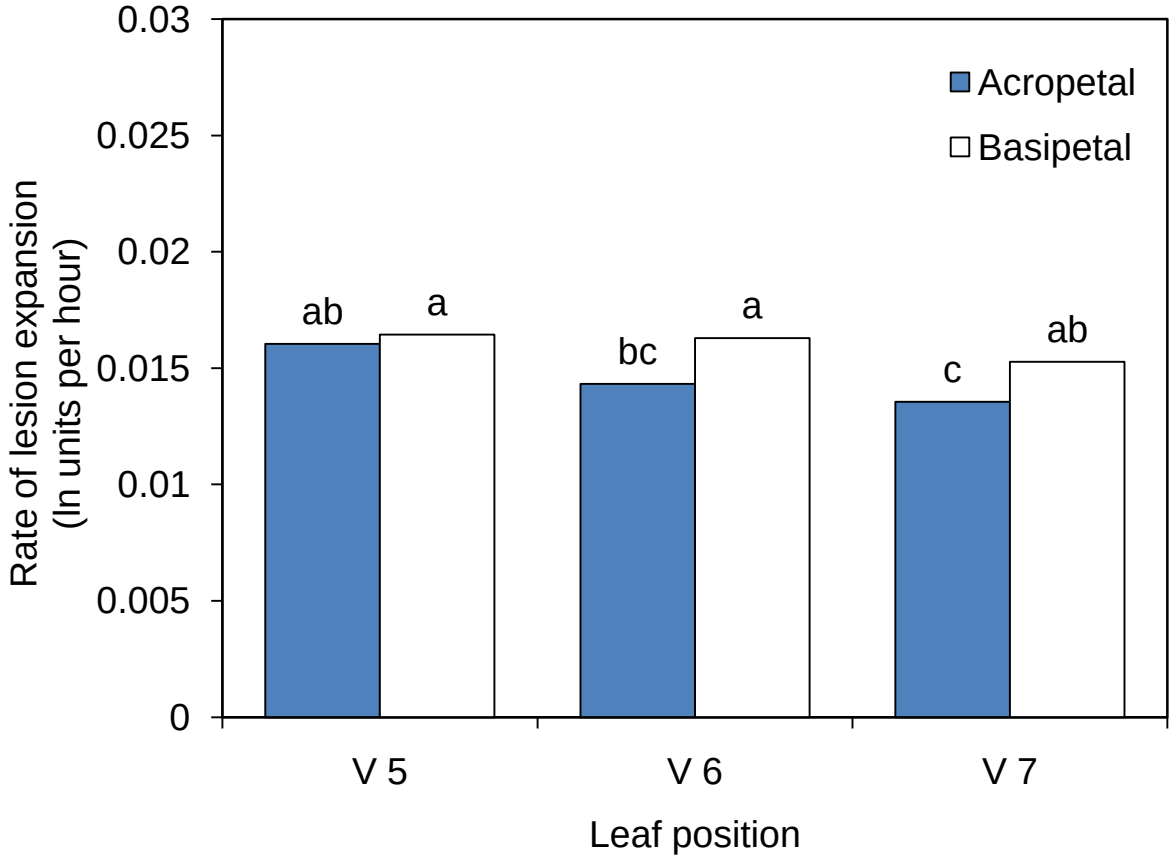

Figure 10. Effect of leaf position on the mean rate of lesion expansion of *Pantoea stewartii* isolates Rif 9A, ES 9211, and ES 9245 averaged over isolates, on the sweet corn variety "Jubilee".

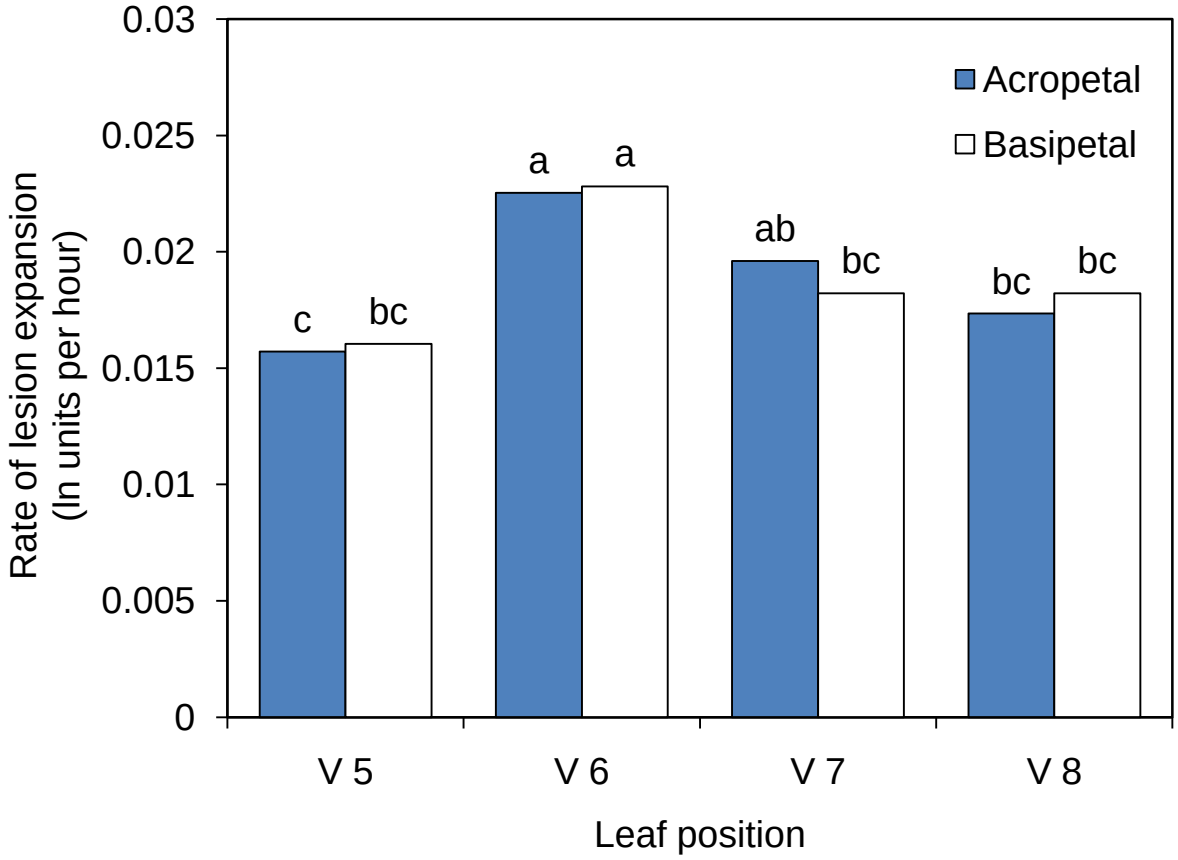

Figure 11. Effect of leaf position and lesion expanding direction on the mean rate of lesion expansion of 13 *Pantoea stewartii* isolates, on the sweet corn variety "Jubilee".

CHAPTER 3.

Quantifying the Spatial and Temporal Spread of *Pantoea stewartii* in the field in Iowa State

ABSTRACT

Stewart's disease, caused by *Pantoea stewartii*, can cause severe economic damage to seed and sweet corn enterprises. Monetary loss in seed corn is due to mandatory phytosanitary regulations that prohibit export, and/or direct yield losses. To date, there is little or no quantitative data on the temporal and spatial spread of this pathogen. Field experiments consisting of two treatments were conducted in 2007 in Ames, IA. Sweet corn seed (variety "Jubilee") treated with insecticide (Cruiser ®) or not treated with insecticide. Sweet corn was planted in plots 6 rows wide (76.2-cm row spacing), with 25 plants per row (91.44-cm within-row spacing). The center six corn plants in each plot were inoculated with a double-marked rifampicin-naldixic acid resistant isolate of *Pantoea stewartii*. To determine if the corn plants within plots were infected with the marked strain of *P. stewartii*, feeding scars caused by the vector (corn flea beetle) were sampled from each plot every 6–10 days, beginning 37 days after inoculation, and continuing until 16 August. The marked strain of *P. stewartii* was first detected on 13 July. The incidence of *P. stewartii* plants infected by the marked isolate was 4% in all of the seed-treated plots, compared to 4% to 6.7% in non-treated plots. By the end of the growing season, mean incidence for treated plots was 14.2 ± 3.15% and mean incidence within non-treated plots was 18.5 ±

1.92%. The exponential model provided the best fit to the pathogen progress data, and there was no statistical difference between the mean rates of pathogen increase over time in insecticide seed-treated plots (0.0089 ± 0.0019 ln units per day) versus nontreated plots (0.0105± 0.0010 ln units per day) (Figure 4 A and B). Spatial patterns based on ordinary runs analyses were random for all plots for all plots and sampling times.

INTRODUCTION

In the late 1880s, T. J. Burrill described a new bacterial disease of corn (Burrill et al., 1887). The bacterium causing this new disease (named Stewart's wilt) was first isolated by F. C. Stewart in Long Island, New York in 1895, however, he did not name the causal agent (Stewart et al., 1897). The pathogen was first named *Pseudomonas stewarti* by E. F. Smith (Smith, 1898), and was later subsequently changed to *Erwinia stewartii* (Smith) by Dye in 1963 (Dye, 1963). More recently, the name *Pantoea stewartii* (Smith) has been proposed based upon the results of DNA-DNA hybridization studies (Mergaert et al., 1993).

Stewart's wilt (also known as Stewart's disease) has been reported to occur in many U.S. states, including Connecticut, Illinois, Indiana, Iowa, Kentucky, Louisiana, Missouri, Nebraska, New York, North Dakota, Ohio, Pennsylvania, and Wisconsin. Stewart's disease has been reported to occur in other parts of the world (Austria, Brazil, Canada, China, Costa Rica, Greece, Guyana, Malaysia, Mexico, Peru, Poland, Puerto Rico, Romania, Russia, Thailand, and Viet Nam) (EPPO, 2008).

In the 1930's, Stewart's disease was reported to cause substantial economic loss to sweet corn production in Connecticut (Stevens, 1934; Elliot, 1935). Stewart's disease has been reported on both field corn and seed corn (Pepper, 1967; Munkvold, et al., 1996), although since the introduction of moderately-resistant field corn hybrids, Stewart's disease has had limited and sporadic impact on the yields of field corn hybrids (Pataky et al., 1990). Stewart's disease, however, continues to have severe negative impacts on the yields of both susceptible sweet corn hybrids and seed corn inbreds. According to Suparyono and Pataky (1989), Stewart's disease was found to cause 40% to 100% yield loss in susceptible sweet corn hybrids when infection occurred at or before the fifth leaf growth stage (V5) of corn development.

In Iowa, the greatest negative economic impact of Stewart's disease is due to the enforcement of zero-tolerance phytosanitary regulations that are imposed by many international trading partners (Pepper, 1967; McGee, 1995). When enforced, all seed corn produced from *P. stewartii*-infected seed corn fields may be: (i) sold as feed corn rather than seed corn (at a greatly reduced price/bu), (ii) harvested and redirected for sale on the domestic seed corn market, or (iii) subject to expensive laboratory tests, to determine if *P. stewartii* can be detected in seed corn lots (Pepper, 1967; McGee, 1995). Since field inspections of inbred seed corn began in Iowa in 1972, Stewart's disease has been detected in 21 of 32 years (through 2003) (Figure 1), with peak prevalence of this disease reported to be as high as 58% (in both 1999 and 2000) (Nutter et al., 1998; Esker and Nutter, 2001; Nutter et al., 2002).

Rand and Cash (1933) reported that the corn flea beetle, *Chaetocnema pulicaria*, was the primary vector of *Pantoea stewartii*. In 1934, Poos and Elliott reported that the bacterium could

overwinter within the gut of corn flea beetles, and that the overwintering generation of *P. stewartii*-infested corn flea beetles was epidemiologically, the most important source of initial inoculum for this disease. This finding was based on the results from testing over 28,500 insect specimens (representing 94 insect species and 76 genera) for the ability to transmit *P. stewartii* (Elliott and Poos, 1934). Based on this extensive research and subsequent studies by Block et al. (1998), the corn flea beetle is now generally accepted as being the primary insect vector for the survival and transmission of *P. stewartii* from season to season.

The corn flea beetle (*Chaetocnema pulicaria*) has two life cycles per year in Iowa, the overwintering generation, and a summer generation that includes 2 or more cycles per season (Esker et al., 2002). In Iowa, there is a beetle-free period that lasts approximately 2 to 4 weeks in June. The beetle-free period begins with the death of the adult overwintering generation, and ends in mid-to-late June with the emergence of the first summer generation. There are generally 2 growth peaks during summer, and the generation time between peaks is approximately 30 days. After corn fields senesce, corn flea beetles migrate back to grassy borders, where they overwinter. The mean acquisition feeding period (for 50% of corn flea beetles to acquire *P. stewartii*) was reported to be 36.5 ± 11.6 h, and the mean transmission period (for 50% of the corn flea beetle to transmit *P. stewartii*) was reported to be 7.6 ± 0.87 h (Menelas et al., 2006).

Based on a study by Esker et al. (2004), it is recommended that yellow sticky cards be placed vertically, 0.3 m above ground, becausee this trap height and orientation captured more corn flea beetles than other trap heights and directions (diagonal, horizontal, vertical). According to Cook et al. (2005), the relationship between corn flea beetle density and Stewart's disease

incidence (%) was best described by the model $y = 4.33 + 0.89(x)$, where y is the incidence of the Stewart's disease at the end of the season within each corn field, and x is the average number of corn flea beetles per trap per day, beginning 1 week after planting.

Quantitative data on the temporal and spatial spread of this bacterial pathogen in sweet corn is currently lacking. For seed corn field inspectors, knowledge concerning the spatial pattern of this disease may help support the development of more efficient sampling designs for this disease (Campbell and Madden, 1990). At present, seed corn field inspectors are simply instructed to "thoroughly" inspect each seed corn field, but are not given specific instructions as to what sampling design or sampling pattern to employ (Nutter and Gaunt, 1996). Finally, the effect of insecticide seed treatment on the temporal and spatial patterns of *P. stewartii* beyond the V5 growth stage, is not known. Therefore, the two objectives of this study were to determine the effect of insecticide seed treatment on the spatial pattern of *P. stewartii*–infected corn plants in field plots, and to quantify the rate of temporal spread of *Pantoea stewartii* in sweet corn.

MATERIALS AND METHODS

Plot preparation. The sweet corn variety “Jubilee” (Syngenta Seeds Inc.) was planted at the Iowa State University Johnson Research Farm in Ames, IA on 14 May 2007. Each experimental plot consisted of 8 rows spaced, 91.44 cm apart, with 29 plants hand-planted at 30 cm intervals per row. Each plot was bordered on all sides by dent corn. Two treatments, with three replications, were arranged in a randomized complete block design. Treatment 1 received an

insecticide seed treatment (Cruiser treated Jubilee seeds, 2.0 mg a.i./kg seed), and the second treatment was a nontreated control. Within each plot, the center six rows and the middle 25 corn plants per row were used to quantify the temporal rates and spatial patterns of *P.* stewartii-infected corn plants over time.

Dipel Dust ® (Voluntary Purchasing Groups, Inc., Bonham, TX) was applied to the plot area on 13 June 2007 to control an infestation of armyworms (*Pseudaletia unipuncta* (Haworth)). Miracle-Gro all purpose fertilizer (15% Nitrogen, 30% Phosphate, and 15% Potash) (Marysville, OH) was applied to sweet corn plants at the rate of 12.3 g/m^2 on 13 June 2007. Orange wooden stakes (30 cm long) were placed (after every 5 seedlings) within the rows on 25 June 2007 to accurately delineate plant positions (Steinlage et al., 2002).

Establishment of point sources of pathogen inoculum. *Pantoea stewartii* isolate Rif 9A was obtained from Dr. Charles B. Block, United States Department of Agriculture (USDA), Plant Introduction Station, Iowa State University, Ames, Iowa. Isolate Rif 9A is a rifampicin- and nalidixic acid-resistant (double-marked) isolate derived from a wild-type isolate of *P. stewartii* (SS104) (Menelas et al 2006). Stock cultures of this isolate were stored at –75ºC in 15% glycerol. Prior to the introduction of point sources of inoculum, cultures of *P. stewartii* isolate Rif 9A were grown for 48 h at 25ºC on nutrient broth yeast extract agar (NBY) amended with cycloheximide (100 μg/ml), rifampicin (50 μg/ml), and nalidixic acid (40 μg/ml (NBY-CRN). Bacterial suspension concentrations were adjusted to approximately 1×10^8 CFU/ml in sterile dd H_2O.

On 7 June 2007, thirty sweet corn plants (growth stage V7, variety Jubilee) were inoculated in the greenhouse with the Rif 9A *P. stewartii* isolate. Inoculated plants were acclimated to the outdoor environment by placing plants outside the greenhouse for 8 hours each day, during daylight hours, for 3 consecutive days. Plants were then placed outdoors for 3 additional days (72 hours). After overwintering adult corn flea beetles were detected in sweet corn plots at the Johnson Farm, six corn plants with visible symptoms of Stewart's disease were transplanted to the 12^{th}, 13^{th} and 14^{th} plant positions within the center two rows (rows 3 and 4) of each plot on 13 June 2007. Thus, there were six *P. stewartii* Rif 9A -inoculated source plants within each plot of 150 plants (disease incidence = 4.0%). After transplanting, source plants were watered twice every day for three days.

Monitoring corn flea beetle population densities. Four 15.2 X 15.2 cm yellow sticky cards were placed in the center 4 rows (one per row) of each plot on 18 May 2007. Sticky cards were mounted vertically, 0.3 m above the ground (Esker and Nutter, 2004). Two of the 4 sticky cards in each plot were placed facing north, and two were placed facing south. The numbers of corn flea beetles on each sticky card were recorded every 2 to 3 days, beginning one week after cards were first placed in sweet corn. Monitoring of sticky cards continued until the end of the season (Esker et al., 2002; Esker and Nutter, 2004). The number of corn flea beetles captured on the four yellow sticky cards in each plot was averaged for each sampling date, and the cumulative (mean) numbers of corn flea beetles in each sweet corn plot were graphed with respect to time of sampling. Area under the pathogen progress curve (AUPPC) (Nutter, 2001) which summarized the change in incidence of *P. stewartii*-infected plants (%) with respect to time was calculated for each corn plot (treatment and replication). Mean AUPPC values were compared for each

treatment (insecticide treated seeds versus the nontreated control) using Tukey's test ($P \leq 0.05$) (SAS 9.1.3, SAS Institute, Inc., Cary, NC).

Selective media preparation. To distinguish the double-marked Rif 9A isolate of *Pantoea stewartii* (Rif 9A) from wild type isolates of *P. stewartii*, a selective medium amended with rifampicin and nalidixic acid was used. This was prepared by amending broth yeast extract agar (NBY) with cycloheximide (100 μg/ml), rifampicin (50 μg/ml), and nalidixic acid (40 μg/ml) (NBY-CRN). Petri dishes (20.32-cm diameter) containing approximately 16 ml NBY-CRN media were poured, allowed to cool, and then stored at 4 ˚C until they were needed for the first sampling date.

Sampling of field isolates. Beginning seven days after *P. stewartii*-infected source plants were first placed within each plot, all plants in the center six rows (including source plants, n=150) were carefully examined for the presence of corn flea beetle feeding scars. Sampling of feeding scars to test for the presence of the Rif 9A (double-marked) isolate of *P. stewartii* began on 13 July and continued every 7 to 10 days until the end of the season. Up to 5 feeding scars per sweet corn plant were sampled by punching out a 0.8 cm diameter leaf disc that consisted of part of the healthy leaf area that surrounded each feeding scar, plus part of the feeding scar itself. Leaf disc samples from each plant were placed in 5 ml tubes containing 4 ml of sterile distilled water. Sample tubes were labeled and kept in a refrigerator (4 ˚C) until they were tested for the presence of the Rif 9A (double-marked) isolate.

Processing of leaf disc samples. Leaf disc samples from each plant were ground using sterile cotton swabs. The suspension from each sample tube was streaked onto NBY-CRN selective

media. Cultures were incubated at 25˚ C for 2-3 days and examined daily for the presence of *P. stewartii* (Rif 9A) colonies. Sweet corn plants were considered positive for the Rif 9A isolate if colonies of *P. stewartii* were recovered from the NBY-CRN media. Colonies were subsampled and tested for pathogenicity by preparing bacterial suspensions (as described previously) and inoculating corn seedlings (var. "Jubilee") at the 3rd leaf stage. Seedlings were then observed for the presence of typical Stewart's disease lesions. The incidence of Stewart's disease within each experimental plot was operationally defined as the number of sweet corn plants that had corn flea beetle feeding scars that tested positive for Rif 9A, divided by the total number of sweet corn plants in each plot (n = 150).

Temporal analyses. To determine the most appropriate pathogen growth model to quantify and compare Stewart's disease epidemics, the percentage of Rif 9A-infected sweet corn plants (incidence) was plotted versus time to obtain pathogen progress curves. For each experimental plot, the change in Stewart's disease (Rif 9A) incidence versus time (*dy/dt*) was also plotted with respect to time (rate curves) (Nutter 1997). Based upon the shapes of the pathogen progress and rate curves, Stewart's disease incidence for each plot was transformed for each sampling date using the exponential population growth model. Four other population growth models (linear, monomolecular, logistic, and Gompertz) were evaluated for model fit. The exponential population growth model best met model evaluation criteria (based on the *F* statistics for the overall model, R^2, and the standard error of estimate for *y* (SE*y*)) (Nutter, 1997). Using this model, rates of pathogen increase over time were calculated and compared for both treated versus nontreated plots. Treatment effects were analyzed using Tukey's test ($P \leq 0.05$) (SAS 9.1.3, SAS Institute, Inc., Cary, NC).

Spatial analyses. Field maps of Rif 9A-positive sweet corn plants within each experimental plot were generated for each sampling date. Both ordinary runs analysis (Gibbons 1971; 1976) and doublets analysis (Van der Plank 1946; 1960) were performed to determine if the spatial patterns for *P. stewartii*-infected plants (within each plot, treatment and replication) were random or clustered over time (Madden et al 1982).

For ordinary runs analyses, a run is defined as a series of like events. In this study, a run was defined as either a sequence of adjacent Rif 9A-infected sweet corn plants, or a sequence of healthy plants. The total number of runs in an experimental plot is represented by the symbol U. The symbol m represents the number of infected plants within a combined row. The symbol N represents the total number of plants in each plot (150). Under the null hypothesis of randomness, the expected value (E) of runs is given by:

$$E(U) = 1+2m(N-m)/N.$$

If the observed number of runs is less than the expected number of runs (E(U)), then the spatial pattern of infected plants is clustered. The standard deviation of U is given by:

$$SU = \{2m(N-m)[2m(N-m)-N]/[N2(N-1)]\}^{1/2},$$

and the standardized U is given by the Z statistic:

$$Z = [U-E(U)]/S.$$

If the Z value is less than -1.64 (for $P < 0.05$), then there is sufficient statistical evidence to conclude the spatial pattern of *P. stewartii*-infected plants in field plots was clustered.

For doublets analyses, A common practice is to combine adjacent rows to accommodate the spatial analysis of an entire plot, with r in the equation representing the number of rows (Van der Plank, 1963). A doublet was defined as the occurrence of two adjacent *P. stewartii* (Rif 9A)-infected plants, and represented by the symbol D. Under the null hypothesis of randomness, when adjacent rows are combined, the expected number of doublets is given by:

$$E(D) = (N-r)m(m-1)/[N(N-1)].$$

The standard deviation of doublets is given by:

$$SD = \{q-q^2+q(m-1)(m-2)/(N-1)\}^{1/2}, \quad q = m(m-1)/N.$$

The standardized D is given by the Z-statistic. If the absolute value of Z-statistics is greater than 1.64, then the spatial pattern of *P. stewartii* (Rif 9A)-infected plants within a plot is considered to be clustered.

RESULTS

Based on model criteria, the exponential model best fit the pathogen progress and rate curves for Stewart's disease (Table 1, Figure 2 and 3, Appendix I). The mean rate of pathogen increase over time in nontreated plots (0.0105 ± 0.0010 log units per day) was not statistically different from the mean rate in insecticide seed treated plots (0.0089 ± 0.0019 log units per day). There was no statistical difference in time of disease detection in treated versus non-treated plots (F = 4.00, P = 0.1161). Insecticide-treated seed did not affect the rate of pathogen expansion in 2007 (F =1.72, P = 0.2597) (Table 1, Figure 4). The final incidence of Stewart's disease in treated (14.2%) versus nontreated plots (18.4%) was not statistically different (F = 3.93, P = 0.1186).

There was no statistical significant difference in AUPPC values for nontreated (1.01) and treated (0.81) sweet corn plots ($F = 3.10$, $P = 0.1531$).

Ordinary runs analysis of the spatial maps (Appendix II) of *P. stewartii* (Rif 9A)-infected corn plants within each sweet corn plot revealed that the spatial patterns of *P. stewartii* (Rif 9A)-infected plants were highly random for each sampling date (Table 2, Appendix III). Doublets analysis indicated most spatial patterns were random over time; one of the three treated plots displayed a weak (but significant) clustered pattern late in the growing season (sampling date 10 August and 16 August) (Table 3, Appendix IV).

The overwintering generation of the corn beetle population (observed from 21 May to 1 June) at the ISU Johnson Farm in Ames, IA was very low in 2007 (Figure 5). The period from 5 June to 1 July was mostly beetle-free, with the exception that 2 beetles were captured on 15 June in one treated plot. Corn flea beetles were captured in all experimental plots on 4 July, which was after the source plants had been transplanted into plots for corn flea beetle feeding scars (13 June). Therefore, the first sampling date (13 July) occurred approximately 10 days after the first summer beetle generation of begun to emerge and feed on *P. stewartii*-infected source plants. The cumulative number of corn flea beetles detected during sampling was very similar for both insecticide seed-treated and nontreated plots (Figure 6). Areas under the curve, calculated for corn flea beetle population density, was not changed by seed treatment (F =1.18, and P = 0.3387).

DISCUSSION

This study was the first to use a double-marked isolate of *P. stewartii* to quantify the rate of plant-to-plant spread of *P. stewartii* within sweet corn. The exponential growth model best fit pathogen progress data here. Perhaps due to the low corn flea beetle population density and the fact that pathogen incidence in the epidemic never exceeded $y = 0.5$ (50%). Therefore, pathogen progress remained in the exponential phase because the proportion of *P. stewartii*–infected plants remained low (thus limiting r) and the proportion of healthy plants ($1 - y$) was well above the inflection point for this model (0.5), indicating that the proportion of plants not yet infected ($1 - y$) was not limiting the exponential rate of *P. stewartii* spread. Another reason for the slow epidemic rate may be related to the length of infectious period of the corn flea beetles. Based upon preliminary research (conducted by our laboratory) on the length of the infectious period (i.e. how long a corn flea beetle remains infectious and can transmit the pathogen), corn flea beetles did not always remain infectious after acquiring the bacterium (*P. stewartii*). However, more experiments are needed to verify this hypothesis.

The lack of statistical difference in the rates of disease progress in insecticide-seed-treated versus nontreated plots may be explained by weather and timing factors. In the 2007 growing season, very dry weather followed a cold winter, and the population of corn flea beetles that survived the 2006-2007 winter was very low in Iowa (Esker et al., 2006). Also, source plants were placed within plots in 13 June, during the beetle-free period (5 June to 1 July); therefore, differences in insect populations could not be observed during this period. Plants in our experiment plots reached V5 growth stage on 1 June. Insecticide seed treatment is thought to be

efficient only until the V5 growth stage (Munkvold et al., 1996); therefore, source plants were introduced after this critical growth stage. Our results confirmed the conclusions reported by Munkvold (1996; 2001), that insecticide seed treatment ceases to be effective in controlling Stewart's disease epidemics after the V5 growth stage. This is further supported by the finding that there was no statistical difference between the cumulative number of corn flea beetles in treated versus nontreated plots during the monitored period.

This was the first study to determine if the spatial pattern of *P. stewartii*-infected sweet corn plants was random or clustered over time. For this experiment, spatial pattern of Stewart's disease was found to be random based on the result of both ordinary runs analysis and doublets analysis. The random spatial pattern may be explained by the acquisition and transmission efficiency of corn flea beetles, and by the moving pattern of the beetles. The average acquisition feeding period (for 50% of the corn flea beetles to acquire *P. stewartii*) is 36.5 ± 11.6 h, while 48 h is needed for 100% of the beetle population to successfully transmit the bacterium (Menelas et al., 2006). We observed that corn flea beetles move (primarily) by jumping, and the jumping distance is about 60 cm. And again, there was a low beetle population in the field in 2007. All these may account for the random spatial pattern of Stewart's disease observed in this study in 2007. Knowledge regarding the random spatial pattern of Stewart's disease can assist farmers and field inspectors to select a random sampling design for seed corn field inspections (Nutter, 2007) to effectively discover the existence of *Pantoea stewartii* infected plants. However, this study needs to be repeated in at least one more year to verify the results.

There was no statistical difference in time of disease detection for the two treatments. However, the rifampicin- and nalidixic acid-resistant strain (Rif 9A) was detected in two of the three nontreated plots on the first sampling date, while Rif 9A was first detected in the remaining four plots (one nontreated and three treated plots) on the second sampling date. More data is required to determine the efficacy of insecticide seed treatment for delaying the spread of Stewart's disease. At least one more year of this experiment is needed to verify the result.

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TABLES AND FIGURES

Table 1. Parameters and statistics for different population growth models (linear, monomolecular, exponential, logistic, and Gompertz), used to describe the change in the incidence of sweet corn plants (variety "Jubilee") testing positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii*, with respect to day of year, for both non-seed-treated and insecticide-seed-treated sweet corn plots located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.

		Johnson 2007				
Plot number	Growth model	p-value	SEEy	R^2	Intercept	Slope
Nontreated plot – Rep 1	Linear	0.01795	0.02501	0.78949	-0.2844	0.00187
Nontreated plot – Rep 2	Linear	0.02460	0.03265	0.75527	-0.3511	0.00221
Nontreated plot – Rep 3	Linear	0.05646	0.04628	0.63866	-0.3873	0.00237
Treated plot – Rep 1	Linear	0.03301	0.02977	0.71866	-0.2853	0.00183
Treated plot – Rep 2	Linear	0.05074	0.01889	0.65605	-0.1408	0.00100
Treated plot – Rep 3	Linear	0.02918	0.03010	0.7346	-0.3028	0.00193
Nontreated plot – Rep 1	Monomolecular	0.02101	0.02918	0.77297	-0.3207	0.00207
Nontreated plot – Rep 2	Monomolecular	0.02663	0.03737	0.74584	-0.3964	0.00246
Nontreated plot – Rep 3	Monomolecular	0.06069	0.05358	0.62643	-0.4416	0.00267
Treated plot – Rep 1	Monomolecular	0.03507	0.03351	0.71055	-0.3185	0.00202
Treated plot – Rep 2	Monomolecular	0.05202	0.02048	0.65206	-0.1536	0.00108
Treated plot – Rep 3	Monomolecular	0.03143	0.03414	0.72509	-0.3392	0.00213
Nontreated plot – Rep 1	Exponential	0.00167	0.06524	0.93393	-2.9917	0.00944
Nontreated plot – Rep 2	Exponential	0.00922	0.11996	0.84741	-3.2930	0.01088
Nontreated plot – Rep 3	Exponential	0.02805	0.17361	0.73950	-3.4058	0.01126
Treated plot – Rep 1	Exponential	0.01719	0.13052	0.79377	-3.1266	0.00986
Treated plot – Rep 2	Exponential	0.03440	0.11145	0.71314	-2.6074	0.00676
Treated plot – Rep 3	Exponential	0.01447	0.1278	0.81019	-3.1811	0.01016
F-statistics [a]				1.72		
P value				0.2597		
Nontreated plot – Rep 1	Logistic	0.00230	0.17904	0.92271	-7.2092	0.02381
Nontreated plot – Rep 2	Logistic	0.01017	0.31204	0.83998	-7.9789	0.02752
Nontreated plot – Rep 3	Logistic	0.03006	0.45086	0.73083	-8.2837	0.02860
Treated plot – Rep 1	Logistic	0.01807	0.33224	0.78880	-7.5178	0.02472
Treated plot – Rep 2	Logistic	0.03533	0.27684	0.70952	-6.1573	0.01666
Treated plot – Rep 3	Logistic	0.01523	0.32603	0.80548	-7.6639	0.02554
Nontreated plot – Rep 1	Gompertz	0.00557	0.0842	0.88061	-2.6739	0.00880
Nontreated plot – Rep 2	Gompertz	0.01417	0.12816	0.81211	-2.9684	0.01026
Nontreated plot – Rep 3	Gompertz	0.03780	0.18304	0.70014	-3.0986	0.01077
Treated plot – Rep 1	Gompertz	0.02193	0.12789	0.7683	-2.7495	0.00897
Treated plot – Rep 2	Gompertz	0.03974	0.09787	0.69303	-2.1837	0.00566
Treated plot – Rep 3	Gompertz	0.01864	0.12648	0.7856	-2.8131	0.00932

[a] F-test on the slopes from the Exponential model for each treatment to compare the effect of treatment.

Table 2. Spatial pattern, based on ordinary runs analysis, of *Pantoea stewartii* (Rif 9A)-infected sweet corn plants (variety "Jubilee") for each sampling date in each plots at the Iowa State University Johnson Research Farm, Ames, Iowa, 2007.

	Spatial pattern from ordinary runs analysis					
	Insecticide seed-treated			Non-insecticide seed-treated		
Sampling date	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
13 Jul	n/a	n/a	n/a	random	random	n/a
23 Jul	random	random	random	random	random	random
2 Aug	random	random	random	random	random	random
10 Aug	random	random	random	random	random	random
16 Aug	random	random	random	random	random	random

Table 3. Spatial pattern, based on doublets analysis, of *Pantoea stewartii* (Rif 9A)-infected sweet corn plants (variety "Jubilee") for each sampling date in each plots at the Iowa State University Johnson Research Farm, Ames, Iowa, 2007.

	Spatial pattern from doublets analysis					
	Insecticide seed-treated			Non-insecticide seed-treated		
Sampling date	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
13 Jul	n/a	n/a	n/a	random	n/a	n/a
23 Jul	random	n/a	random	random	random	random
2 Aug	random	random	random	random	random	random
10 Aug	clustering	random	random	random	random	random
16 Aug	clustering	random	random	random	random	random

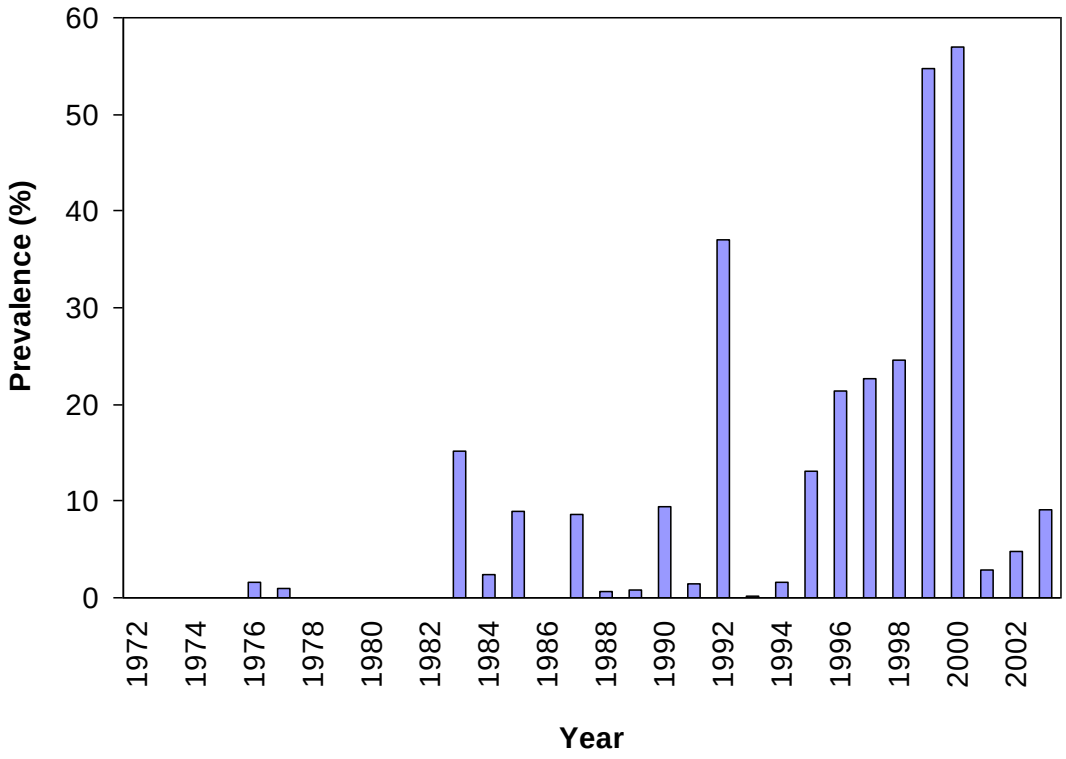


Figure 1. Prevalence of Stewart's disease of corn in Iowa seed corn fields from 1972 to 2003. The number of seed corn fields inspected ranged from approximately 500 seed corn fields in the 1970s to more than 1,300 fields in the latter two decades.

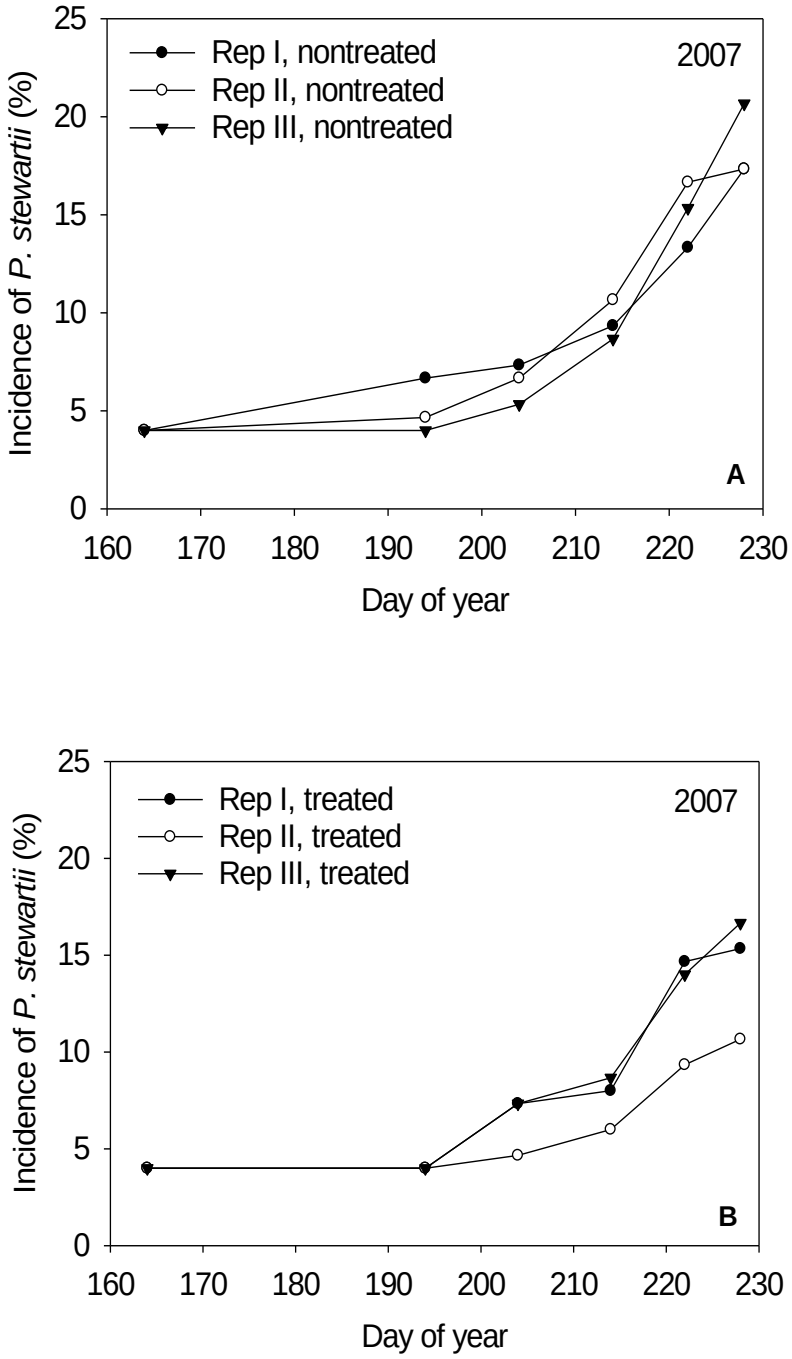


Figure 2. Percentage of sweet corn plants (variety "Jubilee") testing positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to time (day of year) for both (A) non-seed-treated and (B) insecticide-seed-treated sweet corn plots located at the Iowa State University Johnson Research Farm in Ames, Iowa in 2007.

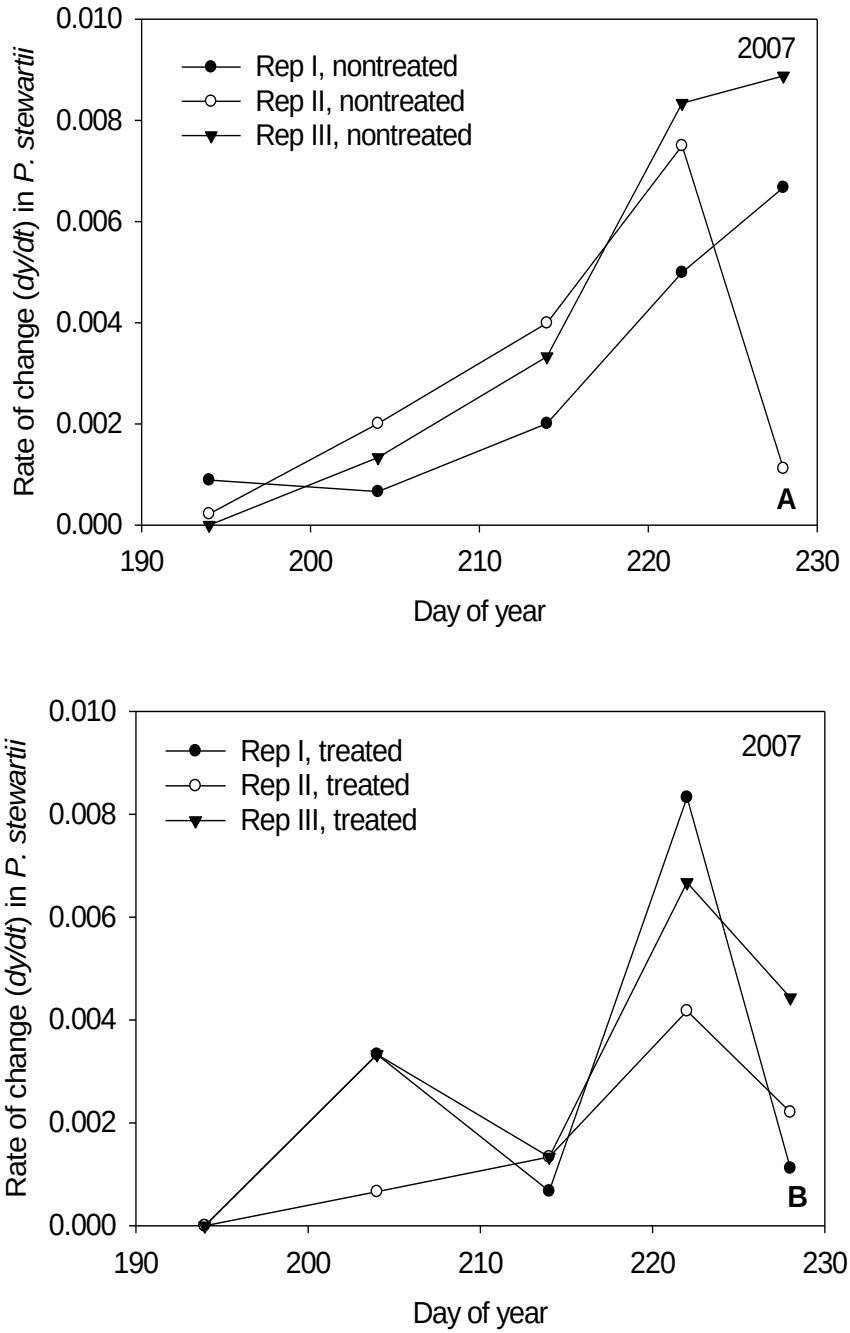


Figure 3. Rate of change (*dy/dt*) in the percentage of sweet corn plants (variety "Jubilee") testing positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to time (day of year) for both (A) non-seed-treated, and (B) insecticide-seed-treated corn plots located at the Iowa State University Johnson Research Farm in Ames, Iowa in 2007.

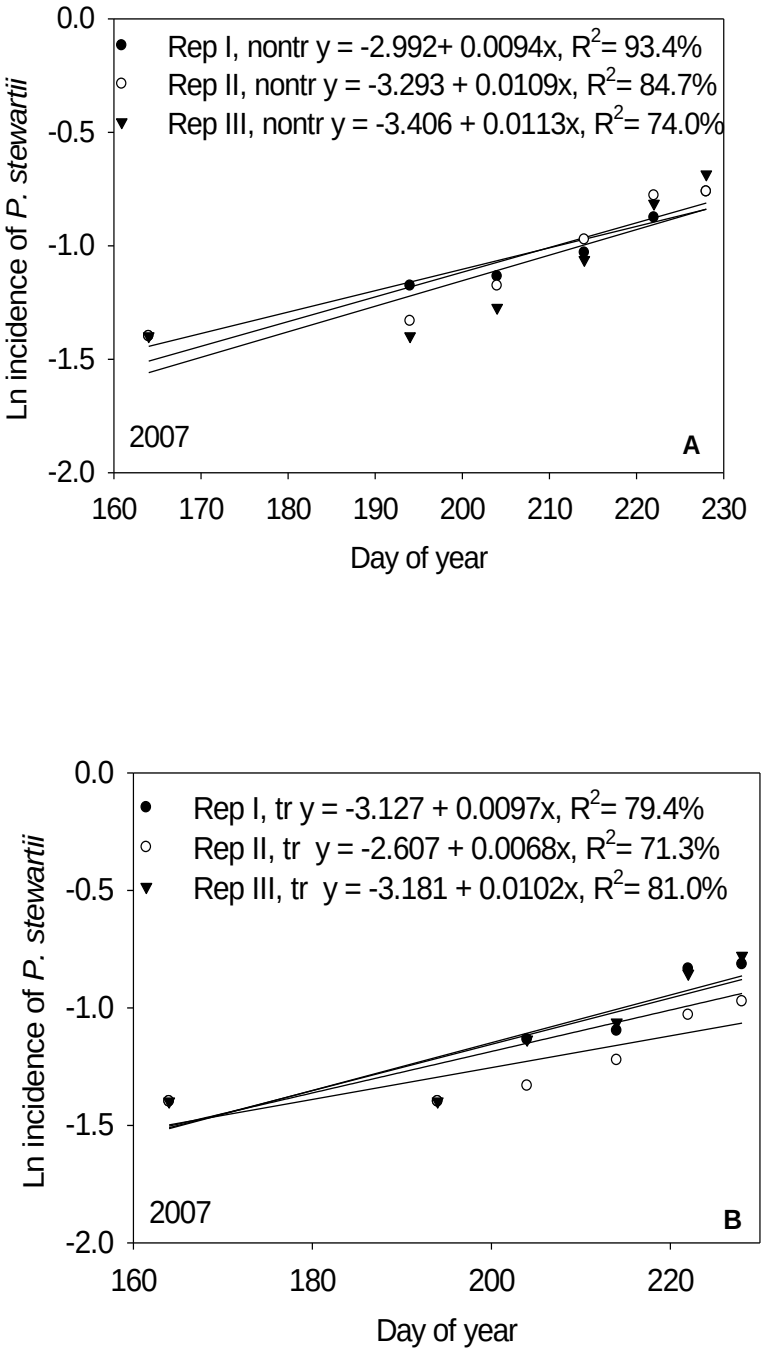


Figure 4. Effect of insecticide seed treatment or no insecticide seed treatment on the exponential growth rate of *P. stewartii* on sweet corn (variety "Jubilee"), using a rifampicin-and nalidixic acid-resistant isolate, for both (A) non-seed-treated and (B) insecticide-seed-treated corn plots located at the Iowa State University Johnson Research Farm in Ames, Iowa in 2007.

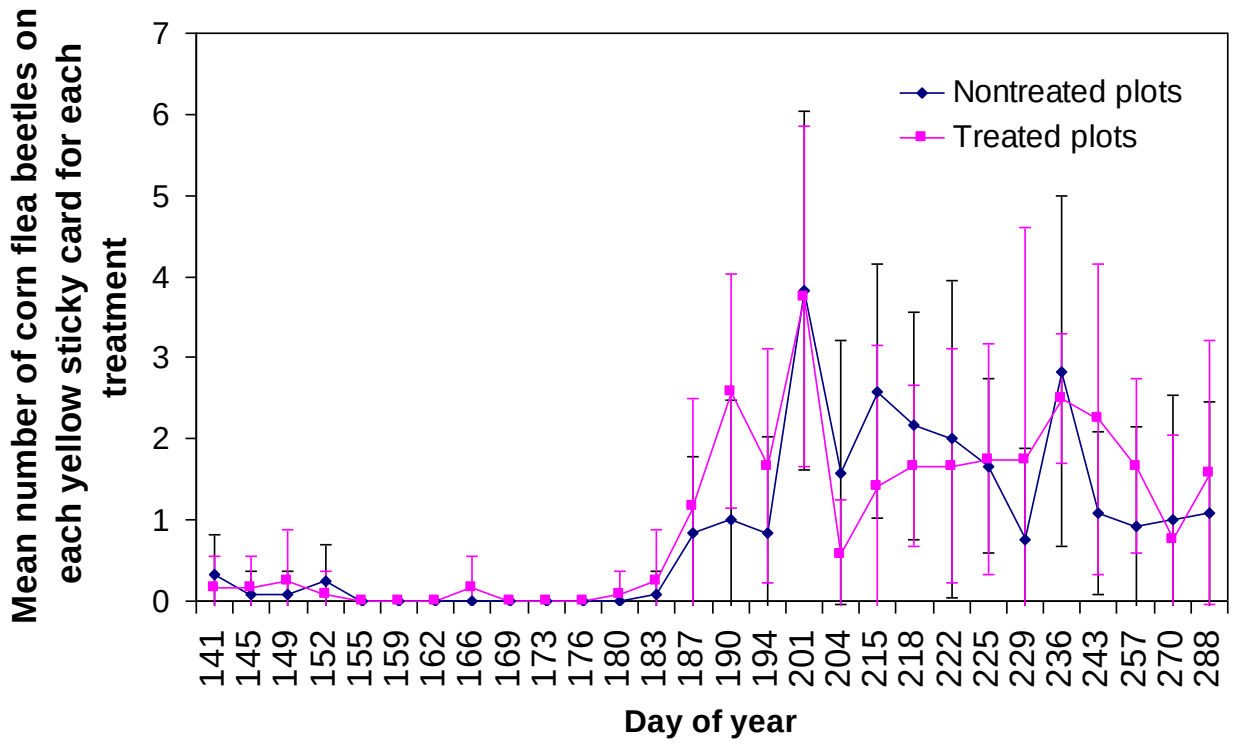


Figure 5. Mean number of corn flea beetles per yellow sticky trap card plotted against time (day of year) in both insecticide seed-treated and nontreated sweet corn plots (variety "Jubilee") at the Iowa State University Johnson Research Farm in Ames, Iowa in 2007.

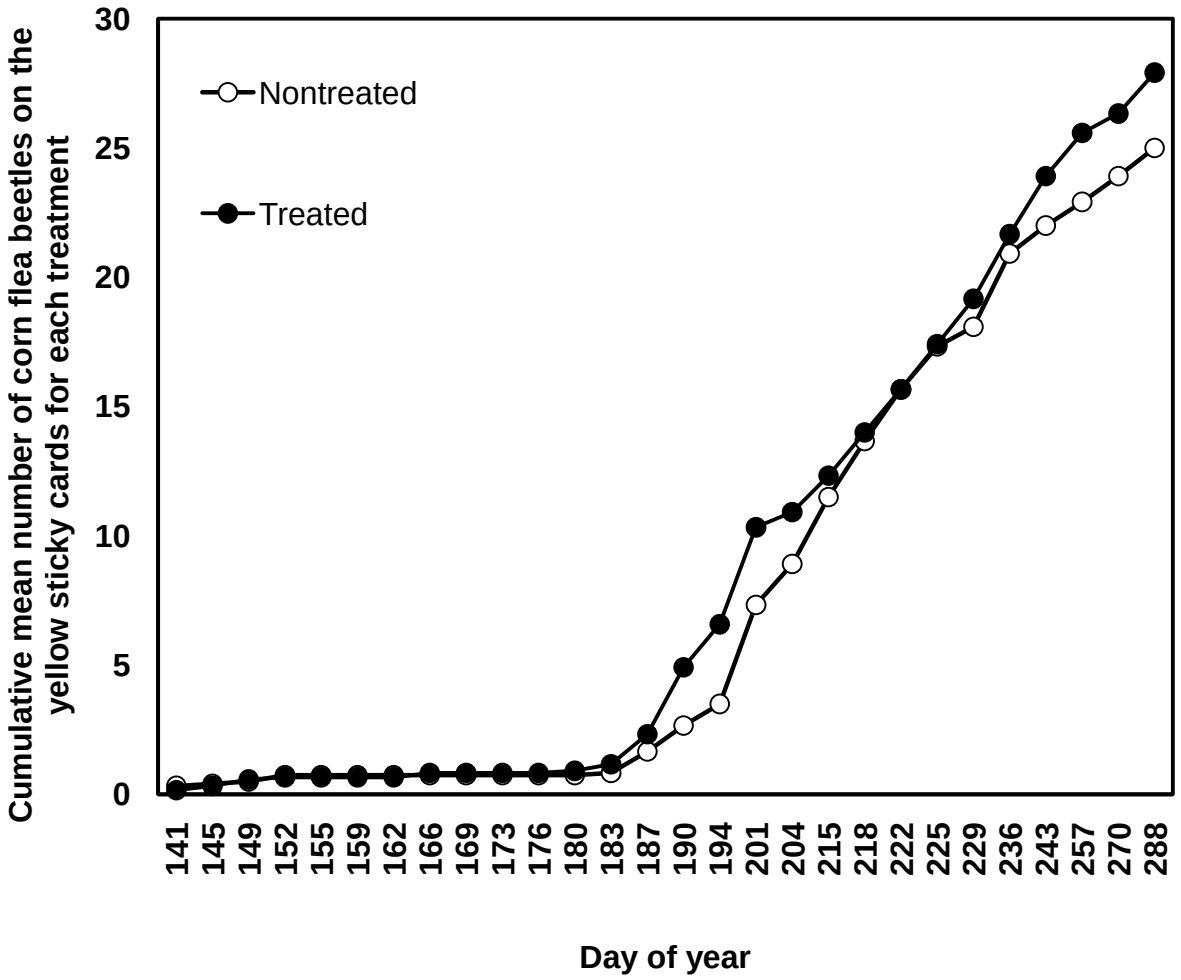


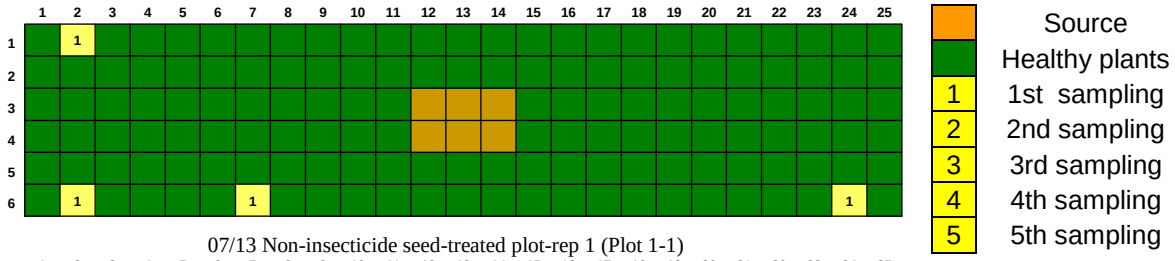
Figure 6. Cumulative number of corn flea beetles on yellow sticky cards in both treated and nontreated corn plots (variety "Jubilee") over time in sweet corn field plots located at the Iowa State University Johnson Research Farm in Ames, Iowa 2007. Black dots depict the number of corn flea beetles trapped per yellow sticky card in treated plots, and white dots depict the number of corn flea beetles trapped in nontreated plots.

APPENDIX I. Percentage of sweet corn plants (variety "Jubilee") testing positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii*, with respect to day of year for both non-seed-treated and insecticide-seed-treated sweet corn plots located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.

		Day of Year					
Treatment	Replication	164	194	204	214	222	228
N[a]	1	0.04	0.06669	0.07331	0.09338	0.13331	0.17334
N	2	0.04	0.04662	0.06669	0.10662	0.16662	0.17334
N	3	0.04	0.04	0.05334	0.08666	0.15338	0.20666
Mean		0.04	0.0511	0.06445	0.09555	0.1511	0.18445
Standard deviation		0	0.0139	0.01017	0.01016	0.01677	0.01923
T[b]	1	0.04	0.04	0.07331	0.08003	0.14666	0.15338
T	2	0.04	0.04	0.04662	0.05997	0.09338	0.10662
T	3	0.04	0.04	0.07331	0.08666	0.14003	0.16662
Mean		0.04	0.04	0.06442	0.07555	0.12669	0.14221
Standard deviation		0	0	0.01541	0.0139	0.02904	0.03152

[a] N = nontreated plots (Cruiser treated Jubilee seeds).
[b] T = treated plots (Cruiser treated Jubilee seeds).

APPENDIX II A. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 1 of the non-insecticide seed-treated corn treatment (plot 1-1) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.



07/13 Non-insecticide seed-treated plot-rep 1 (Plot 1-1)

07/23 Non-insecticide seed-treated plot-rep 1 (Plot 1-1)

08/02 Non-insecticide seed-treated plot-rep 1 (Plot 1-1)

08/10 Non-insecticide seed-treated plot-rep 1 (Plot 1-1)

08/16 Non-insecticide seed-treated plot-rep 1 (Plot 1-1)

APPENDIX II B. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 2 of the non-insecticide seed-treated corn treatment (plot 1-2) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.

07/13 Non-insecticide-seed treated plot-rep 2 (Plot 1-2)

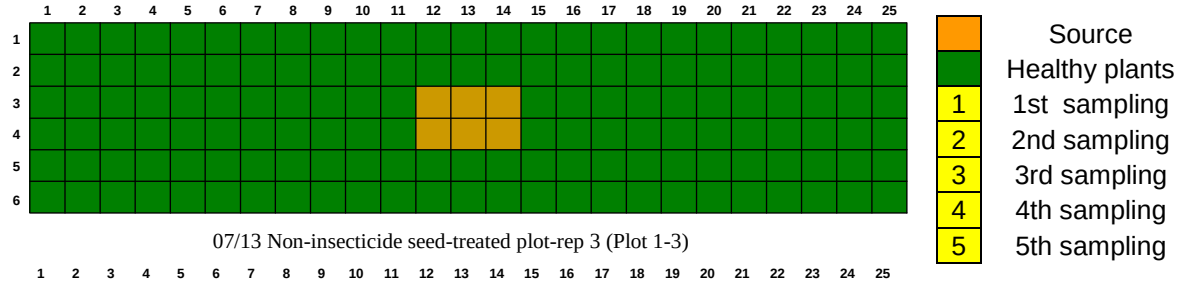
07/23 Non-insecticide seed-treated plot-rep 2 (Plot 1-2)

08/02 Non-insecticide seed-treated plot-rep 2 (Plot 1-2)

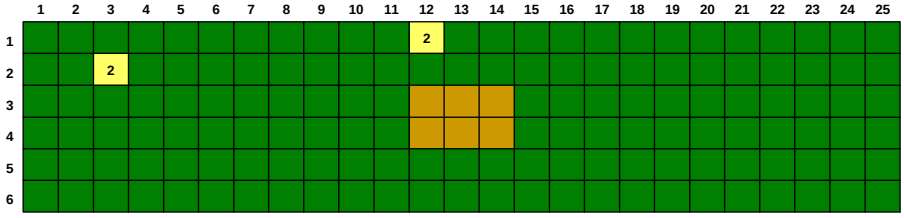
08/10 Non-insecticide seed-treated plot-rep 2 (Plot 1-2)

08/16 Non-insecticide seed-treated plot-rep 2 (Plot 1-2)

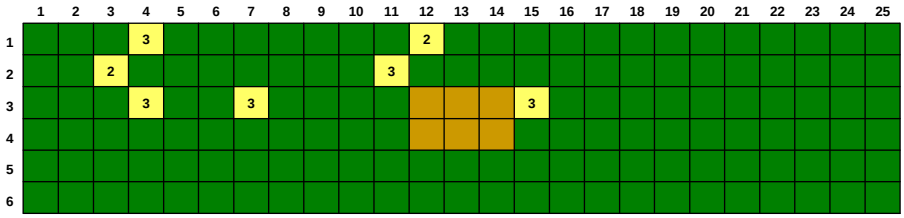
APPENDIX II C. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 3 of the non-insecticide seed-treated corn treatment (plot 1-3) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.



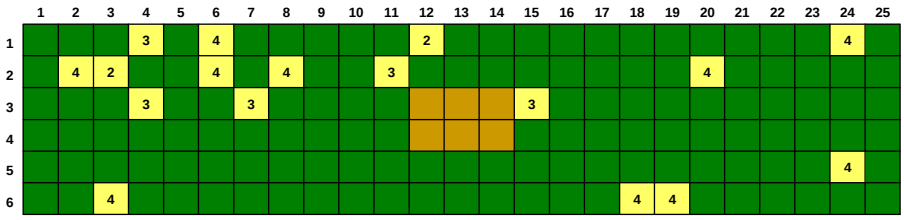
07/13 Non-insecticide seed-treated plot-rep 3 (Plot 1-3)



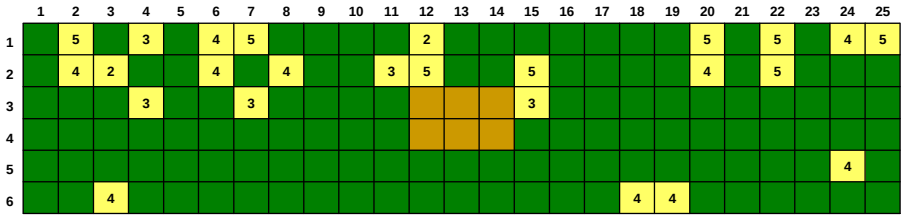
07/23 Non-insecticide seed-treated plot-rep 3 (Plot 1-3)



08/02 Non-insecticide seed-treated plot-rep 3 (Plot 1-3)

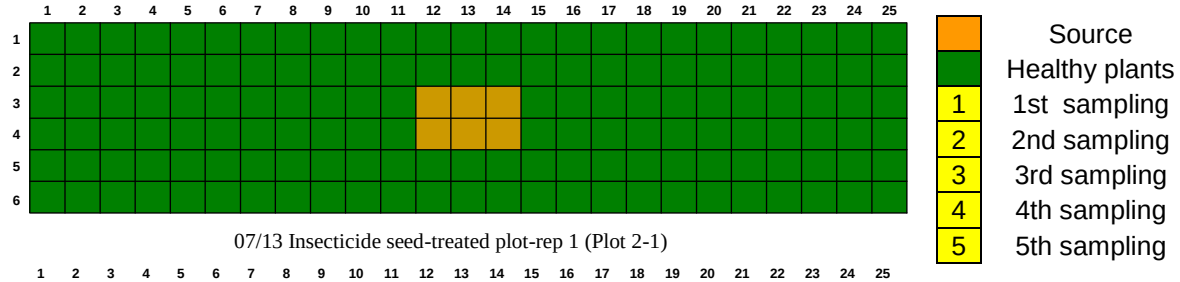


08/10 Non-insecticide seed-treated plot-rep 3 (Plot 1-3)



08/16 Non-insecticide seed-treated plot-rep 3 (Plot 1-3)

APPENDIX II D. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 1 of the insecticide seed-treated corn treatment (plot 2-1) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.



07/13 Insecticide seed-treated plot-rep 1 (Plot 2-1)

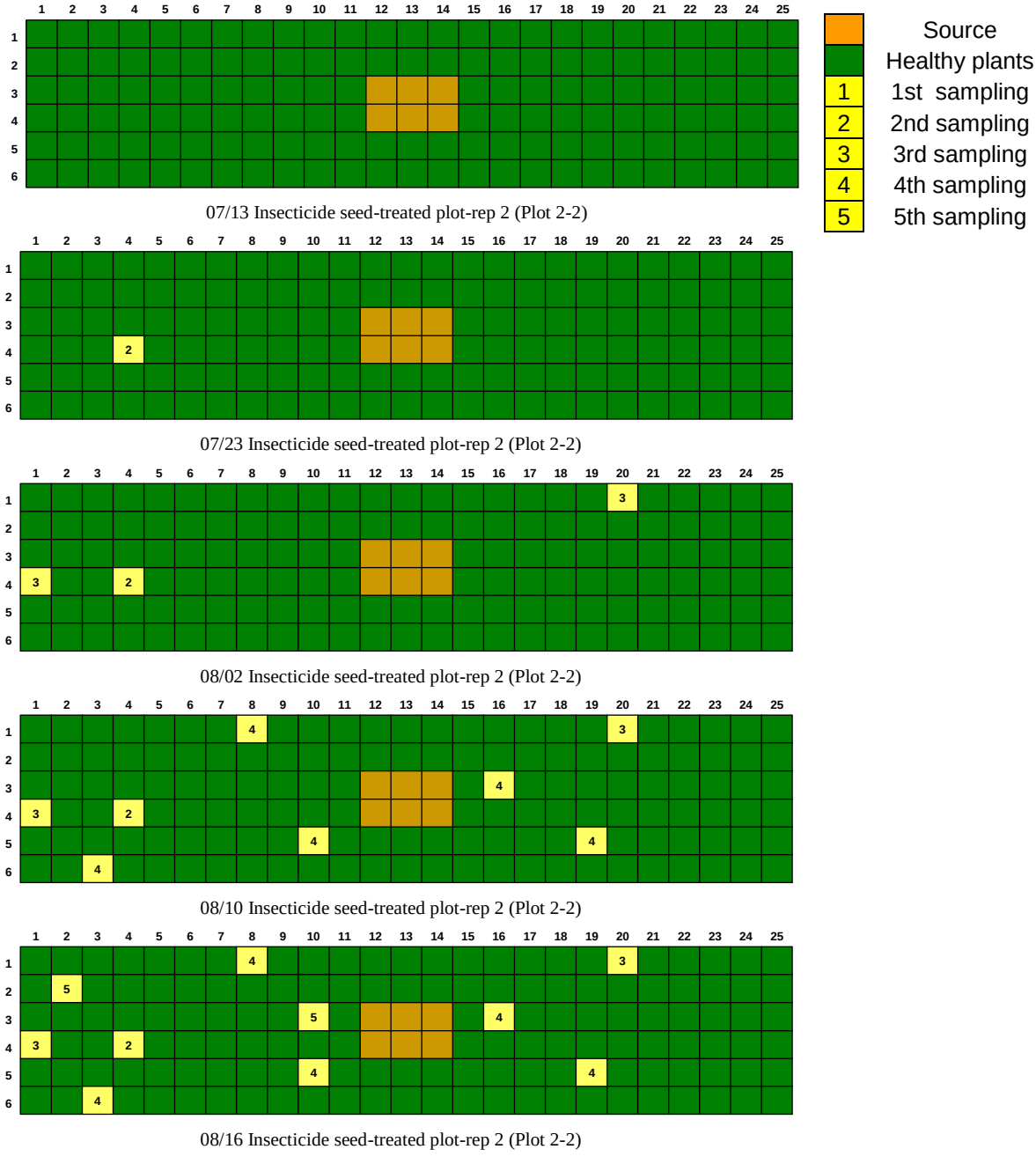
07/23 Insecticide seed-treated plot-rep 2 (Plot 2-1)

08/02 Insecticide seed-treated plot-rep 1 (Plot 2-1)

08/10 Insecticide seed-treated plot-rep 1 (Plot 2-1)

08/16 Insecticide seed-treated plot-rep 1 (Plot 2-1)

APPENDIX II E. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 2 of the insecticide seed-treated corn treatment (plot 2-2) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.



07/13 Insecticide seed-treated plot-rep 2 (Plot 2-2)

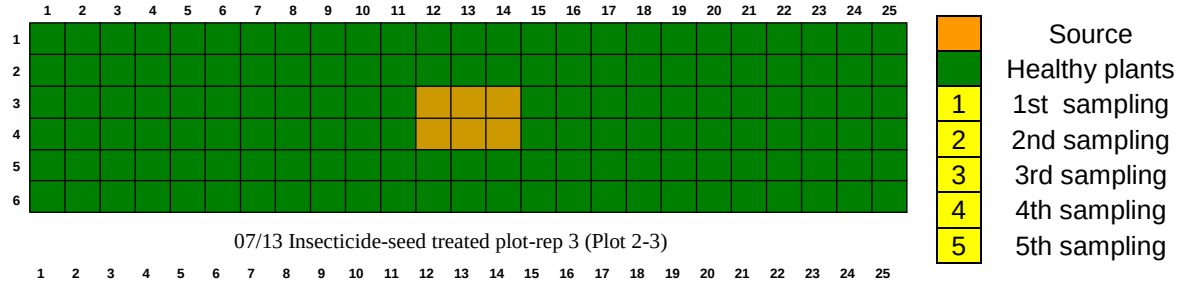
07/23 Insecticide seed-treated plot-rep 2 (Plot 2-2)

08/02 Insecticide seed-treated plot-rep 2 (Plot 2-2)

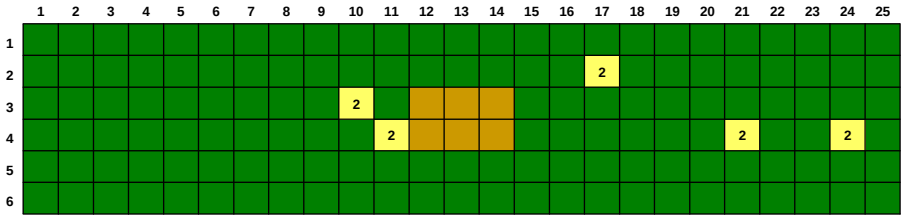
08/10 Insecticide seed-treated plot-rep 2 (Plot 2-2)

08/16 Insecticide seed-treated plot-rep 2 (Plot 2-2)

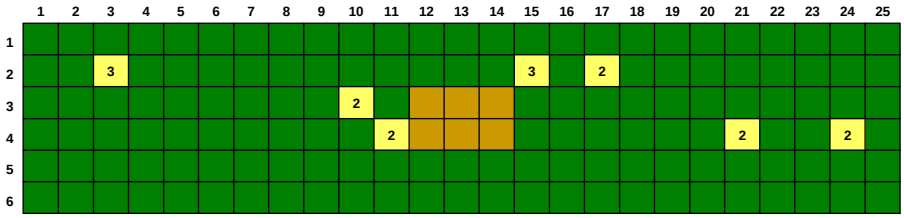
APPENDIX II F. Spatial maps of corn plants (variety "Jubilee") that tested positive for the presence of the rifampicin- and nalidixic acid-resistant isolate of *Pantoea stewartii* with respect to date of sampling in rep 3 of the insecticide seed-treated corn treatment (plot 2-3) located at the Iowa State University Johnson Research Farm, Ames, Iowa in 2007.



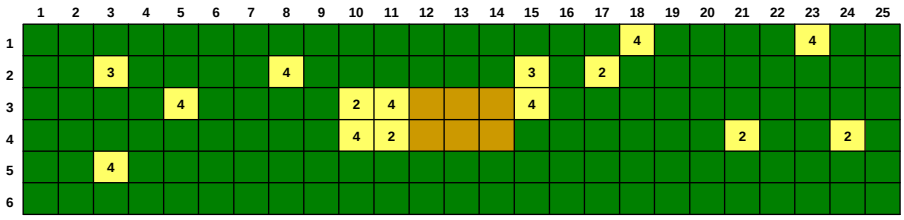
07/13 Insecticide-seed treated plot-rep 3 (Plot 2-3)



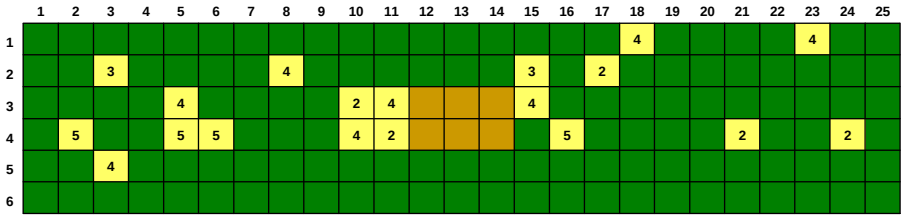
07/23 Insecticide seed-treated plot-rep 3 (Plot 2-3)



08/02 Insecticide seed-treated plot-rep 1 (Plot 2-3)



08/10 Insecticide seed-treated plot-rep 3 (Plot 2-3)



08/16 Insecticide seed-treated plot-rep 3 (Plot 2-3)

APPENDIX III. Spatial pattern of *Pantoea stewartii* (Rif 9A) over time based on the results of ordinary runs analysis from each sampling date for each corn plot (variety Jubilee) on Johnson Research Farm of Iowa State University, Ames, Iowa 2007.

	Number of infected corn plans (m)					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	4	0	0	0	1	0
23-Jul	5	1	2	5	4	5
2-Aug	8	3	7	6	10	7
10-Aug	14	8	17	16	19	15
16-Aug	20	10	25	17	20	19

	Expected number of runs (E(U))					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	8.78	1.00	1.00	1.00	2.99	1.00
23-Jul	10.65	2.99	4.94	10.65	8.78	10.65
2-Aug	16.11	6.88	14.32	12.50	19.61	14.32
10-Aug	26.28	16.11	30.99	29.44	33.99	27.88
16-Aug	35.44	19.61	42.32	30.99	35.44	33.99

	Actual number of runs (U)					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	9	1	1	1	3	1
23-Jul	11	3	5	10	9	11
2-Aug	17	7	15	12	19	15
10-Aug	23	17	31	28	37	25
16-Aug	31	21	41	30	39	31

	Standard deviation of number of runs (S(U))					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	0.61	0.00	0.00	0.00	0.12	0.00
23-Jul	0.76	0.12	0.28	0.76	0.61	0.76
2-Aug	1.22	0.45	1.07	0.92	1.51	1.07
10-Aug	2.07	1.22	2.47	2.34	2.72	2.21
16-Aug	2.84	1.51	3.41	2.47	2.84	2.72

	Z-statistics					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	0.37	n/a	n/a	n/a	0.12	n/a
23-Jul	0.45	0.12	0.19	-0.85	0.37	0.45
2-Aug	0.73	0.28	0.64	-0.54	-0.40	0.64
10-Aug	-1.58	0.73	0.01	-0.62	1.11	-1.30
16-Aug	-1.57	0.92	-0.39	-0.40	1.25	-1.10

APPENDIX IV. Spatial pattern of *Pantoea stewartii* (Rif 9A) over time based on the results of doublets analysis from each sampling date for each corn plot (variety Jubilee) on Johnson Research Farm of Iowa State University, Ames, Iowa 2007.

	Number of infected corn plans (m)					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	4	0	0	0	1	0
23-Jul	5	1	2	5	4	5
2-Aug	8	3	7	6	10	7
10-Aug	14	8	17	16	19	15
16-Aug	20	10	25	17	20	19

	Expected number of doublets (E(D))					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	0.08	0.00	0.00	0.00	0.00	0.00
23-Jul	0.13	0.00	0.01	0.13	0.08	0.13
2-Aug	0.38	0.04	0.28	0.20	0.60	0.28
10-Aug	1.22	0.38	1.82	1.61	2.29	1.41
16-Aug	2.55	0.60	4.02	1.82	2.55	2.29

	Actual number of doublets (D)					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	4	0	0	0	1	0
23-Jul	5	1	2	5	4	5
2-Aug	8	3	7	6	10	7
10-Aug	14	8	17	16	19	15
16-Aug	20	10	25	17	20	19

	Standard deviation of number of doublets (S(D))					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	0.28	0.00	0.00	0.00	0.00	0.00
23-Jul	0.36	0.00	0.12	0.36	0.28	0.36
2-Aug	0.59	0.20	0.52	0.44	0.74	0.52
10-Aug	1.02	0.59	1.22	1.16	1.35	1.09
16-Aug	1.41	0.74	1.70	1.22	1.41	1.35

	Z-statistics					
Sampling date	Plot 1-1	Plot 2-2	Plot 1-3	Plot 2-1	Plot 1-2	Plot 2-3
13-Jul	-0.28	n/a	n/a	n/a	n/a	n/a
23-Jul	-0.37	n/a	-0.11	-0.37	-0.28	-0.37
2-Aug	-0.63	-0.20	-0.54	-0.46	0.54	-0.54
10-Aug	1.74	-0.63	0.15	0.34	-0.96	1.46
16-Aug	1.74	-0.81	0.58	0.15	-1.10	1.27

APPENDIX V. Cumulative number of corn flea beetles on yellow sticky cards in both treated and nontreated corn plots (variety "Jubilee") over time in sweet corn field plots located at the Iowa State University Johnson Research Farm in Ames, Iowa 2007. Black dots depict the number of corn flea beetles trapped per yellow sticky card in treated plots, and white dots depict the number of corn flea beetles trapped in nontreated plots.