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PEST RISK ANALYSIS

The risk of introducing *Erwinia stewartii* in maize seed

for

The International Seed Federation
Chemin du Reposoir 7
1260 Nyon, Switzerland

by

Jerald Pataky
Professor of Plant Pathology
University of Illinois
Department of Crop Sciences
1102 S. Goodwin Ave.
Urbana, IL 61801 USA

Robert Ikin
Biosecurity Consultant
Box 148
Taigum QLD 4018
Australia

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PREFACE

Maize is one of the most important agricultural crops worldwide and there is considerable international trade in seed. A high volume of this seed originates in the United States, where much of the development of new varieties occurs. *Erwinia stewartii* (*Pantoea stewartii*) is a bacterial pathogen (pest) of maize that occurs primarily in the US. In order to prevent the introduction of this bacterium to other areas, a number of countries have instigated phytosanitary measures on trade in maize seed for planting.

This analysis of the risk of introducing *Erwinia stewartii* in maize seed was prepared at the request of the International Seed Federation (ISF) as an initiative to promote transparency in decision making and the technical justification of restrictions on trade in accordance with international standards. In 2001 a consensus among ISF (then the International Seed Trade Federation (FIS)) members, including representatives of the seed industry from more than 60 countries developed a first version of this PRA as a qualitative assessment following the international standard, FAO Guidelines for Pest Risk Analysis (Publication No. 2, February 1996). The global study completed Stage 1 (Risk initiation) and Stage 2 (Risk Assessment) but did not make comprehensive Pest Risk management recommendations (Stage 3) that are necessary for trade to take place. With the adoption of the International Standards for Phytosanitary Measures No 11 “Pest risk analysis for quarantine pests” (FAO 2002) the IFS took the opportunity to revise the initial pest risk analysis in line with this standard, and to provide more detailed pest risk management options. In addition, because of the interest in trade into Europe, a risk assessment was conducted using the regional standard developed by EPPO. This was completed using the pest data sheets developed for the global pest risk analysis.

Technical information on the pest and vector is summarized in Appendix 1 – the CABI Crop Protection Compendium data sheet for *Pantoea stewartii* (revised) and Appendix 2 – the CABI Crop Protection Compendium data sheet for *Chaetocnema pulicaria*. Appendix 3 presents brief answers requested by the EPPO Pest Risk Assessment decision-making scheme. Appendix 4 describes the ELISA-based seed health test for *E. stewartii* that was developed at Iowa State University and approved by the USDA National Seed Health System. Appendix 5 presents recommendations on sampling seed for *Erwinia stewartii.* The CABI Crop Protection Compendium and additional references were used in preparation of this analysis. References are summarized succinctly throughout the analysis. Sources are documented in the list of references for the reader who wishes to examine and to interpret independently the information being summarized.

This report focuses on the key issues of the seed-borne nature of the pest. Although *Erwinia stewartii* is seed-borne in maize, the role of infected seed is insignificant in the epidemiology of Stewart’s wilt in areas of North America where the disease is endemic. The pathogen appears to have become specialised to exist in two specific hosts, *Zea mays* and *C. pulicaria*. Levels of Stewart’s wilt infection in US fields under good agricultural practice (GAP) are affected by the resistance of the host plant (i.e., the particular variety) and the prevalence of the corn flea beetle vector, *Chaetocnema pulicaria*, in which the bacteria also overwinters.

In terms of international trade in seed for planting, the probability of introducing (entry and establishment) *E. stewartii* to a new area as a result of seed transmission is much lower than previously reported. Previous calculations of rates of transmission in the general plant pathological literature from 1940 to 1990 are based on a small number of experiments in which relatively few samples of seed from highly susceptible, open-pollinated cultivars were tested. As a result of the wide acceptance of inferences from this sparse and out-of-date research, phytosanitary restrictions were placed on maize seed. It is now considered that restrictions on trade in seed that are based on this earlier work are no longer valid and should be re-evaluated in the manner of this report.

This report provides the technical justification and an assessment of the risk level that may be posed by trade in commercial seed, and suggests field and laboratory phytosanitary risk management procedures (measures) that can be applied in accordance with international standards under the IPPC and the WTO SPS Agreement. It is considered that the implementation of these recommended procedures, done to an international standard, will lead to the removal of unjustified restrictions. In the spirit of the harmonization of phytosanitary processes we urge the consideration of this report which identifies the levels of risk and also the appropriate management options which if implemented by the seed industry will permit trade without compromising phytosanitary security.

Jerald K. Pataky and Robert Ikin
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1. STAGE 1 - INITIATION

1.1 PRA INITIATED BY REVIEW OF POLICY: NEW INFORMATION IMPACTS ON AN EARLIER DECISION

A pest risk analysis for *Erwinia stewartii* was undertaken for the International Seed Trade Association in 2000 to indicate the risk of the importation of the pathogen. A revision of this PRA is warranted due to new detection technologies and new information about seed transmission of this bacterium that impacts the PRA and the recommendations made earlier.

Until recently, estimates of rates of seed transmission of *E. stewartii* were based primarily on information from research done in the first half of the 20th century. In these studies, relatively small samples of seed from highly susceptible maize cultivars were evaluated with techniques that are now out of date (Frutchey, 1936; Ivanhoff, 1933; Rand & Cash, 1933; Smith, 1914). Subsequently, summaries of this research were used to promulgate the notion that rates of seed transmission of *E. stewartii* are about 2% (Elliott, 1941, Robert, 1955, Pepper, 1967). Recently, several researchers have re-evaluated seed transmission of *E. stewartii* using modern techniques to detect seed-borne *E. stewartii* (Block et al., 1998; Block et al., 1999; Lamka et al., 1991; Khan et al., 1996; Michener et al., 2002a, 2002b). Based on this recent work, it is apparent that seed transmission of *E. stewartii* occurs at much lower rates than those reported in the first half of the century. In modern maize hybrids and inbreds with improved levels of host resistance, seed transmission of *E. stewartii* is very low, if it occurs at all.

Previous studies on seed transmission of *E. stewartii* also relied on early techniques such as plating seed on agar media and greenhouse and/or field grow-out trials to detect the bacterium. The development in the past decade of an enzyme-linked immunosorbent assay (ELISA) for *E. stewartii* greatly enhanced the ability to detect this bacterium. Other modern techniques such as DNA probes with specific primers for *E. stewartii* also are being developed. These modern techniques should be considered when establishing regulatory guidelines for *E. stewartii*.

1.1.1 THE PEST

Erwinia stewartii (Syn. *Pantoea stewartii*), the causal agent of Stewart's bacterial wilt, is endemic in portions of the United States Corn Belt where maize seed is produced. The bacterium is vectored by and overwinters in the corn flea beetle, *Chaetocnema*

ISF Secretariat Chemin du Reposoir 7 1260 Nyon Switzerland +41 22 365 44 20 isf@worldseed.org

pulicaria. The bacterium may be seed-borne when maize seed is produced on seed parent plants that are infected systemically. Stewart's wilt and *E. stewartii* have been reported infrequently from various areas of the world where maize is produced, but the bacterium has not become established beyond the area to which it is endemic in the United States. Consequently, many countries place quarantine restrictions on maize seed produced in the United States in order to prevent the introduction of *E. stewartii.* These phytosanitary regulations are based primarily on out-of-date information assembled from research done in the first half of the 20th century. Research done in the past decade using modern techniques to detect *E. stewartii* in modern corn inbreds and hybrids indicates that seed transmission of *E. stewartii* occurs at rates substantially lower than those reported in the 1930s. A pest risk analysis for *Erwinia stewartii* in maize seed is warranted due to new detection technologies and new information about seed transmission of this bacterium. This PRA impacts earlier phytosanitary regulations based on outmoded information.

1.2 Identification of PRA Area

The PRA area includes any country in the world that trades in maize seed for planting and where *E. stewartii* is not present.

1.3 Information

(See technical information in CABI data sheets, Appendices 1 and 2; and list of additional references, section 4.2).

1.3.1 Previous PRA

A previous version of this PRA was posted on the International Seed Federation web site (http://www.worldseed.org/seed_health.htm). The previous PRA was prepared in December 2000 using the FAO / IPPO "Guidelines for Pest Risk Analysis" (Publication No. 2, February 1996). This revision of that PRA follows the FAO International Standards for Phytosanitary Measures No 11 "Pest risk analysis for quarantine pests" adopted in April 2001.

1.4 Conclusion of Initiation

The pest is *Erwinia stewartii*. Its vector is *Chaetocnema pulicaria*. The pathway is maize seed for planting. The PRA area is any country outside of the US where the pests are not established, particularly Europe.

2. STAGE 2 – PEST RISK ASSESSMENT

2.1 – A: Pest Categorization

2.1.1 Identity of the Pest

Erwinia stewartii (E. F. Smith) Dye, 1963

Syn. *Pseudomonas stewartii* E. F. Smith, 1898
Bacterium stewartii E. F. Smith, 1914
Aplanobacter stewartii (E. F. Smith) McCulloch, 1918

Bacillus stewartii (E. F. Smith) Holland, 1920
Phytomonas stewartii (E. F. Smith) Bergey et al., 1923
Xanthomonas stewartii (E. F. Smith) Dowson, 1939
Pantoea stewartii (E. F. Smith) Mergaert et al., 1993

2.1.2 Presence or Absence in PRA Area

Erwinia stewartii is endemic throughout a large portion of the maize growing regions of the eastern and midwestern United States and it occurs intermittently in Canada. The bacterium is or has been present in other countries in the Western Hemisphere and in restricted regions elsewhere in the world (CABI; McGee, 1988; Pepper, 1967). *Erwinia stewartii* has been reported by CABI from Italy, Romania, and Poland. According to the National Seed Institute of Austria, *E. stewartii* also occurred sporadically in a very restricted manner in Niederösterreich, Burgenland, and Steiermark where damage was noted only on sweet corn (P. Heffer, FIS, *personal communication*). Although Stewart's wilt and *E. stewartii* have been reported infrequently from various areas of the world where maize is produced, the bacterium has not become established beyond the area to which it is endemic in the United States.

2.1.3 Regulatory Status

Over 60 countries place quarantine restrictions on maize seed produced in North America in order to prevent the introduction of *E. stewartii.* Specific restrictions vary among countries.

2.1.4 Potential for Establishment and Spread in PRA Area

Based on the rates of plant-to-seed and seed-to-seedling transmission, the probability of transmitting *E. stewartii* is extremely remote when seed is produced on resistant or moderately resistant seed parent plants. Plant-to-seed transmission is less than 0.3% for moderately resistant plants and less than 0.03% for resistant plants. When susceptible plants are systemically infected through natural methods, plant-to-seed transmission is about 10% or less. Thus, few seed lots are likely to have 35% or more infected kernels that have resulted in the highest rates of seed-to-seedling transmission. Seed-to-seedling transmission probably is very low (e.g. less than 0.06%) for seed with less than 10% infected kernels, if *E. stewartii* is transmitted in these seed at all.

An insect vector is necessary for *E. stewartii* to be transferred from an infected seedling to other plants. The corn flea beetle, *C. pulicaria*, is the only known vector of importance. It is unlikely that a suitable vector of *E. stewartii* occurs in the PRA area.

Potential for Adaptation of Pathogen

Adaptation of *E. stewartii* is unlikely. *Erwinia stewartii* appears to be a relatively homogeneous organism. One hundred and twenty-four isolates of *E. stewartii* originating from sweet corn or flea beetles collected in the northeastern, midwestern and mid-Atlantic states of the US had homogeneous metabolic profiles at 93% similarity (Wilson et al., 1999). Two-thirds of the isolates formed 18 separate groups

with the same metabolic profile, while one-third of the isolates had distinct profiles. This phenotypic homogeneity was interpreted as an indication that the pathogen has been streamlined to exist in particular hosts (i.e., *Zea mays* and *Chaetocnema pulicaria*). Infection is not severe in other hosts, and *E. stewartii* is not transmitted efficiently by other vectors. The authors suggested that a considerably greater amount of diversity would be expected in an organism that survived more ubiquitously in the environment (Wilson et al., 1999).

METHOD OF SURVIVAL AND POTENTIAL FOR SPREAD IF INTRODUCED

In North America, *E. stewartii* is disseminated by the insect vector, *C. pulicaria*. There are no known examples of widespread, prolonged occurrences of Stewart's wilt in the absence of this insect. Although the disease has been reported infrequently from various parts of the world, *E. stewartii* has never become established outside of the region of North America to which it is endemic, presumably because of the lack of an adequate vector and overwintering host. The potential for establishment and spread of *E. stewartii* in the absence of corn flea beetles is extremely unlikely. If populations of *C. pulicaria* are not substantially large, Stewart's wilt will not become established and *E. stewartii* will not survive.

2.1.5 POTENTIAL FOR ECONOMIC CONSEQUENCES IN PRA AREA

The economic impact of introducing *E. stewartii* to the PRA area will be inconsequential unless the bacterium becomes established due to the presence of an insect vector. If vectors are present, the economic impact depends on the level of resistance or susceptibility of the maize cultivars being grown in the PRA area.

Economic losses in maize due to Stewart's wilt have been inconsequential in North America for the past 50 years except for a few, small sporadic outbreaks (Pataky et al., 2000; Pepper, 1967). The lack of economic importance of this disease is due primarily to adequate levels of resistance incorporated into maize hybrids that are grown where the disease occurs. In sweet corn, economic losses can be significant when susceptible or moderately susceptible hybrids are grown where flea beetles occur.

Relatively little information is available concerning Stewart's wilt reactions of most of the maize cultivars grown outside of the United States. In a recent evaluation of an international collection of maize germplasm, accessions collected from most areas of the world had moderate reactions to Stewart's wilt (Pataky, et al., 2000). In some instances, levels of resistance were sufficient to prevent or minimize economic losses due to Stewart's wilt. For example, several maize cultivars grown in the Republic of South Africa were unaffected by Stewart's wilt due to sufficient levels of resistance (Michener & Pataky, 2002).

2.1 – B: CONCLUSION OF PEST CATEORIZATION

Erwinia stewartii causes Stewart's bacterial wilt, a disease of maize that can be of economic importance on maize cultivars that lack sufficient levels of host resistance. Stewart's wilt is endemic in some areas of the United States Corn Belt where seed is produced. This area coincides with the occurrence of the insect vector and overwintering host, *C. pulicaria*. The vector is incapable of surviving the winter if the

average daily temperature for December, January and February is below –3 C (i.e., if the mean of [[daily high + daily low]/2] is below –3 C). Thus, the exact area of occurrence varies from year to year as affected by winter weather. The bacterium can be seed borne if seed parent plants are infected systemically, but rates of seed transmission are very low. *Erwinia stewartii* has the potential to be a quarantine pest in the pathway of seed imported for sowing. It is seed-borne under certain circumstances, although it requires a vector to spread and to establish. It has the potential to cause economic losses in maize varieties that are not resistant. It is possible that varieties in the PRA area are susceptible as they do not currently need resistance to this pathogen.

2.2 Assessment of the Probability of Introduction and Spread

2.2.1 Probability of Entry

2.2.1.1 Identification of Pathways

Erwinia stewartii could be introduced to the PRA area through infected maize plant tissue, infested insect vectors, or infected seed; however, seed is the most probable pathway. Infected plant tissue is a highly unlikely source of entry because seed shipments should be free of plant debris. Introduction through infected maize plant tissue is more likely to occur if leaf or stalk tissue is being imported for other purposes (e.g., research purposes). Likewise, seed shipments should be free of *C. pulicaria*. Prior to harvest, the insect migrates from maize fields to grass borders where it overwinters. Thus, introduction of *E. stewartii* through the insect vector is more likely to occur in other ways by which the corn flea beetle could be introduced rather than with seed.

2.2.1.2 Probability of the Pest being Associated with Pathway at Origin

The probability that seed harbors *E. stewartii* varies among locations and years depending on the prevalence of the overwintering vector and the severity of infection of seed parent plants. After cold winters (average below –3 C), flea beetle populations are small, and therefore, the incidence of Stewart's wilt infected plants is low. A low percentage of seed produced on susceptible inbred seed parents may harbor *E. stewartii*, but seed produced on moderately resistant and resistant inbred parents should not be infected by the bacterium because systemic infection of seed parent plants did not occur. In years following warmer winters (above 0 C), the level of vector survival will result in a larger proportion of plants systemically infected and the incidence of seed-borne *E. stewartii* will be correspondingly higher.

2.2.1.3 Probability of Survival during Transport or Storage

It is highly probable that seed-borne *E. stewartii* would survive during storage and transport of seed even though the number of viable bacteria per seed may decrease during this period.

Using an ELISA-based seed health test, *E. stewartii* was detected from seed for up to 3 years (Pataky, *personal communication*). The pathogen has been recovered from seed for up to 5 months after harvest (Rand & Cash, 1933). Guo et al. (1987)

reported that *E. stewartii* survived in stored maize longer at low temperatures, but disappeared after 200-250 days in storage at 8-15℃ (Guo et al., 1987).

2.2.1.4 Probability of Surviving Exisitng Pest Management Procedures

Phytosanitary procedures are the only management measures applied to seed-borne *E. stewartii* primarily because Stewart's wilt is controlled in North America by growing resistant hybrids or by controlling the insect vector. Hence, seed-borne *E. stewartii* is unimportant epidemiologically in the areas where Stewart's wilt is endemic. The probability of introducing *E. stewartii* in seed in spite of phytosanitary procedures is a function of sampling.

2.2.1.5 Probability of Transfer to a Suitable Host

Maize seed that might harbor *E. stewartii* could be planted throughout the PRA area. In order for the bacterium to be transferred to a suitable host within the PRA area, seed-to-seedling transmission must occur, the insect vector *C. pulicaria* must acquire the bacterium by feeding on an infected seedling, and the vector must transmit *E. stewartii* to maize or other host plants.

Seed-to-seedling transmission of *E. stewartii* is a relatively rare event. Of over 408,000 seedlings grown from seed harvested from seed parent plants that were inoculated or naturally infected with *E. stewartii*, only 51 seedlings were infected, i.e., 0.0125% transmission from seed harvested from infected plants (Block et al. 1998; Khan et al., 1996; Michener et al. 2002). Only a single incidence of transmission occurred in seed lots with less than 10% kernel infection. In seed lots with greater than 10% kernel infection, the rate of *E. stewartii*-transmission from infected seed was about 0.02% for infected seed produced on naturally-infected plants and 0.04 to 0.14% for infected seed produced on seed parent plants that were inoculated with *E. stewartii.* Therefore, *E. stewartii* is not likely to be transmitted when seed lots have 1% or fewer infected kernels. Seed-to-seedling transmission may occur at low rates in seed lots with greater than 1% kernel infection.

An insect vector is necessary for *E. stewartii* to be transferred from an infected seedling to other plants. The corn flea beetle, *C. pulicaria*, is the only known vector of importance. A few other insects transmitted *E. stewartii* in greenhouse trials, of which *C. denticulata* was most efficient, but none were vectors of importance in field conditions (Elliot & Poos, 1940*). Chaetocnema pulicaria* and *C. denticulata* have never been reported in Europe or the Palearctic region (Gruev & Deberl, 1997). It is unlikely that a suitable vector of *E. stewartii* occurs in the PRA area.

All types of maize can be infected by *E. stewartii*. Severity of infection is dependent on the level of resistance or susceptibility of the maize cultivar. Several other plant species also are infected when they are inoculated with this bacterium. If *E. stewartii* is introduced and a suitable vector is present in the PRA area, all maize plants in the PRA area and a few other plant species could potentially be infected.

2.2.2 Probability of Establishment

2.2.2.1 Availability of Suitable Hosts, Alternate Hosts and Vectors in PRA Area

All maize can be infected by *E. stewartii* although the severity of infection is dependent on host resistance or susceptibility. Several species of plants also can serve as a host for this bacterium. If the bacterium is introduced and a suitable vector is present, host plants should be plentiful in the PRA area.

An insect vector is necessary for *E. stewartii* to be transferred from an infected seedling to other plants. The corn flea beetle, *C. pulicaria*, is the only known vector of importance. A few other insects transmitted *E. stewartii* in greenhouse trials, of which *C. denticulata* was most efficient, but none were vectors of importance in field conditions (Elliot & Poos, 1940*). Chaetocnema pulicaria* and *C. denticulata* have never been reported in Europe or the Palearctic region (Gruev & Deberl, 1997).

2.2.2.2 Suitability of Environment

Stewart's wilt and *E. stewartii* do not have strict environmental limitations during the growing season. Conditions that are favorable for the growth and development of maize are suitable for this disease. However, Stewart's wilt is rarely epidemic in the southernmost portions of the United States, which may indicate that prolonged periods of warm temperatures adversely affect the insect vector, *C. pulicaria*, or the bacterium. The bacterium survives the winter in association with *C. pulicaria*. The vector is unable to survive from season to season when the average daily temperature in December, January and February is below –3 C.

2.2.2.3 Cultural Practices and Control Measures

In areas of the United States where Stewart's wilt is endemic, incidence of systemic infection of susceptible sweet corn hybrids has been reduced 50% to 85% by controlling corn flea beetles with seed treatment insecticides, e.g., imidacloprid, thiamethoxam, and clothianidin (Kuhar et al., 2002; Munkvold et al., 1996; Pataky et al., 2000). Seed treatment insecticides could be used to reduce the probability of the establishment of *E. stewartii* in the PRA area or to control the disease if the bacterium becomes established. Occasionally, insecticides are applied in-furrow at planting or as foliar sprays after planting to control corn flea beetles.

Usually, Stewart's wilt is controlled throughout North America by planting resistant maize hybrids. Resistance is inherited relatively simply and could be incorporated into maize cultivars grown in the PRA area. Resistance restricts the movement of *E. stewartii* in the vascular system of plants (Braun, 1982, 1990). Frequency of systemic infection is related to levels of resistance or susceptibility (Michener et al., 2003). Maize cultivars grown in the PRA area may possess levels of resistance sufficient to prevent economic consequences due to Stewart's, particularly if maize lines were derived from US Corn Belt dent corn (e.g., an Iowa Stiff Stalk Synthetic pedigree). For example, several maize lines grown in the Republic of South Africa have adequate levels of resistance to prevent damage from Stewart's wilt in the event *E. stewartii* and *C. pulicaria* are introduced to the RSA (Michener & Pataky, 2002).

2.2.2.4 Other Characteristics of the Pest Affecting the Probability of Establishment

In contrast to other species of *Erwinia* that are more ubiquitous in nature and are adapted to multiple hosts, *E. stewartii* appears to be adapted only to maize and *C. pulicaria*.

2.2.3 Probability of Spread after Establishment

Erwinia stewartii is unlikely to become established without a suitable vector. If a vector is present, the probability of spread after establishment will coincide directly with spread of the vector. There is no alternate mechanism of in-season dispersal of *E. stewartii*.

2.2.4 Conclusion of the Probability of Introduction and Spread

The probability of introducing *Erwinia stewartii* on maize seed and the probability of *E. stewartii* becoming established in the PRA area can be examined in three sequential steps: i.) the probability of seed harboring *E. stewartii*, ii.) the probability of transmitting *E. stewartii* from seed to seedlings, and iii.) the probability of insect vectors that allow for the establishment and spread of *E. stewartii* in the PRA area.

The probability of seed harboring *E. stewartii* can be assessed from visual inspections of seed production fields and/or an ELISA-based seed health test of seed lots. The probability of seed-borne *E. stewartii* from fields without symptoms of Stewart's wilt is virtually zero. The only possibility that seed from these fields harbor *E. stewartii* is through contamination of seed lots with seed from other fields, or misdiagnosis of Stewart's wilt. For seed produced in fields with symptoms of Stewart's wilt but very few or no systemically infected plants (i.e., less than 25% severity of Stewart's wilt), there is a remote probability that seed may harbor *E. stewartii*. When 100% of Stewart's wilt resistant maize hybrids had non-systemic symptoms, about 8 of 64,000 kernels were infected with *E. stewartii* (i.e., about 0.0013% kernel infection). When 100% of maize hybrids with moderate Stewart's wilt reactions were infected with *E. stewartii* (i.e., an occasional systemically infected plant), about 20 of 18,000 kernels were infected with *E. stewartii* (i.e., about 0.1% kernel infection). For seed produced in fields with several systemically infected plants, there is a distinct probability that seed may harbor *E. stewartii*. When 100% of maize hybrids with susceptible Stewart's wilt reactions were infected with *E. stewartii* (i.e., systemically infected plants were frequent and severity of infection was above 25%), about 1,100 of 11,500 kernels were infected with *E. stewartii* (i.e., about 10% kernel infection).

If seed is suspected for harboring *E. stewartii* based on visual inspection of seed production fields or if seed production fields are not inspected, an ELISA-based seed health test can be used to demonstrate with a known probability that the percentage of kernel infection is below a certain threshold level. For example, an ELISA-based seed health test of 400 randomly sampled kernels (4 100-kernel samples) has a 98.2% probability of detecting seed-borne *E. stewartii* if kernel infection is 1% or greater. Likewise, an ELISA-based seed health test of 800 randomly sampled kernels (8 100-kernel samples) has a 99.97% probability of detecting seed-borne *E. stewartii* if kernel infection is 1% or greater.

The probability of transmitting *E. stewartii* from seed to seedlings appears to differ qualitatively among seed lots in which kernel infection is infrequent and frequent. Only a single incident of transmission has been documented in seed lots with less than 10% kernel infection; whereas, in seed lots with greater than 10% kernel infection, the rate of *E. stewartii*-transmission from infected seed is about 0.02% when seed is produced on naturally-infected plants. Therefore, there is an extremely low probability that *E. stewartii* will be transmitted when seed lots have less than 1% infected kernels, but seed-to-seedling transmission may occur at low rates in seed lots with greater than 10% kernel infection. For example, in a seed lot with 10% kernel infection, it would be reasonable to find 1 infected seedling per 50,000 plants. In a seed lot with less than 1% kernel infection, *E. stewartii* probably will not be transmitted successfully from seed to seedlings, but if this occurs, it would be reasonable to find less than 2 infected seedlings per 1 million plants.

Erwinia stewartii will not become established or spread in the PRA area unless the vector is present. There is no evidence that any insect other than *C. pulicaria* is capable of efficiently vectoring *E. stewartii*. *Chaetocnema pulicaria* has not been reported from the PRA area. Therefore, it is highly unlikely that an occasional introduction of *E. stewartii* on maize seed will result in the establishment of Stewart's wilt in areas where it does not presently occur.

2.3 Assessment of Potential Economic Consequences

2.3.1 Direct Pest Effects

If *E. stewartii* is introduced to and established in the PRA area, it will only affect maize. The economic consequences in the PRA area can be compared to the economic consequences of Stewart's wilt on maize grown in the United States where the disease is endemic.

Economic losses in maize due to Stewart's wilt have been inconsequential in North America for the past 50 years except for a few, small sporadic outbreaks (Pataky et al., 2000; Pepper, 1967). The lack of economic importance of this disease is due primarily to adequate levels of resistance incorporated into maize hybrids that are grown where the disease occurs. Stewart's wilt caused substantial economic losses in the 1930s prior to the development of resistant cultivars (Pepper, 1967). In sweet corn, economic losses can be significant when susceptible or moderately susceptible hybrids are grown in areas where flea beetles occur. Sweet corn yield losses due to Stewart's wilt are affected by the level of resistance or susceptibility of the cultivar and by the growth stage at which plants are infected (Suparyono & Pataky, 1989). In sweet corn, yield losses due to Stewart's are associated with systemic infection with about an 0.8% reduction in yield for each 1% incidence of plants systemically infected as seedlings (Freeman & Pataky, 2001). Losses do not occur or are minimal in resistant and moderately resistant hybrids; however, losses frequently range from 40 to 100% when susceptible sweet corn hybrids are grown under epidemic conditions and are infected prior to the 5-leaf stage.

The economic impact of introducing *E. stewartii* to the PRA area will be inconsequential if the bacterium fails to become established due to the absence of an insect vector. If the vector is present and *E. stewartii* becomes established, the

geographic distribution and prevalence of Stewart's wilt within the PRA area will depend on the dissemination and survival of the corn flea beetle. The economic impact will depend on the level of resistance or susceptibility of the maize cultivars being grown in the area. Relatively little information is available concerning Stewart's wilt reactions of maize cultivars grown outside of the United States. If cultivars being grown are closely related to maize hybrids grown in the Corn Belt of the United States (e.g., genetic backgrounds with Iowa Stiff Stalk Synthetic or Mo17), levels of resistance should be sufficient to prevent or minimize economic losses due to Stewart's wilt. For example, Stewart's wilt reactions of some maize hybrids sold commercially in the Republic of South Africa were adequate to prevent economic losses due to Stewart's wilt (Michener & Pataky, 2003).

Stewart's wilt usually is controlled in the United States by growing resistant hybrids. The disease also can be managed by controlling the insect vector with insecticides Resistance to Stewart's wilt is relatively simply inherited (Blanco et. al, 1979; Ming et al., 1999; Parker & Hooker, 1993; Smith, 1971) and can be selected easily in a breeding program. Within a few generations, maize germplasm can be improved considerably for resistant reactions to Stewart's wilt. Thus, any substantial economic impact of introduction and establishment of *E. stewartii* to new areas would be temporary; however, resources would need to be allocated for breeding and selecting for resistance. Seed treatment insecticides at a cost of about US$3 per hectare can be used to reduce the incidence of systemic Stewart's wilt by about 50% to 90%.

2.3.2 Indirect Pest Effects

Introduction and establishment of *E. stewartii* could result in phytosanitary regulations imposed by trading partners. Also, as mentioned previously, additional resources would be necessary to screen germplasm and breed maize for resistance in order to control Stewart's wilt in the field.

2.3.2.1 Time and Place Factors

Based on demonstrated rates of seed transmission, the introduction and establishment of *E. stewartii* is most likely to occur as isolated events rather than as multiple events at many points. Spread of the disease from a focus would coincide with the dispersal of *C. pulicaria*. The temporal and spatial distributions of *C. pulicaria* in the midwestern United States are being examined presently (Cook, 2003; Esker & Nutter, 2002, 2003). The pattern of *C. pulicaria* dispersal in the US could be used to estimate the temporal and spatial impact of *E. stewartii* in the PRA area. Based on preliminary observations in the midwestern United States, it appears that an overwintering generation of flea beetles emerges at planting (about early April). This generation has the potential for the greatest economic impact because this generation infects plants as seedlings. This generation appears to be distributed primarily in the area where the insect overwintered. Flea beetle populations decline following the emergence of the overwintering generation but populations increase as a second generation peaks prior to crop anthesis (about late-June). The first summer generation appears to be distributed about 300 km further north than the overwintering generation (Cook, 2003). This generation has a minimal economic impact because most plantings of maize are at a growth stage beyond which

Stewart's wilt affects yield (i.e., infection from the second generation causes the leaf blight phase of the disease and plants are not infected systemically). Subsequent generations (the second summer generation and possibly a third) appear to be distributed another 300 km northward (Cook, 2003), but *E. stewartii* transmitted by these generations has no impact on yield. However, these later generations of flea beetles are important because they become the overwintering generation that infect the next year's crop. Thus, during the entire growing season, the geographic distribution of corn flea beetles appears to increase about 600 km, but the economic impact of Stewart's wilt is primarily limited to the area where the vector overwinters. Each winter the range of occurrence recedes southward about 300 to 600 km because the insect is unable to survive winter temperatures averaging -3 C.

Following the introduction and establishment of *E. stewartii*, any major economic impact of Stewart's wilt should be limited initially to an area proximal to the point of introduction. Any primary economic impact should occur for only 5 years or less because maize cultivars will be screened for resistance, and control measures (e.g., resistant cultivars, insecticides) can be deployed as necessary within 5 years.

2.3.2.2 Analysis of Commercial Consequences

Stewart's wilt has never affected the demand or prices for maize grain in the United States where the disease is endemic. It is highly improbably that the disease would have much macroeconomic effect in the PRA area. On a microeconomic scale, individual producers sustain losses in profits if they grow susceptible maize hybrids (field corn or sweet corn) when Stewart's wilt is prevalent. For most producers, the costs of control are minimal or non-existent since the cost of seed of resistant and susceptible hybrids does not differ. Therefore, there is no increase in production costs when producers employ the most effective and efficient method to control Stewart's wilt. When producers choose to grow susceptible hybrids (usually sweet corn producers), an additional production cost of about US$3 per hectare results from the use of seed treatment insecticides to control corn flea beetles.

2.3.3 Conclusion of the Assessmentof Economic Consequences

Stewart's wilt has little economic impact on maize grown in the United States where the disease is endemic except for situations in which extremely susceptible hybrids are grown for specialty purposes (e.g., processing sweet corn). The disease probably would have a similar minimal impact on maize in the PRA area.

If *E. stewartii* is introduced to a new area, the economic impact of Stewart's wilt is likely to be less than 2% of the value of the maize crop during the first 10 years following the introduction and establishment of *E. stewartii.* During the initial growing season following introduction and in the subsequent two or three seasons, the disease probably would occur in a limited area. The economic impact may be significant to producers in that area if the cultivars being grown are susceptible to Stewart's wilt, but the overall impact in the PRA area would be minimal because the disease would not be widespread. Following the initial occurrence of Stewart's wilt in the PRA area, maize cultivars grown throughout the area should be screened for reactions to the disease. By the third or fourth year after introduction when the geographic distribution of Stewart's wilt may be enlarging, cultivars with moderate to high levels of resistance should be identified and grown. Within another three or four

years, resistance should be incorporated into most of the maize germplasm used where the bacterium has been introduced. Thus, by the time Stewart's wilt becomes widespread, a solution to the problem is in place.

2.3.3.1 Endangered Area

Ananlyses at this point should be specific for the particular area for which the PRA is being undertaken, generally a country or a region. Information relevant to environmental and other conditions at the point of entry, establishment and spread should be considered. Issues of climate mapping and the use of CLIMEX as a means of matching climatic data might be undertaken.

2.4 Degree of Uncertainty

An analysis of the risk of introducing *E. stewartii* in maize seed is influenced substantially by rates of seed transmission, the ability to detect *E. stewartii* in seed, and the presence of an insect vector. Recent research on seed transmission has resulted in quantitative data from which rates of plant-to-seed and seed-to-seedling can be assessed relatively accurately. Likewise, the ability of an ELISA-based seed health test to detect seed-borne *E. stewartii* is relatively certain with known probabilities based on binomial distributions. Thus, there is a relatively high degree of certainty about conclusions made concerning the introduction of the bacterium on seed. Establishment of *E. stewartii* in the PRA area requires an insect vector. Absence of the insect vector in the PRA area is a logical conclusion based on information in the literature and past experience. The degree of uncertainty associated with the occurrence of the insect is higher than the uncertainty associated with rates of seed transmission and ELISA-based seed health test, partly because the question being asked is impossible to prove. While it is possible to demonstrate that a vector occurs in an area, it is impossible to prove that a vector is not present.

The assessment of the economic consequences that could result from the introduction and establishment of *E. stewartii* are based on extrapolations of the impact of Stewart's wilt in the United States, including assessments of temporal and spatial distributions of the vector and implementation of control practices.

2.5 Conclusion of the Pest Risk Assessment Stage

Seed is the most probable pathway of introducing *E. stewartii*, however, establishment requires the insect vector, *C. pulicaria*. The probability that seed harbors *E. stewartii* varies among locations and years depending on prevalence of the pathogen in the overwintering vector and the severity of infection of seed parent plants. The potential of introducing *E. stewartii* in maize seed is exceedingly remote except when seed is produced on plants that are severely, systemically infected. If symptoms of systemic infection are not present in seed production fields, seed will not harbor *E. stewartii*. Kernel infection (i.e., plant-to-seed infection) has been greater than 10% only when highly susceptible plants are systemically infected.

If kernels are infected with *E. stewartii*, the bacterium must be transmitted from seed to seedlings in order to become established. Only a single instance of seed-to-seedling transmission of *E. stewartii* has been documented when kernel infection was below 10% and seed was produced on naturally-infected seed parent plants. When

seed parent plants were inoculated with *E. stewartii*, kernel infection has been as high as 50% and rates of seed to seedling transmission ranged from 0.038% to 0.14%. It seems unlikely that *E. stewartii* will be successfully established in the PRA area if a threshold of kernel infection is established at 1% or less. At this threshold, *E. stewartii* will be transmitted to less than 2 in 1,000,000 seedlings, if it is transmitted at all.

Erwinia stewartii will not become established unless the insect vector feeds on infected seedlings, acquires *E. stewartii*, and transmits the bacterium to other plants. There is no other method of dissemination or survival. Although it is impossible to prove that *C. pulicaria* does not occur in the PRA area, it is reasonable to conclude that the occurrence of corn flea beetles is extremely rare in the PRA area because they have never been found there. Thus, it is not likely that the vector will feed on a rarely infected seedling that might occur as the result of *E. stewartii* introduced from seed.

The overall economic impact of Stewart's wilt should be minimal if *E. stewartii* is introduced and becomes established in the PRA area because the initial occurrence will be in a limited area and effective controls can be implemented before the disease is widespread. However, the economic impact could be substantial to a few producers in the area of introduction if they are growing highly susceptible maize cultivars.

3. STAGE 3 – PEST RISK MANAGEMENT

3.1 LEVEL OF RISK

The probability of introducing *E. stewartii* on maize seed, the probability of *E. stewartii* becoming establishing in the PRA area, and the probability of substantial economic consequences if *E. stewartii* becomes established in the PRA area are all very low. There is almost no chance of introducing *E. stewartii* in seed from fields that show no visual symptoms of Stewart's wilt, or from fields that have no systemically infected plants. For seed produced in fields with many systemically infected plants, seed-borne *E. stewartii* may occur at relatively moderate rates (e.g., 0.2% to 10%). Rates of seed-to-seedling transmission from infected seed produced on naturally-infected plants are about 0.02%. If introduced on seed, *Erwinia stewartii* will not become established or spread in the PRA area unless the vector is present. There are no documented occurrences of *C. pulicaria* outside of the Nearctic region. In fact, when Stewart's wilt occurred infrequently outside North America, *E. stewartii* failed to establish, probably due to the lack of a vector. If *E. stewartii* becomes established in the PRA area, economic impact would be minimal because adequate control measures (i.e., host resistance) can be implemented before the bacterium is distributed widely within the PRA area.

3.2 TECHNICAL INFORMATION REQUIRED

REASON FOR INITIATING THE PROCESS: New detection technologies and new information about seed transmission of *E. stewartii* impact earlier phytosanitary regulations based on outmoded information.

ESTIMATION OF THE PROBABILITY OF INTRODUCTION TO THE PRA AREA: Nearly zero for seed produced in fields with no symptoms or non-systemic symptoms of Stewart's wilt. Less than 2 in 1,000,000 for seed with less than 1% kernel infection.

EVALUATION OF POTENTIAL ECONOMIC CONSEQUENCES: Stewart's wilt has little economic impact on maize grown in the United States where the disease is endemic except for situations in which extremely susceptible hybrids are grown for specialty purposes (e.g., processing sweet corn). The disease probably would have a similar minimal impact on maize in the PRA area. During the initial growing season following introduction and in the subsequent two or three seasons, the disease probably would occur in a limited area. The economic impact may be significant to producers in that area if the cultivars being grown are susceptible to Stewart's wilt, but the overall impact in the PRA area would be minimal because the disease would not be widespread. By the time Stewart's wilt became widespread, cultivars with moderate to high levels of resistance should have been identified and could be grown in the PRA area.

3.3 ACCEPTABILITY OF RISK

The risk of introducing *E. stewartii* by the pathway identified in this PRA should be equated to the risk that is posed by other pathways that are in place and are managed, e.g., import of maize grain for processing and for feed. This comparison should be specific for the each area for which the PRA is being conducted.

3.4 IDENTIFICATION AND SELECTION OF APPROPRIATE RISK MANAGEMENT OPTIONS

1 Area freedom	
1.1 Visual survey during growing season - systemic infection	Based on rates of plant-to-seed and seed-to-seedling transmission, the probability of introducing *E. stewartii* is extremely remote when seed is produced on resistant or moderately resistant seed parent plants. Plant-to-seed transmission is less than 0.2% for moderately resistant plants and less than 0.025% for resistant plants. When susceptible plants are systemically infected through natural methods, plant-to-seed transmission is about 10% or less. Based on these rates of plant-to-seed transmission and rates of seed-to-seedling transmission of about 0.02%, a reasonable probability of transmitting *E. stewartii* occurs only when seed parent plants are systemically infected. Visual inspection of seed production fields for systemic Stewart's wilt followed by laboratory confirmation (ooze

test) will identify seed lots that have any reasonable probability of introducing *E. stewartii*.

1.2 Uninspected field - Testing with ELISA

Seed from fields with systemically-infected plants may harbor *E. stewartii*. An ELISA-based seed health test of 400 kernels randomly sampled from a seed lot has a 98.2% probability of detecting *E. stewartii* if kernel infection is 1% or greater. An ELISA-based seed health test of 800 kernels randomly sampled from a seed lot has a 99.97% probability of detecting *E. stewartii* if kernel infection is 1% or greater [See Appendix 5. Sampling seed for *Erwinia stewartii* using ELISA]. Transmission of *E. stewartii* has been documented only in seed lots with greater than 10% kernel infection except for a single plant grown from a seed lot with 9 ± 3.3% kernel infection. A detection threshold of 0.5% kernel infection provides adequate assurance that *E. stewartii* will not be transmitted in seed.

1.3 Infected field – Testing seed with ELISA

A threshold of 0.5% for estimates of kernel infection from the ELISA-based seed health test ensures that seed lots have less than 1% *E. stewartii*-infected kernels which represents a reasonable risk considering rates of seed-to-seedling transmission and the absence of an insect vector. Based on rates of seed-to-seedling transmission of 0.02%, the 0.5% threshold would ensure less than 1 in 1,000,000 infected seedlings. Using the ELISA-based seed health test with four 100-kernel replicate samples, binomial probabilities for group sampling can be used to estimate the proportion of infected seed [See Appendix 5. Sampling seed for *Erwinia stewartii* using ELISA]. If zero or one of four 100-kernel samples is positive, the best estimate of kernel infection is 0.29% or less, and the seed lot would be accepted. If more than one positive sample is found in the initial seed health test of four 100-kernel samples, seed lots should be re-tested. Re-tested seed lots should be accepted if the estimated percentage of infected kernels is below 0.5%. [See Appendix 5. Sampling seed for *Erwinia stewartii* using ELISA].

1.4 Certification

Issue of an International Phytosanitary Certificate with the Additional declaration of

	inspection in year of production of freedom from *E stewartii* with details of inspection or test results.
2. Seed treatment	*Erwinia stewartii* must be acquired and transmitted by an insect vector in order to become established. Seed treatment insecticides (e.g., imidacloprid, thiomethoxam, and clothianidin) killed flea beetles and reduced the incidence of systemic Stewart's by 50% to 85% in field trials. Application of seed treatment insecticides to seed that may harbor *E. stewartii* would drastically reduce the probability of successful transmission of the bacterium by an insect vector. Seed treatment insecticides control Stewart's wilt effectively through early seedling stages (e.g., 4- to 5-leaf stage).
2.1 Certification	Issue of an International Phytosanitary Certificate with the Additional declaration of treatment of specific chemicals for *E stewartii* with details of rates of treatment, periods and temperatures.
3 Certification of freedom from plant parts (trash)	Issue of an International Phytosanitary Certificate with the Additional declaration that the seed has been inspected and found free of trash. Alternatively an ISTA certificate of cleanliness may be submitted and attached to the PC.
4 Inspection on arrival	Inspection on arrival by the NPPO which – o Checks the compliance of the certification with the import requirements in terms of declarations. Also the compliance with the source of material and quantity. Any non-compliance will result in the refusal of the import, re-consignment or destruction.
5 Post entry quarantine	In cases where the inspection and treatments of consignments are not acceptable because they do not meet the appropriate level of protection of the NPPO, the seed would be grown in post entry quarantine and the first generation of seed harvested and released if found healthy. Note this is not suitable for imports of hybrid seed lines.

3.5 CONCLUSION OF PEST RISK MANAGEMENT

Seed transmission of *E. stewartii* is insignificant in the epidemiology of Stewart's wilt in areas where the disease is endemic. The probability of introducing *E. stewartii* to the PRA area on maize seed, the probability of *E. stewartii* becoming established in the PRA area, and the probability of substantial economic consequences if *E. stewartii* becomes established in the PRA area are all very low.

The probability of seed harboring *E. stewartii* can be assessed from visual inspections of seed production fields and/or an ELISA-based seed health test of seed lots. There is nearly no chance of introducing *E. stewartii* in seed from fields without symptoms of Stewart's wilt or from fields without systemically infected plants. For seed produced in fields with many systemically infected plants, seed-borne *E. stewartii* may occur at relatively low rates, i.e., less than 10%. Rates of seed transmission are even lower because the rate of seed-to-seedling transmission is about 0.02% or lower when infected seed is produced on naturally infected plants. Thus, in a worse case scenario, it would be very unlikely that more than 20 seedlings would be infected from a million seed (i.e. 10% infected kernels x 0.02% seed-to-seedling transmission). If introduced on seed, *Erwinia stewartii* will not become established or spread in the PRA area unless the vector, C. pulicaria, is present. There are no documented occurrences of *C. pulicaria* outside of the Nearctic region. In fact, when Stewart's occurred infrequently outside of North America, *E. stewartii* failed to become established probably due to the lack of a vector. If *E. stewartii* becomes established in the PRA area, economic impact would be minimal because adequate control measures (i.e., host resistance) can be implemented before the geographic distribution of the bacterium is widespread within the PRA area.

Previous documented occurrences of Stewart's wilt in other countries (e.g., Italy, Romania and Poland), without the widespread establishment of *E. stewartii,* is strong circumstantial evidence that an adequate insect vector does not occur in these countries. It is doubtful that *E. stewartii* could become established in the absence of an insect vector suitable for overwintering and dissemination.

If quarantine regulations against *E. stewartii* in maize seed are continued, they should reflect recent research on seed transmission of *E. stewartii,* changes in maize seed production, and improved methodology for detecting seed-borne bacterial pathogens. The probability of introducing *E. stewartii* to a new area as a result of seed transmission is much lower than one would expect based on rates of transmission that became accepted in the general plant pathological literature in the first half of the 20th century. Based on research done since 1990 that re-evaluates the importance of seed transmission of *E. stewartii*, it appears extremely unlikely that *E. stewartii* is seed transmitted except when seed parent plants are systemically infected. Seed transmission of *E. stewartii* has been demonstrated for only one plant from seed lots for which the level of kernel infection was less than 10%; and in this unique instance, the best estimate of kernel infection was 9 ± 3.3%. Therefore, a 1% threshold of *E. stewartii*-kernel infection provides excellent assurance that seed transmission will not occur. An ELISA-based seed health test of 400 kernels has a 98.2% probability of detecting *E. stewartii*-kernel infection above 1%. An 0.5% threshold for estimates of kernel infection from the ELISA-based seed health test ensures that seed lots have less than 1% *E. stewartii*-infected kernels which represents a reasonable risk

considering rates of seed-to-seedling transmission and the absence of an insect vector. For seed lots that may harbor low levels of *E. stewartii* (e.g., less than 1%), seed treatment insecticides provide additional assurance of preventing *E. stewartii* from being acquired by a vector in PRA areas.

Recommendations, in order of priority, are:

- re-evaluate quarantine restrictions against *E. stewartii* in maize seed

or,

- develop a quarantine protocol that accounts for rates of seed transmission of *E. stewartii* and the ability to detect seed-borne *E. stewartii* with an ELISA-based seed health test, as follows:
  - no restriction on seed produced in areas where *E. stewartii* does not occur
  - no restriction on seed produced in fields in which fewer than 1% of plants are systemically infected with Stewart's wilt based on visual inspection
  - no restriction on seed for which *E. stewartii* was not detected from an ELISA-based seed health test of 400 randomly selected kernels [four 100-kernel samples]
  - require seed treatment insecticides on seed for which *E. stewartii*-infection of kernels is positive but estimated* to be less than 0.5%
  - prohibit seed from being imported if kernel infection is estimated to be 0.5% or above

*The number of 100-kernel samples tested is unlimited but the estimate of the percentage of kernel infection must be calculated from all samples tested using the binomial equation for group sampling:

$$p = 1 - (1 - Q)^{1/n}$$

where, p = the best estimate of the proportion of infected kernels
Q = the proportion of positive samples, and
n = 100, the number of seed per sample (See Appendix 5)

4. DOCUMENTATION OF PEST RISK ANALYSIS

4.1 DOCUMENTATION REQUIREMENTS

PURPOSE: new detection technologies and new information about seed transmission of *E. stewartii* impacts earlier phytosanitary regulations based on outmoded information

PEST: *Erwinia stewartii* (Syn. *Pantoea stewartii*)

PATHWAY: maize seed

PRA AREA: all areas importing maize seed from North America where *E. stewartii* occurs, with a particular emphasis on Europe and the Palearctic region

SOURCES OF INFORMATION: CABI Crop Protection Compendium and references listed below (See Item 4.2).

CONCLUSIONS OF RISK ASSESSMENT: The probability of introducing *Erwinia stewartii* on maize seed and the probability of *E. stewartii* becoming established in the PRA area can be examined in three sequential steps: i.) the probability of seed harboring *E. stewartii*, ii.) the probability of transmitting *E. stewartii* from seed to seedlings, and iii.) the probability of insect vectors that allow for the establishment and spread of *E. stewartii* in the PRA area. The probability of seed harboring *E. stewartii* can be assessed from visual inspections of seed production fields and/or an ELISA-based seed health test of seed lots. The probability of seed-borne *E. stewartii* is nearly zero for seed produced in fields with no symptoms or non-systemic symptoms of Stewart's wilt. The expected number of infected seedlings is less than 2 in 1,000,000 for seed with less than 1% kernel infection. Based on binomial distributions and group samples, an ELISA-based seed health test can detect with a known probability the percentage of infected kernels.

Stewart's wilt has little economic impact on maize grown in the United States where the disease is endemic except for situations in which extremely susceptible hybrids are grown for specialty purposes (e.g., processing sweet corn). The disease probably would have a similar minimal impact on maize in the PRA area. During the initial growing season following the introduction of *E. stewartii* and in the subsequent two or three seasons, the disease probably would occur in a limited area. The economic impact may be significant to producers in that area if the cultivars being grown are susceptible to Stewart's wilt, but the overall impact in the PRA area would be minimal because the disease would not be widespread. By the time Stewart's wilt becomes widespread, cultivars with moderate to high levels of resistance could be identified and grown in the PRA area.

RISK MANAGEMENT: The following options were identified:

- Re-evaluate quarantine restrictions
- Inspect seed production fields for the absence of systemic infection by *E. stewartii*
- Require that seed from uninspected fields and from fields with systemic Stewart's wilt be assayed using an ELISA-based seed health test with four 100-kernel replicate samples
- Require that seed tested by the ELISA-based seed health test have an estimated percentage of infected kernels of 0.5% or
- Apply systemic insecticide seed treatments to seed that may harbor *E. stewartii*

The following options were recommended, in order of priority:

- Re-evaluate quarantine restrictions against *E. stewartii* in maize seed

or,

- develop a quarantine protocol that accounts for rates of seed transmission of *E. stewartii* and the ability to detect seed-borne *E. stewartii* with an ELISA-based seed health test, as follows:
    - no restriction on seed produced in areas where *E. stewartii* does not occur

- o no restriction on seed produced in fields in which fewer than 1% of plants are systemically infected with Stewart's wilt based on visual inspection
- o no restriction on seed for which *E. stewartii* was not detected from an ELISA-based seed health test of 400 randomly selected kernels [four 100-kernel samples]
- o require seed treatment insecticides on seed for which *E. stewartii*-infection of kernels is positive but estimated* to be less than 0.5
- o prohibit seed from being imported if kernel infection is 0.5% or above

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APPENDIX 1: CABI CROP PROTECTION COMPENDIUM DATA SHEET FOR *PANTOEA STEWARTII* (SYN. *ERWINIA STEWARTII*)

Selected texts for *Pantoea stewartii*

NAMES AND TAXONOMY

Preferred Name
Pantoea stewartii (Smith 1898) Mergaert et al. 1993

Trade Names
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae

Other Names Used
Pantoea stewartii subsp. *stewartii* (Smith 1898) Mergaert et al. 1993
Aplanobacter stewartii (Smith) McCulloch 1918
Bacillus stewartii (Smith) Holland 1920
Bacterium stewartii (Smith) Smith 1905
Erwinia stewartii (Smith 1898) Dye 1963
Phytomonas stewartii (Smith) Bergey et al. 1923
Pseudobacterium stewartii (Smith) Krasil'nikov 1949
Pseudomonas stewartii Smith 1898
Xanthomonas stewartii (Smith) Dowson 1939

BAYER CODE: ERWIST

Common Names
English:
bacterial wilt of maize
Stewart's wilt
Stewart's bacterial wilt
Stewart's disease
French:
flétrissement bactérien du maïs
maladie de Stewart

NOTES ON TAXONOMY AND NOMENCLATURE

Considerable debate on the taxonomy of this pathogen in the first half of the twentieth century was resolved by the proposal of *Erwinia stewartii* (Smith) Dye as the correct name (Dye, 1963). Recently, the nomenclature of the genus *Erwinia* has been modified based on chemotaxonomic and molecular approaches, however the taxonomic complexity of this group has not been completely resolved and a dual system of nomenclature is in use presently (Kwon et al., 1997). The genus *Pantoea* was proposed for some strains of the *Erwinia herbicola-Enterobacter agglomerans* complex, including *E. stewartii* (Gavini et al., 1989, Mergaert et al., 1993). Members of the genus *Pantoea* form a homogeneous taxon, however separation of this group from other *Erwinia* species is not fully supported by some approaches, such as 16S RNA sequence analysis (Kwon et al., 1997). Thus, the pathogen continues to be referred to by two Latin binomials, *Pantoea stewartii* and *Erwinia stewartii*.

HOST RANGE

All types of maize (*Zea mays*) are hosts of *P. stewartii*. The bacterium also has been isolated from teosinte (*Zea mexicana*) and eastern gamma grass (*Tripsacum dactyloides).* Plants of many genera can been artificially inoculated successfully with *P. stewartii* and may serve as weak secondary hosts (Bradbury, 1967; Pepper, 1967; Poos, 1939).

Primary hosts: *Zea mays* (maize), *Zea mexicana* (teosinte).

Secondary hosts: *Agrostis gigantea* (bent couch), *Coix lacryma-jobi, Cucumis sativus* (cucumber), *Dactylis glomerata* (orchardgrass), *Digitaria*, *Euchlaena perennis*, *Panicum capillare* (tumble panicgrass), *Panicum dichotomiflorum*, *Poa pratensis* (Junegrass), *Schlerachne punctata*, *Setaria lutescens* [*Setaria pumila*], *Sorghum sudanense* (Sudan grass), *Tripsacum dactyloides*, *Tripsacum zea*, *Triticum aestivum* (wheat).

Affected Plant Stages: Seedling stage, vegetative growing stage, flowering stage, and fruiting stage.

Affected Plant Parts: Whole plant, leaves, stalks, roots, inflorescence, and seeds.

Maize plants are infected by *P. stewartii* as a result of feeding wounds made by corn flea beetles. Following initial infection, the bacterium moves in the xylem of plants. Severity of infection and the relative degree of resistance or susceptibility of a plant is associated with intra-plant movement of *P. stewartii*. In plants with highly susceptible reactions, infection is systemic and *P. stewartii* can be isolated from tissues throughout the plant, including seed. In plants with resistant reactions, the bacterium usually is restricted to tissues near the site of infection, i.e., insect feeding wounds (Braun, 1982, 1990).

GEOGRAPHIC DISTRIBUTION

Notes on distribution

In spite of several instances of isolated occurrence throughout the world, *P. stewartii* has never become established outside of the area to which Stewart's wilt is endemic in the United States. *Pantoea stewartii* occurs throughout the maize growing regions of the eastern and midwestern United States and intermittently in Canada. The bacterium is or has been present in other countries in the Western Hemisphere and in restricted regions elsewhere in the world (Pepper, 1967, McGee, 1988).

An earlier record from Switzerland is now viewed as doubtful (CMI, 1987). The former USSR is now reported to be free from *P. stewartii.* Although previous distribution maps have included the former Yugoslavia, the disease has never occurred there (CMI, 1987). Although now absent, *P. stewartii* has previously been recorded in Italy (Anon., 1983; Mazzuchi, 1984), Poland, Romania, Henan (China), Malaysia, Thailand and Vietnam (CMI, 1987).

Pantoea stewartii occurs throughout the midwestern and eastern United States (Pepper, 1967). Stewart's wilt is endemic in the mid-Atlantic and Ohio River Valley regions and in the southern portion of the Corn Belt including portions of the following states: Arkansas, Delaware, Illinois, Indiana, Kentucky, Maryland, Missouri, New Jersey, New York, Ohio, Pennsylvania, Tennessee, Virginia, and West Virginia. The occurrence of Stewart's wilt in other eastern and midwestern states coincides with the occurrence of the corn flea beetle, *Chaetocnema pulicaria*, the insect vector and overwintering host of *P. stewartii*.

Pantoea stewartii has not been reported from Alaska, Hawaii, or most of the western United States including: Arizona, Colorado, Montana, Nevada, Oregon, Utah, and Wyoming (Pepper, 1967). The pathogen has not become established in Idaho or Washington although it was reported in 1920 in Washington and on a few plants in Idaho in 1964. Failure of the

pathogen to persist in this region presumably is due to dry climatic conditions and absence of the corn flea beetle (Pepper, 1967).

Stewart's wilt occurs over a wide range of environmental conditions. Weather affects the survival of the insect vector but does not appear to have substantial impact on the bacterium. In North America, Stewart's wilt usually does not occur in areas where the average daily winter temperature (i.e., the average for December, January, and February) is below –3 C (Stevens, 1934; Stevens and Haenseler, 1941) because *C. pulicaria* does not survive under these conditions.

See also CABI/EPPO (1998, No. 265).

List of countries

Europe
Austria: restricted distribution (EPPO, 2002 (footnote 1))
Croatia: absent, never occurred (EPPO, 2002 (footnote 6))
Greece: absent, not established (EPPO, 2002)
Italy: absent, not established (Anon., 1983; Massuchi, 1984; EPPO, 2002)
Poland: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 9))
Romania: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002)
Switzerland: absent, invalid record (EPPO, 2002 (footnote 5))
Yugoslavia: absent, never occurred (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 14))

Asia
[China]
Henan: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002)
India: present, no further details (Pepper, 1967)
Malaysia: absent, not established (Bradbury, 1967; CMI, 1987)
Peninsular Malaysia: absent, not established (CMI, 1987; EPPO, 2002)
Thailand: absent, not established (Bradbury 1967; CMI 1987; EPPO, 2002 (footnote 11))
Vietnam: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 13))

Western Hemisphere
Bolivia: present, no further details (EPPO, 2002 (footnote 2))
[Brazil]
Sao Paulo: present, no further details (Bradbury, 1967; CMI, 1987; EPPO, 2002)
[Canada]
Alberta: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 3))
British Columbia: absent, not established (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 4))
Ontario: present, no further details (Bradbury, 1967; CMI, 1987; EPPO, 2002)
Costa Rica: present, no further details (Bradbury, 1967; CMI, 1987; EPPO, 2002)
Guyana: present, no further details (Bradbury, 1967; CMI, 1987; EPPO, 2002)
Mexico: restricted distribution (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 7))
Paraguay: absent, invalid record (EPPO, 2002 (footnote 10))
Peru: restricted distribution (Bradbury, 1967; CMI, 1987; EPPO, 2002 (footnote 8))
Puerto Rico: present, no further details (Bradbury, 1967; CMI, 1987; EPPO, 2002)
Trinidad and Tobago: absent, intercepted only (EPPO, 2002)
USA: widespread (Bradbury 1967; CMI 1987; EPPO, 2002 (footnote 12))
Alabama: present, no further details (Pepper, 1967)
Arkansas: present, no further details (Pepper, 1967)
California: present, no further details (Pepper, 1967)
Connecticut: present, no further details (Pepper, 1967; EPPO, 2002)
Delaware: present, no further details (Pepper, 1967)
Florida: present, no further details (Pepper, 1967)

Georgia (USA): present, no further details (Pepper, 1967)
Idaho: present, no further details (Pepper, 1967)
Illinois: present, no further details (Pepper, 1967; Parker & Hooker, 1993; EPPO, 2002)
Indiana: present, no further details (Pepper, 1967; EPPO, 2002)
Iowa: present, no further details (Pepper, 1967; EPPO, 2002)
Kansas: present, no further details (Pepper, 1967)
Kentucky: present, no further details (Poneleit & Evans, 1972; EPPO, 2002)
Louisiana: present, no further details (Kang, 1987; EPPO, 2002)
Maine: present, no further details (Pepper, 1967)
Maryland: present, no further details (Pepper, 1967)
Massachusetts: present, no further details (Pepper, 1967)
Michigan: present, no further details (Pepper, 1967)
Mississippi: present, no further details (Pepper, 1967)
Missouri: present, no further details (Pepper, 1967; EPPO, 2002)
Nebraska: present, no further details (Pepper, 1967; EPPO, 2002)
New Hampshire: present, no further details (Pepper, 1967)
New Jersey: present, no further details (Pepper, 1967)
New Mexico: present, no further details (Pepper, 1967)
New York: present, no further details (Straub & Heath, 1983; Dillard & Kline, 1989; EPPO, 2002)
North Dakota: present, no further details (Pepper, 1967; EPPO, 2002)
Ohio: present, no further details (Pepper, 1967; EPPO, 2002)
Oklahoma: present, no further details (Pepper, 1967)
Pennsylvania: present, no further details (Castor et al., 1975; EPPO, 2002)
Rhode Island: present, no further details (Pepper, 1967)
South Carolina: present, no further details (Pepper, 1967)
South Dakota: present, no further details (Pepper, 1967)
Tennessee: present, no further details (Pepper, 1967)
Texas: present, no further details (Pepper, 1967)
Vermont: present, no further details (Pepper, 1967)
Virginia: present, no further details (Pepper, 1967)
Washington: present, no further details (Pepper, 1967)
West Virginia: present, no further details (Pepper, 1967)
Wisconsin: present, no further details (Pepper, 1967; EPPO, 2002)

Footnotes from EPPO, 2002. PQR database (version 4.1). Paris, France: European and Mediterranean Plant Protection Organization:

(1) No severe economic damage.
(2) RS 97/203: associated with symptoms of bacterial wilt of maize, together with *Erwinia chrysanthemi*.
(3) The Canadian NPPO declares that the sole report in Alberta was from a trial plot planted with seed originating from USA. The disease did not reappear in subsequent years (Canadian Pest Data Sheet, 1986).
(4) Recorded in 1951 (Foster & MacSwain, Rep. BC Dep. Agric. 1951) in two growers' crops near Victoria. There are no more recent records. The Canadian NPPO declares that *P. stewartii* does not now occur in British Columbia.
(5) The original publication (from the late 1940s) only claims bacterial wilt symptoms. In view of the absence of any later information, this record should be considered erroneous.
(6) Officially declared never to occur, but no specific supporting evidence available.
(7) Present in México (Toluca valley), Oaxaca, Tabasco, Tlaxcala, Veracruz. De la Isla, B.M.L. (1984) Fitopatología futura, p. 84. México.
(8) Coast.
(9) There are no further details on the supposed earlier record of the EPPO data sheet; the Polish Plant Protection Service does not confirm.

(10) Information from COSAVE suggested that there was an erroneous or doubtful record from Paraguay. In the absence of any more specific information, the record should be considered erroneous.
(11) Probably on crops grown from imported seed. No records since 1960s, so probably did not establish.
(12) EPPO Reporting Service 500/04: first major outbreak in New York State since 1933 occurred in 1986.
(13) Probably on crops grown from imported seed. No records since 1960s, so probably did not establish.
(14) This record, as the one in EPPO DS 1982, is an error. Hadzistevic, D. (1986) (Bacterial wilt of maize caused by *Erwinia stewartii* not yet recorded in Yugoslavia). Zastita Bilja 37, 87-91 (in Serbo-Croat).

BIOLOGY AND ECOLOGY

Transmission and Survival

It is doubtful that *P. stewartii* could become established in the absence of an insect vector suitable for overwintering and dissemination (Claflin, 1999). The corn flea beetle, *Chaetocnema pulicaria*, is generally recognised as the only important insect vector of *P. stewartii* (Pepper, 1967). There are no known examples of widespread, prolonged occurrences of Stewart's wilt in the absence of this insect. Although the disease has been reported infrequently from various parts of the world, *P. stewartii* has never become established outside of the region of North America to which it is endemic, presumably because of the lack of an adequate vector and overwintering host. The potential for establishment and spread of *P. stewartii* in the absence of corn flea beetles is extremely unlikely.

The pathogen overwinters in the alimentary tract of this insect, which emerges from hibernation and feeds on young maize plants (Pepper, 1967). Dill (1979) sampled regurgitated material and feces of *C. pulicaria* fed *P. stewartii*-infected plants. A sufficient quantity of bacteria were present in both types of samples to incite disease, but based on significantly more bacteria in fecal material, Dill concluded that the probable mode of transmission was from fecal contamination rather than regurgitated material. In a study of survival of the pathogen in association with insect vectors (Elliot and Poos, 1934), *P. stewartii* was recovered in pure culture from 75% of surface-disinfected adult flea beetles collected in April. Maize inoculated with these cultures by wounding or insect feeding developed characteristic wilt symptoms. The pathogen was not recovered from 39 other insect species examined (Poos and Elliot, 1935). The pathogen was recovered from *Diabrotica undecimpunctata howardi* and *Diabrotica longicornis*, but they were considered to be inefficient vectors (Ivanoff, 1933; Rand and Cash, 1933).

Over 28,500 insects representing 94 species and 76 genera were tested as vectors of *P. stewartii* (Elliott and Poos, 1934; Poos and Elliott, 1936; Elliott and Poos, 1940). A few insect species other than *C. pulicaria* transmitted *P. stewartii* in greenhouse trials, of which *Chaetocnema denticulata* was most frequent, but none were considered vectors of importance under field conditions (Elliott & Poos, 1940). *Delia platura* and *Agriotes mancus* also transmitted *P. stewartii* to maize plants in cage experiments (Rand and Cash, 1933; Frutchey, 1936). It is generally accepted that *C. pulicaria* provides the only means for overwintering and transmission of the pathogen (Elliot and Poos, 1934, 1940; Pepper, 1967). Stewart's wilt occurs in North America only when flea beetles are present; however, outbreaks of Stewart's wilt in other areas, such as the Po Valley of Italy (Anon., 1983), may have occurred in the absence of *C. pulicaria*.

In the USA, the occurrence of Stewart's wilt can be predicted on the basis of the average daily temperature in December, January and February which affects the survival of *C.*

pulicaria (Boewe, 1949; Castor et al., 1975; Pepper, 1967; Stevens, 1934). If the average daily temperature during this period is above freezing, flea beetles survive and Stewart's wilt is likely to be severe on susceptible hybrids. If the average daily temperature is less than –3 C, flea beetles are less likely to survive and it is unlikely that Stewart's wilt will be severe. Modifications to the Stevens-Boewe system of forecasting Stewart's wilt have been proposed (Esker and Nutter., 2002).

In 1975 in Connecticut, two cycles of infection were observed in 14 successive plantings of the Stewart's wilt susceptible sweetcorn cultivar 'Jubilee' (Heichel et al., 1977). The first cycle of Stewart's wilt apparently resulted from *P. stewartii* transmitted by overwintering flea beetles. The second cycle appeared to result from bacteria transmitted by a summer generation of the insect. Recent evaluations of flea beetle population dynamics and the occurrence of Stewart's wilt corroborate these observations and provide additional secondary evidence that corn flea beetles are the only vector of epidemiological importance (, Cook, 2003; Esker and Nutter, 2002, 2003).

Pantoea stewartii survives in living host plants, insect vectors and seed. There is no evidence that *P. stewartii* is capable of overwintering in soil or crop residues (Pepper, 1967). The insect vector, *C. pulicaria* is the primary overwintering host. The bacterium also may survive in seed produced on seed parent plants that were systemically infected. Seed transmission of *P. stewartii* is associated closely with the severity of infection of the plant on which seed is produced which is related to the susceptibility or resistance of the seed parent plant (Block et al., 1999; Khan et al., 1996; Michener et al., 2002a, 2002b). Some research on *P. stewartii* in the first half of the twentieth century did not consider reactions of seed parent plants and/or the occurrence of vectors; threfore, conclusions about rates of seed transmission of *P. stewartii* were in error. Although the seed-borne nature of *P stewartii* is unequivocal; seed transmission of *P. stewartii* plays an insignificant role in the epidemiology of Stewart's wilt in areas where the disease is endemic. Based on recent evaluations of rates of plant-to-seed and seed-to-seedling transmission (Block et al. 1998, Block et al., 1999, Khan et al., 1996, Michener et al., 2002a, 2002b), the probability of transmitting *P. stewartii* in seed is extremely remote when seed is produced on resistant or moderately resistant seed parent plants (see Seed Transmission under SEEDBORNE ASPECTS).

Physiology and Virulence

Anatomical changes associated with disease development were studied in resistant and susceptible maize by using light and transmission electron microscopy (Braun, 1982). Pathogen populations increased at similar rates in leaves of resistant and susceptible hosts. When leaves of plants at the tassel stage were inoculated with *P. stewartii*, lesions expanded 3-4 times more rapidly in susceptible plants than in resistant plants. Pit membranes became coated with material resembling bacterial exopolysaccharide while pathogen populations in vessels remained very low. Many vessels become totally occluded with bacterial cells and exopolysaccharide as populations of *P. stewartii* increased (Braun, 1982). Exopolysaccharide production and virulence are correlated (Braun, 1990, Coplin et al., 1992). Capsular polysaccharide synthesis and virulence in *P. stewartii* appears to require quorum-sensing regulatory proteins (Minogue et al., 2002). A bacterial agglutinin was extracted from ground maize seed and the activities of this agglutinin against 22 strains of *P. stewartii* varying in virulence were assessed. Specific agglutination (agglutination titre/mg protein per mL) values were correlated negatively with virulence ratings (Bradshaw-Rouse et al., 1981). Virulence of strains of *P. stewartii* has been studied extensively at the molecular level. A 24-kb pathogenicity gene cluster in *P. stewartii* is required for water-soaked lesion formation and wilting of maize seedlings, but this gene cluster may not be required for initial growth of the bacterium (Coplin et al., 1992; Frederick et al., 2001).

Pantoea stewartii appears to be a relatively homogeneous organism. One hundred and twenty-four isolates of *P. stewartii* originating from sweet corn or flea beetles collected in the

northeastern, midwestern and mid-Atlantic states of the US had homogeneous metabolic profiles at 93% similarity (Wilson et al., 1999). Two-thirds of the isolates formed 18 separate groups with the same metabolic profile, while one-third of the isolates had distinct profiles. This phenotypic homogeneity was interpreted as an indication that the pathogen has been streamlined to exist in particular hosts (i.e., *Z. mays* and *C. pulicaria*). Infection is not severe in other hosts, and *P. stewartii* is not transmitted efficiently by other vectors. The authors suggested that a considerably greater amount of diversity would be expected in an organism that survived more ubiquitously in the environment (Wilson et al., 1999).

Means of Movement and Dispersal

Plant parts liable to carry the pest in trade/transport:
- Fruits: borne internally; visible to naked eye.
- Flowers/inflorescences/cones/calyx: borne internally; borne externally; visible to naked eye.
- Leaves: borne internally; visible to naked eye.
- Seedlings/micropropagated plants: borne internally; visible to the naked eye.
- Roots: borne internally; not visible to naked eye but usually visible under light microscope.
- Stems (above ground)/shoots/trunks/branches: borne internally; borne externally; visible to naked eye.
- True seeds (inc. grain): borne internally; borne externally; not visible to naked eye.

Plant parts not known to carry the pest in trade/transport:
- Bark
- Bulbs/tubers/corms/rhizomes
- Wood.

Transport pathways for long distance movement:
- Mail: Transfer of seeds.

SEEDBORNE ASPECTS

Incidence

Pantoea stewartii is seed-borne. *Pantoea stewartii* was detected in the chalazal region of maize kernels, the aleurone layer and between endosperm cells, but not in the embryo or on the seed coat (Ivanhoff, 1933; Rand and Cash, 1933). The pathogen was recovered from seed for up to 5 months after harvest (Rand and Cash, 1933). In China, *P. stewartii* survived in stored maize longer at low temperatures, but could not be recovered after 200-250 days in storage at 8-15℃ (Guo et al., 1987). The bacteriu m has been detected by ELISA in 3-yr-old seed.

Incidence of seed infected with *P. stewartii* is related to severity of infection of seed parent plants. Kernel infection (i.e., plant-to-seed transmission) is associated with the level of host resistance or susceptibility of the seed parent plant which affects severity. Resistance confines the movement of *P. stewartii* in the vascular system of plants (Braun, 1982, 1990), thus limiting systemic infection and restricting infection of seed. In highly resistant lines, symptoms are distinct within a few cm of flea beetle feeding wounds, but there is no evidence of further spread of the bacterium. In moderately resistant lines, *P. stewartii* may be recovered up to 10 cm from feeding wounds, but plants are not infected systemically. Plant-to-seed transmission has not been observed when seed parent plants are infected non-systemically (Block et al., 1999; Khan et al., 1996). Kernel infection was below 0.025% when seed parent plants were rated as resistant and below 0.2% when seed parent plants were rated as moderately resistant (Michener et al., 2002b). Plant-to-seed transmission usually ranged from about 0.2 to 12% for seed produced on highly susceptible, systemically-infected plants (Block et al., 1999; Khan et al., 1996; Michener et al., 2002b), although in one

instance, up to 35% incidence of infection was observed in seed harvested from naturally infected plants (Block et al, 1998; McGee, 1996).

Effect on Seed Quality

Seeds harvested from severely infected ears often are deformed, shrunken and discoloured. Germination often is adversely affected (Pepper, 1967; Block, 1996).

Seed Transmission

Pantoea stewartii is seed transmitted. The rate of seed transmission of *P. stewartii* is associated with the susceptibility or resistance of the host and the severity of infection of the seed parent plant (Block et al., 1998; Block et al., 1999; Khan et al., 1996: Michener et al., 2002a, 2002b). Host resistance confines the movement of *P. stewartii* in the vascular system of plants (Braun, 1982, 1990), thus limiting systemic infection and restricting infection of seed.

The first research on seed transmission of *P. stewartii*, in which seed from highly susceptible maize cultivars were evaluated, failed to account for possible infection of plants by corn flea beetles. Seed transmission was first hypothesised from circumstantial evidence when Stewart's wilt developed in about 9% of plants grown in the field or greenhouse from seed obtained from infected plants (Stewart, 1897; Smith, 1909). In other early reports of seed transmission that did not account for transmission of *P. stewartii* by corn flea beetles, the bacterium was reported to be transmitted by seed to as many as 85% of plants grown in a greenhouse (Thomas, 1924). In subsequent research in the 1930s after the discovery of the flea corn beetle vector, the disease was reported to be transmitted at rates from 2% to 13% in severely damaged seed harvested from severely infected plants (Frutchey, 1936; Rand and Cash, 1933). These rates of seed transmission usually were derived from relatively small samples (e.g., less than 100 kernels). Ivanhoff (1933) suggested that wounding of the subterranean parts of the host plant at early stages of development by insects or other agencies may be an important factor in transmission of the disease through seed. Based on these studies, a 2% rate of seed transmission was reported in several bulletins and reports on Stewart's wilt (Elliott, 1941; Robert, 1955, Pepper, 1967). References to a low rate of seed transmission of *P. stewartii* (e.g., 2%) has been repeated frequently in plant pathological literature, but the quantitative aspects of seed transmission of this bacterium were not evaluated thoroughly until the 1990s.

Recent studies have re-evaluated seed transmission of *P. stewartii*. The transmission process has been divided into two phases: plant-to-seed transmission (i.e., kernel infection) and seed-to-seedling transmission. Severity of infection of seed parent plants also has been considered in recent research because most inbred seed parents grown today have considerably higher levels of resistance to Stewart's wilt than did the open-pollinated cultivars that were evaluated previously. As noted previously (see Incidence), kernel infection (i.e., plant-to-seed transmission) is associated with the level of host resistance or susceptibility.

Seed-to-seedling transmission has been evaluated for seed harvested from naturally infected plants, for seed from plants on which leaves were inoculated by the pinprick method (Blanco et. at., 1977; Chang et al.1977), and for seed from plants in which *P. stewartii* was injected into ear shanks. Seed transmission was not observed among more than 75,000 seedlings grown from seed harvested from inoculated plants for which incidence of Stewart's wilt was 100%, but relatively few plants were systemically infected (Khan et al., 1996). Among 18 seed lots for which kernel infection ranged from 1 to 72% , *P. stewartii* was transmitted from seed to seedlings in 6 seed lots with greater than 35% kernel infection, but only a single instance of seed transmission was observed among more than 35,000 plants grown from seed lots in which kernel infection was less than 35% (Block et al., 1998). Seed-to-seedling

transmission was 0.14% from infected kernels harvested from pinprick-inoculated plants and 0.022% (1 in 4,563) from infected kernels harvested from naturally infected plants (Block et al., 1998). Seed-to-seedling transmission was 0.038% when infected kernels produced by ear shank inoculations were planted in field trials in Illinois and Wisconsin (Michener et al., 2002a). Since results from these studies generally concur, the best estimate of seed-to-seedling transmission of *P. stewartii* can be calculated from data from all studies. A total of 51 infected seedlings were observed from an estimated 82,595 infected seed which corresponds to 0.062% seed-to-seedling transmission of *P. stewartii* (Michener et al., 2002a).

Based on these rates of plant-to-seed and seed-to-seedling transmission, the probability of transmitting *P. stewartii* is extremely remote when seed is produced on resistant or moderately resistant seed parent plants. Plant-to-seed transmission is less than 0.3% for moderately resistant plants and less than 0.03% for resistant plants. When susceptible plants are systemically infected through natural methods, plant-to-seed transmission is about 10% or less. Thus, few seed lots are likely to have 35% or more infected kernels that have resulted in the highest rates of seed-to-seedling transmission. Seed-to-seedling transmission probably is very low (i.e., less than 0.06%) for seed with less than 10% infected kernels, if *P. stewartii* is transmitted in these seed at all.

Seed Treatments

Several physical treatments, chemicals such as mercuric chloride (Smith, 1909) and antibiotic treatments were tested from the 1930s to the 1950s (Rich, 1956; Williams, 1957; Pepper, 1967). Some controlled wilt symptoms on seedlings, but not on adult plants. Many were phytotoxic. Antibiotic sprays reduced disease incidence, but did not improve yields (Lockwood and Williams, 1956).

In a study in China, soaking maize seeds with several antibiotics at 40-47℃ for 1.5 h was sufficient to destroy *P. stewartii* and also stimulated seed germination. Soaking the seeds with the same antibiotics at room temperature did not completely eliminate the bacteria and required 18 h for the treatment (Guo et al., 1991).

Seed treatment insecticides (e.g., imidacloprid, thiomethoxam, and clothianidin) are effective against the insect vector of *P. stewarti* (Munkvold et al., 1996; Pataky et al., 2000b) and they reduce the incidence of Stewart's wilt infected seedlings by about 75% (Kuhar et al., 2002; Pataky et al., 2000b). Seed treatment insecticides do not appear to directly affect *P. stewartii*, but they effectively inhibit secondary spread of Stewart's wilt by controlling *C. pulicaria*. If seedlings are infected through seed transmission, seed treatment insecticides can lower the probability of dissemination of *P. stewartii*.

Seed Health Tests

Several test have been proposed or used to detect seed-borne *P. stewartii.* An ELISA-based seed health test was selected as the most reliable assay for seed-borne *P. stewartii* when tests were reviewed recently in the USA by the National Seed Health System.

An ELISA for the presence of *P. stewartii* in seed was developed in the 1990s (Lamka et al., 1991) and is available commercially (AgDia Inc., Elkhart, IN 46514 USA). Based on this ELISA, a seed health test for *P. stewartii* was developed at the Iowa State University Seed Science Center. This ELISA-based seed health test, which uses a sample of 400 kernels, was the method recommend by a National Seed Health System technical panel reviewing seed health tests for *P. stewartii*. While it is impossible to demonstrate that a seed lot is not infected with *P. stewartii*, the ELISA-based seed health test can be designed to demonstrate with a known probability that the percentage of seed infected with *P. stewartii* is below a certain threshold level. For example, in an ELISA-based assay of 400 randomly sampled

kernels from a seed lot, there is a 98.2% probability of detecting *P. stewartii* if kernel infection is 1% or greater and infected seed contain a threshold level of bacteria (10^5 cells per ml). Other seed health test procedures reviewed by the panel were considered undesirable except for the possibility of a developing a test based on a DNA probe for *P. stewartii* developed from PCR with arbitrary primers (Blakemore et al., 1992; Blakemore et al., 1999). Also, other PCR assays have been developed to identify *P. stewartii* with primers from the sequences of *hrpS, cpsDE* and the 16S rRNA ITS region (Coplin et al., 2002).

Nigrosine medium was developed in China for isolation of *P. stewartii*. The medium (pH 6.7) contains yeast extract (1 g/L distilled water); 30 mL glycerol; 200 µg/mL nystatin; 3 g sodium taurocholate; 15 g NaCl; 1% aqueous nigrosine solution (20 mL); 17 g agar (Guo et al., 1982a). When incubated at 30℃ for 5-7 days, colonies of *P.stewartii* are circular, slightly convex, smooth and glistening with a characteristic black-pigmented centre and a wide, transparent margin. This medium was superior to others tested for the isolation of *P. stewartii* from diseased maize seed (Guo et al., 1982b) but it was ineffective as a selective medium because of difficulty in distinguishing various bacteria based on morphology of colony growth (Blakemore et al., 1999).

A seedling grow-out procedure, in which 400 seeds are germinated on blotters, was used routinely for several years to test for *P. stewartii* in seed (McGee, 1982). Based on recent studies of seed transmission of *P. stewartii* (Block, 1998; Michener et al., 2002a), a sample of 400 seeds does not give a realistic chance of detecting the pathogen in grow-out tests.

ECONOMIC IMPACT

Economic losses in maize due to *P . stewartii* have been inconsequential in North America for the past 50 years except for a few, small sporadic outbreaks and a few extensive epidemics on susceptible sweet corn hybrids (Anderson, 1986; Anderson et al., 1986; Pepper, 1967; Pataky et al., 1996; Pataky et al., 2000b). The lack of economic importance of this disease in North America is due primarily to adequate levels of resistance incorporated into maize hybrids that are grown where the disease occurs. Stewart's wilt caused substantial economic losses in the 1930s prior to the development of resistant cultivars (Pepper, 1967). Severe losses due to Stewart's wilt were reported in Italy in the 1940s and the disease reoccurred there as an important problem in the 1980s (Anon., 1983; Mazzucchi, 1984).

In sweet corn, economic losses can be significant when susceptible or moderately susceptible hybrids are grown in an area where flea beetles occur. Yield losses in sweet corn due to Stewart's wilt are affected by the level of resistance or susceptibility of the cultivar and by the growth stage at which plants are infected (Suparyono and Pataky, 1989). Yield losses are associated with systemic infection with about an 0.8% reduction in yield for each 1% incidence of plants infected systemically as seedlings (Freeman and Pataky, 2001). Losses do not occur or are minimal in resistant and moderately resistant hybrids; however, losses frequently range from 40 to 100% when susceptible sweet corn hybrids grown under epidemic conditions are infected prior to the 5-leaf stage (Pataky and Eastburn, 1993).

Stewart's wilt can have an economic effect as a result of phytosanitary regulations imposed by trading partners. The economic effects of phytosanitary regulations primarily affect seed commerce. Also, in areas where corn flea beetles and *P. stewartii* occur, resources must be used to screen germplasm and breed maize for resistance in order to control Stewart's wilt.

PHYTOSANITARY RISK

Economic Importance: Low in dent maize, moderate in sweetcorn
Distribution: Worldwide
Seedborne Incidence: Low
Seed Transmitted: At extremely low levels
Seed Treatment: None

SYMPTOMS

Two phases of Stewart's bacterial wilt, a seedling wilt phase and a leaf blight phase, are differentiated by the time at which infection occurs. When susceptible cultivars are infected as seedlings, plants may wilt rapidly. Pale-green to yellow linear streaks with irregular or wavy margins occur on leaves. Symptoms run parallel to veins and may extend the entire length of the leaf on susceptible cultivars. Systemic infection occurs on susceptible and moderately susceptible cultivars and distinct leaf symptoms occur on new leaves emerging from the plant whorl. In resistant cultivars, symptoms usually are limited to within 2 to 3 cm surrounding flea beetle feeding wounds and systemic infection occurs rarely, if ever. If infection of seedlings occurs within a week of seedling emergence, main stalks can be killed resulting in profuse growth of tillers (Pataky et al., 1996).

Systemically infected plants may produce premature, bleached and dead tassels. Cavities may form in the stalks near the soil line. In such plants, bacteria spread throughout the vascular system, sometimes infecting the kernels. Bacterial exudate may ooze through stomata of the inner husks in cases of severe infection. The surface of the enveloped kernels may then be covered with bacterial slime (Pepper, 1967).

The leaf blight phase of Stewart's wilt occurs after tassels form. Leaf symptoms are similar to those of the seedling wilt phase. Short to long, irregular, pale-green to yellow streaks occur on the leaves. Symptomatic tissue dies and becomes straw-coloured to brown. Leaf symtpoms originate from feeding wounds of *C. pulicaria*. Like leaf symptoms of the seedling wilt phase, necrotic tissues may extend the entire length of leaves or symptoms may be limited to a few cm depending on the resistance or susceptibility of the cultivar. Premature leaf death due to Stewart's wilt predisposes the weakened plant to stalk rot and reduced yields (Pepper, 1967).

Descriptors: Whole plant: dwarfing; seedling blight. Leaves: necrotic areas; abnormal colours; yellowed or dead. Roots: reduced root system. Inflorescence: discoloration panicle; blight; necrosis. Stalks: brown cavaties at base of stalk; ooze. Seeds: bacterial infection.

MORPHOLOGY

The bacterium is a facultative anaerobic, gram-negative, nonflagellate, nonspore-forming, nonmotile rod measuring approximately 0.4 to 0.8 x 0.9 to2.2 μm. Colonies on yeast extract-dextrose-calcium carbonate agar are yellow and convex. Colonies on nutrient-glucose agar are cream-yellow to orange-yellow. *P. stewartii* produces extracellular polysaccharides that are associated with pathogenicity, i.e. virulence (Bradbury, 1967; Pepper, 1967).

SIMILARITIES TO OTHER SPECIES

Necrotic leaf symptoms of both the seedling wilt and leaf blight phases of Stewart's wilt can resemble multiple, coalesced lesions of northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*. Seedlings wilted by *P. stewartii* also may resemble plants suffering from drought stress, nutritional deficiency or insect injury (Pepper, 1967). A simple microscopic examination of leaf tissue for bacterial ooze can easily differentiate Stewart's wilt and NCLB lesions. Bacterial ooze from symptomatic leaf tissue also is a simple diagnostic method to differentiate the seedling wilt phase from drought or other seedling stresses.

DETECTION AND INSPECTION METHODS

Pale-green to yellow, linear streaks with irregular or wavy margins occur parallel to leaf veins and may extend the length of the leaf. This tissue senesces and typical necrotic symptoms occur within a few weeks after infection. When examined microscopically, bacterial ooze is apparent from vascular bundles of cut sections of symptomatic leaf tissue placed in a drop of water.

Conducting vessels become plugged. If stalks of systemically infected plants are cut in cross section, large yellow to brown cavities may be observed at the base of the stalk and yellow slime may exude. Masses of bacteria stream from the vascular bundles of cut edges of symptomatic leaf tissue. When a cut edge of diseased tissue is placed in a drop of water, the drop can become cloudy quickly. Wilting and stunting occur when infection is systemic. Main stalks die and tillers grow profusely if the primary growing point is infected soon after seedlings emerge.

DIAGNOSTIC METHODS

A simple method to confirm a diagnosis of Stewart's wilt based on symptomatic leaf tissue is to observe with the aid of a microscope bacterial ooze streaming from vascular bundles of cut sections of symptomatic leaf tissue placed in a drop of water. An ELISA test also is available for the detection of *P. stewartii* from infected plant tissues including seed and from the insect vector, *C. pulicaria* (Cook, 2003; Esker and Nutter, 2002, 2003; Lamka et al., 1991; McGee, 1996). *Pantoea stewartii* also may be identified in infected maize tissue through the use of a stem-printing technique. Cross sections of stems, cut near the soil line, are pressed onto agar media (McGee, 1996).

Colonies on nutrient agar are small, round, slow-growing and yellow. Nutrient agar streaks vary from thin, yellow, moist and fluid, to thin, dry, orange-yellow and not fluid. Broth culture shows feeble growth, a whitish ring, and yellow precipitate (Bradbury, 1967). In a nigrosine medium selective for *P. stewartii*, colonies incubated at 30℃ for 5-7 days are circu lar, slightly convex, smooth and glistening. They demonstrate a characteristic black-pigmented centre and a wide transparent margin. The medium (pH 6.7) contains yeast extract (1 g/L distilled water); 30 mL glycerol; 200 µg/mL nystatin; 3 g sodium taurocholate; 15 g NaCl; 1% aqueous nigrosine solution (20 mL); 17 g agar (Guo et al., 1982a).

The optimum temperature for growth of *P. stewartii* ranges from 27 to 30℃; the maximum temperature for growth varies between 32 and 40℃. The organism is oxidase-negative and catalase-positive; acid but no gas is produced from fructose, galactose, D-glucose, beta-metylglucoside, arabinose, xylose, lactose, mannose, mannitol, glycerol and sucrose. *Pantoea stewartii* utilizes acetate, fumarate, gluconate, malate and succinate, but not benzolate, oxalate, or propionate as carbon- and energy-yielding sources (Bradbury, 1967; Krieg and Holt, 1984). It exhibits slow growth on gelatin, but no liquefaction. Nitrate is not reduced to nitrite; hydrogen sulphide is not produced (Bradbury, 1967).

CONTROL

Forecasting

In the USA, the occurrrence of Stewart's wilt is forecast on the basis of average daily temperature during December, January and February which is associated with the survival of *C. pulicaria* (Pepper, 1967; Castor et al., 1975; ; Stevens, 1934, Boewe, 1949). If the mean for this period is above freezing, C*. pulicaria* overwinter and Stewart's may be present. If the mean is below –3 C, few flea beetles survive and Stewart's wilt is not likely to occur. Growers use this forecast information when deciding whether to plant susceptible or wilt-

resistant maize varieties and whether or not to apply suitable vector control measures, such as seed treatment insecticides.

Host-Plant Resistance

Stewart's wilt is controlled effectively throughout North America by planting resistant maize hybrids. Resistance to Stewart's wilt is inherited relatively simply (Blanco et al., 1979; Ming et al., 1999; Kang 1990; Parker and Hooker, 1993, Smith, 1971) and can be selected easily in a breeding program. Within a few generations, maize germplasm can be improved considerably for resistant reactions to Stewart's wilt. Resistance restricts the movement of *P. stewartii* in the vascular system of plants (Braun, 1982, 1990). Frequency of systemic infection is related to levels of resistance or susceptibility (Michener et al., 2003). Maize germplasm collected throughout the world includes various levels of resistance to *P. stewartii* (Pataky et al., 2000a), and cultivars grown in certain areas, such as the Republic of South Africa, have sufficient resistance to prevent economic losses due to Stewart's wilt (Michener and Pataky, 2002).

Chemical Control

In field experiments during 1975 and 1976, maize plots planted with a susceptible hybrid and treated with carbofuran to control the beetle vector, *C. pulicaria*, consistently had fewer plants with symptoms of *P. stewartii* than non-treated plots (Ayers et al., 1979). Carbofuran banded over the row and incorporated at planting substantially reduced feeding by *C. pulicaria* and hence the incidence of Stewart's wilt (Heichel et al., 1977). Currently, various foliar applied insecticides are used on occasion by sweet corn producers in North America to control flea beetles and subsequently Stewart's wilt when Stewart's wilt susceptible hybrids are grown in areas where flea beetles occur.

In areas of the United States where Stewart's wilt is endemic, the incidence of systemic infection has been reduced 50% to 85% by controlling corn flea beetles by applications of seed treatment insecticides, e.g., imidacloprid, thiamethoxam, and clothianidin (Munkvold et al., 1996; Pataky et al., 2000b; Kuhar et al., 2002). Seed treatment insecticides also can be used to reduce the establishment of *P. stewartii* in areas where the bacterium might be introduced on seed by reducing the probablity of acquisition by an insect vector.

In a recent study in China, soaking maize seeds with several antibiotics at 40-47℃ for 1.5 h was sufficient to destroy *P. stewartii* and also stimulated seed germination. Soaking the seeds with the same antibiotics at room temperature did not completely eliminate the bacteria and required 18 h for the treatment (Guo et al., 1991).

Disease-free seed

Seed produced in areas where Stewart's wilt does not occur will insure that *P. stewartii* is not introduced to areas where it is absent. Visual inspections for symptoms of Stewart's wilt in seed production fields also can provide a qualitative assessment of whether or not seed may harbor *P. stewartii*. Since plant-to-seed transmission of *P. stewartii* requires systemic infection of seed parent plants, field inspections for the absence of Stewart's wilt or the absence of systemically-infected plants should insure that seed is free of *P. stewartii*. Visual inspections can be grouped into three categories: no Stewart's wilt present, leaf blight symptoms of Stewart's wilt present but no systemically-infected plants, and some plants systemically-infected. Seed-borne *P. stewartii* will not occur in fields in which symptoms are not present. Maize is not infected asymptomatically by this bacterium. In fields in which the leaf blight phase of Stewart's wilt occurs but plants are not systemically infected, seed will not harbor *P. stewartii* unless kernels are infected on an undetected systemically-infected plant. An extremely low percentage of seed may harbor *P. stewartii* due to sampling errors associated with visual inspections of fields, but significant levels of kernel infection (e.g. >1%)

are highly improbable when severity of the leaf blight phase of Stewart's wilt is low, e.g., less than 25% severity (Block et al., 1999). *Pantoea stewartii* may be seed-borne in fields in which plants are systemically infected. Infected seed was detected by Block et al. (1999) from plants with greater than25% of the leaf area systemically infected; however, only 18 of 63 seed lots from fields with systemically-infected plants were infected with *P. stewartii*. Thus, systemic infection of seed parent plants indicates that seed can be infected, but it does not ensure that seed is infected.

If symptoms of Stewart's wilt are detected in seed production fields, detection of *P. stewartii*-infected seed can be done prior to export or at the port of entry. For seed production fields that are not inspected or those in which systemic infection is observed, an ELISA-based seed health test using an appropriately large sample of seed could be used to ensure at a known probability that *P. stewartii*-kernel infection is below an accepted threshold. Since seed-to-seedling transmission is frequent only when seed lots have a relatively large percentage of infected kernels (i.e., >35%), an ELISA-based seed health test of 400 kernels that has a 98% probability of detecting 1% kernel infection is an appropriate test. Seed-to-seedling transmission is an extremely remote possibility in a seed lot with less than 1% kernel infection.

Biological Control

Biological controls for *P. stewartii* have not been developed adequately to be used. A bacteriophage of *P. stewartii* was isolated from *C. pulicaria* (Woods et al., 1981). The phage was partially characterized according to host range, one-step growth experiment behaviour and morphology. The host range was limited to 8 of 13 strains of *P. stewartii* and one strain of *Erwinia herbicola* var. *herbicola*. Woods et al. (1981) suggested that under field conditions, *P. stewartii* might be effectively reduced or eliminated within its beetle vector by virulent bacteriophages.

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APPENDIX 2: CABI CROP PROTECTION COMPENDIUM DATA SHEET FOR *CHAETOCNEMA PULICARIA*

Chaetocnema pulicaria Melsheimer, 1847

Taxonomic groups
Domain: Eukaryota
Kingdom: Metazoa
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Chrysomelidae
Subfamily: Alticinae

BAYER CODE: CHAEPU

Common Names
English:
corn flea beetle
flea beetle, corn
French:
altise du maïs
altise du mais
Germany:
Erdflih, mais-

NOTES ON TAXONOMY AND NOMENCLATURE

Chaetocnema pulicaria was first described by Frederick E. Melsheimer in 1847. He placed the corn flea beetle in the family Chrysomelidae, subfamily Alticinae (Halticinae), and that status remains unchanged (Arnett et al. 2001)

HOST RANGE (Poos and Elliott 1936, Poos 1955)

Zea mays (corn) is the preferred host of *Chaetocnema pulicaria*, but is reported to feed on several secondary hosts. Only feeding on *Zea mays* is considered economically important.

Primary host: *Zea mays* (corn)

Secondary hosts:
Agrostis alba (Redtop), *Avena sativa* (Oat), *Cynodon dactylon* (Bermuda grass), *Cyperus esculentis* (Chufa), *Cyperus strigosus* (Strawcolored sedge), *Dactylis glomerata* (Orchard grass), *Digitaria ischaemum* (Smooth crabgrass), *Digitaria sanquinalis* (Crabgrass), *Echinochloa crusgalli* (Barnyard grass), *Eleusine indica* (Goosegrass), *Eragrostis pectinacea* (Nees), *Elymus virginicus* (Virginia wildrye), *Hordeum distichon* (Barley), *Lolium multiflorum* (Italian ryegrass), *Panicum capillare* (witchgrass), *Panicum dichotomiflorum* (fall panicum), *Pleum pretense* (Timothy), *Poa pretenses* (Kentucky bluegrass), *Setaria faberi* (giant foxtail), *Setaria lutenscens* (Yellow foxtail), *Sorghum vulgare* var. *sudanense* (Sudan grass), *Triticum aestivum* (Wheat)

Affected Plant Stages: Seedling, vegetative growing, flowering, and fruiting stages.

Affected Plant Parts: Whole plant, leaves, and roots.

NOTES ON HOST RANGE

Poos and Elliott (1936) and Poos (1939, 1955) several secondary hosts of *C. pulicaria*.

GEOGRAPHIC DISTRIBUTION

Notes on geographic distribution

Native to the western hemisphere, more specifically to the eastern-half of the United States (Metcalf et al. 1962; Dill 1979). *Chaetocnema pulicaria* is distributed in most areas east of the Rocky Mountains. Based on available fact sheets and control recommendations, the corn flea beetle is present in areas along the east coast (from Maine south to Florida), the United States-Canadian border (from Maine west to North Dakota), and the Gulf coast (from Florida to Texas). Corn flea beetles are present in all the Midwestern states and the central plains states of South Dakota, Nebraska, Kansas, and Oklahoma. The corn flea beetle has also been identified in New Mexico, Utah, and Washington.

List of countries

Western Hemisphere
North America: present, no further details (present in states east of the Rocky Mountains)
USA: unconfirmed record (CABABSTRACTS, 1984)
- Alabama: present, no further details (Flanders, 2002)
- Arkansas: present, no further details (Johnson et al., 2003)
- Colorado: present, no further details (Downie and Arnett, 1996)
- Connecticut: present, no further details (Downie and Arnett, 1996)
- Delaware: present, no further details (USDA, 2001)
- Florida: present, no further details (Downie and Arnett, 1996)
- Georgia:
- Illinois: widespread (Forbes, 1894; USDA, 2001; Cook, 2003)
- Indiana: widespread (Dill, 1979; Downie and Arnett, 1996)
- Iowa: widespread (Esker, 2001; USDA, 2001)
- Kansas: present, no further details (Lingafelter, 1998; USDA, 2001)
- Kentucky: present, no further details (Bessin, 2001; USDA, 2001)
- Louisiana: unconfirmed record (CABABSTRACTS, 1987)
- Maine:
- Maryland: present, no further details (USDA, 2001)
- Massachusetts:
- Michigan:
- Minnesota: present, no further details (USDA, 2001; Hines and Hutchinson, 2002)
- Mississippi: present, no further details (USDA, 2001)
- Missouri: widespread (Hall, 1978)
- Nebraska: present, no further details (USDA, 2001; Stack et al., 2002)
- New Hampshire:
- New Jersey: present, no further details (USDA, 2001)
- New Mexico: present, no further details (Foster et al., 1991)
- New York: present, no further details (Downie and Arnett, 1996; USDA, 2001)
- North Carolina: present, no further details (Downie and Arnett, 1996)
- North Dakota:
- Ohio: present, no further details (Downie and Arnett, 1996; USDA, 2001)
- Oklahoma: present, no further details (Foster et al., 1991; Lingafelter, 1998)
- Pennsylvania: present, no further details (Calvin, 2002)
- Rhode Island: present, no further details (Sikes, 1999)
- South Carolina:
- South Dakota:
- Tennessee: present, no further details (Lingafelter, 1998; USDA, 2001)
- Texas: present, no further details (Lingafelter, 1998; Foster et al., 1991)
- Utah: present, no further details (Lingafelter, 1998)
- Vermont: present, no further details
- Virginia: present, no further details
- Washington: present, no further details (Lingafelter, 1998)
- West Virginia: present, no further details (USDA, 2001)
- Wisconsin: present, no further details. (Anon., 2002)

Canada:
- Ontario: present, no further details (Anon., 2003)
- Quebec: present, no further details (Anon., 2003)

Central America:
- Belize: present, no further details (Kovarik et al., undated)

BIOLOGY AND ECOLOGY

Chaetocnema pulicaria overwinters as an adult near corn fields at the base of plants or in the soil. Adults become active in the spring when soil surface temperatures reach 18-21° C (Poos and Elliott, 1936; Poos, 1955). Adults begin feeding on corn and other available hosts, mate, and lay eggs at the base of plants or just beneath the soil surface. Corn is the preferred host for oviposition, but eggs are also deposited at the base of other grasses (Poos, 1955).

Yellowish-white and semi-translucent, eggs are approximately 0.41mm in length (Poos, 1955) and hatch in approximately 6 days (Poos, 1955; Dill, 1979). Tiny white larvae with brown heads feed on host plant roots and reach full growth in 14 days, completing 3 instars and a prepupal stage (Poos, 1955; Dill, 1979). Pupation requires 5.5 to 7 days at 31° C and 6.5 to 8 days at 25° C; adults are ready to mate approximately 7 days after emergence (Dill, 1979).

Poos (1955) reported the generation time of *C. pulicaria* as 29.7 days, but Dill (1979) indicated higher temperatures result in faster developmental rates. In the laboratory, Dill (1979) found a linear relationship between developmental rate and temperatures ranging from 20° C to 31° C. The life span of *C. pulicaria* is reported to range from 30 days to 1 year (Poos, 1955).

Two or more summer generations of beetles develop and feed on corn plants. The number of generations is dependent on the length of the growing season and seasonal weather conditions. Heichel et al. (1977), Hall (1978), Dill (1979), Adams and Los (1986), Esker (2001), and Cook (2003) have provided overviews of the seasonal dynamics of the corn flea beetle. Heichel et al. (1977), Esker (2001), and Cook (2003) detailed two generations of the corn flea beetle and patterns of subsequent Stewart's wilt in Connecticut, Iowa, and Illinois, respectively. Three generations of the corn flea beetle were reported by Hall (1978) in Missouri.

Newly emerged adult flea beetles feed on the upper and lower surfaces of corn leaves and remove leaf tissue (Poos and Elliott, 1936). High densities and heavy feeding can result in the skeletonization of leaves and death of seedlings. Although lower densities cause little direct injury to plants, *C. pulicaria* serves as the primary overwintering host and vector of *Pantoea stewartii* (syn = *Erwinia stewartii*), the bacterium that causes Stewart's wilt (Rand and Cash, 1933; Poos and Elliott, 1936; Pepper, 1967). This bacterium is found in the gut of the corn flea beetle (Elliott and Poos, 1934), and although the entire gut harbors *P. stewartii*, the hind gut contains significantly more bacteria than either the fore or mid-guts (Dill 1979). The bacterium is not transmitted transovarially (Poos, 1955; Dill, 1979).

Overwintered adults transmit the bacteria that cause early-season Stewart's wilt infection (Elliott and Poos, 1940). Subsequent generations of *C. pulicaria* acquire *P. stewartii* from previously infected corn plants and spread the disease within and among fields. Dill (1979) sampled regurgitated material and feces of corn flea beetles that fed on Stewart's wilt-diseased plants. He reported that sufficient bacteria were present in both types of samples to incite disease, but there were significantly more bacteria in fecal material. From this he concluded the probable major mode of transmission was from fecal contamination rather than regurgitated foregut contents.

Robert (1955), found 10 to 20 percent of *C. pulicaria* that became active in the spring carried *P. stewartii*; estimates reached as high as 75 percent by mid-July. Esker (2001) found that the proportion of corn flea beetles infested with the bacterium fluctuated throughout the season, but increased with each generation in Iowa. Infestation was highest in August, coinciding with peak populations of the second summer generation of the corn flea beetle. Flea beetle infestation also varied throughout each season in Illinois, peaking as high as 60 and 76% in August in 2001 and 2002, respectively (Cook, 2003).

When temperatures begin to decrease in late summer and fall, *C. pulicaria* adults go into dormancy until temperatures rise in the spring. Flea beetle survival is dependent on winter temperatures. When mean winter temperatures are below 0° C, fewer flea beetles survive than when mean winter temperatures are above 0° C (Elliott and Poos, 1940). Hall (1978) sampled overwintering flea beetles in Missouri and estimated that the population experienced 56% mortality (44% survival of original population) over the course of the winter months, with the highest mortality occurring in January.

MEANS OF MOVEMENT AND DISPERSAL

Chaetocnema pulicaria is noted for its remarkable jumping ability due to its enlarged hind femora. It may also walk, fly, or move on wind/air currents. Unpublished work by P.A. Glick found *C. pulicaria* in airplane collections made at altitudes ranging from 6 to 15,240 meters (Poos and Elliott, 1936). Movement by *C. pulicaria* also was shown to occur well above the plant canopy in data from suction traps 7.6 meters in height at locations throughout the state of Illinois (Cook, 2003).

NATURAL ENEMIES

Notes on natural enemies

Dill (1979) observed an unidentified nematode parasitizing female adults and feeding on ovarial tissue. He also found a parthenogenic species of *Microtonus* (Hymenoptera: Braconidae).

ECONOMIC IMPACT

Chaetocnema pulicaria injures corn plants by removing leaf tissue and by transmitting pathogenic bacteria. Feeding by *C. pulicaria* rarely causes economic damage. High densities and heavy feeding may result in the skeletonization of leaves and death of seedlings (Poos, 1955). Lower densities that cause minimal direct injury to plants also are a concern because *C. pulicaria* serves as the primary overwintering host and vector of *P. stewartii*, the organism that causes Stewart's wilt. Depending on the severity of infection, yield and crop quality may be affected (Pepper, 1967).

Corn plants may become infected by *P. stewartii* at any time during plant growth, but plants infected at an early stage are usually affected most severely. The effects of Stewart's wilt on yield are described in detail in the CABI Crop Protection Compendium worksheet for *Pantoea stewartii*. Yield reduction is significantly higher due to early season systemic infection in susceptible and moderately susceptible hybrids (Suparyono and Pataky, 1989; Freeman and Pataky, 2001). When plants were inoculated 3 to 5 weeks after planting, Pataky et al. (1988), observed losses of up to 60 percent in susceptible sweet corn hybrids. Suparyono and Pataky (1989) observed losses ranging from 40 to 100 percent when susceptible and moderately susceptible hybrids were inoculated from the V3 to V5 leaf stages. When infection occurred at later growth stages, (V7 to V9 or later), yield was not reduced as much. Yields of resistant or moderately resistant hybrids were rarely affected when infection occurred after the V3 stage (Suparyono and Pataky, 1989).

ENVIRONMENTAL IMPACT

PHYTOSANITARY RISK

The corn flea beetle is not considered to be a major risk to corn, but the bacterium transmitted by this insect is regulated. The presence of Stewart's wilt may limit the export of corn produced where the flea beetle carries this pathogen.

SYMPTOMS

Feeding injury by *C. pulicaria* on corn leaves appears as fine scratches that are white in color and irregular in shape (Poos and Elliott, 1936). The insect eats through the epidermis of the corn leaf, leaving a transparent line parallel to the leaf veins; this injury is often referred to as a "windowpane" effect. *P. stewartii* enters the plant at these feeding wounds and is carried throughout the vascular system. Stewart's wilt symptoms begin at the sight of the feeding scars.

MORPHOLOGY

Eggs
Eggs of *C. pulicaria* are yellowish-white and semi-translucent. Eggs are laid singularly or in groups up to ten. Dill (1979) reported that 25 eggs averaged 0.208 ± 0.027 mm wide and 0.447 ± 0.031 mm long.

Larvae

Tiny, white larvae with brown heads complete 3 instars and a prepupal stage. Dill (1979) reported the following measurements:

1st instar:	head capsule width = 0.16 (± 0.006) mm
	head capsule length = 0.2 (± 0.014) mm
	body width = 0.201 (± 0.012) mm
	body length = 0.951 (± 0.078) mm
2nd instar:	head capsule width = 0.214 (± 0.006) mm
	head capsule length = 0.287 (± 0.016) mm
	body width = 0.52 (± 0.09) mm
	body length = 3.17 (± 0.27) mm
3rd instar:	head capsule width = 0.282 (± 0.007) mm
	head capsule length = 0.337 (± 0.0310) mm
	body width = 0.73 (± 0.06) mm
	body length = 4.18 (± 0.28) mm
Prepupal:	head capsule width = 0.282 (± 0.007) mm
	head capsule length = 0.337 (± 0.031) mm
	body width = 0.77 (± 0.07) mm
	body length = 2.92 (± 0.1) mm

Larvae reside in the soil, feed on host plant roots, and reach full growth, 1.3 to 2.5 mm long, in 14 days.

Pupae

Pupae are white initially and 1.6 to 4.8 mm long. They darken with age.

Adults

Chaetocnema pulicaria is approximately 1.3 to 3.5 mm long, shiny, and black, with enlarged hind femora that allow it to jump. It is oval, slightly oblong in shape, with faint green or blue-bronze luster. Antennomeres, tibia, and tarsi are reddish or brownish yellow; its pronotum is subopaque, alutaceous, and sparsely punctuate. Elytra have convex intervals each with a row of minute punctures (Downie and Arnett, 1996).

SIMILARITIES TO OTHER SPECIES

Chaetocnema pulicaria is most similar to *Chaetocnema parcepunctata* Crotch. The primary difference is the appearance of the pronotum. The pronotum of the corn flea beetle is subopaque and distinctly alutaceous, whereas *C. parcepunctata* has a shiny pronotum, slightly alutaceous. *Chaetocnema confinis* differs from *C. pulicaria* in that the sides of its pronotum are obliquely truncate at the front angles, whereas the pronotum of *C. pulicaria* is regularly curved, with no angulation. (Downie and Arnett, 1996)

DETECTION AND INSPECTION METHODS

Estimating densities of corn flea beetles is accomplished by direct inspection of corn plants, sweeping plants with a net, and using yellow sticky traps (Heichel et al., 1977; Hall, 1978; Dill, 1979; Adams and Los, 1986; Esker, 2001; Cook, 2003). Adults also have been collected from plants using a vacuum or aspirator (Cook, 2003). Symptoms of *C. pulicaria* injury can be seen on plant leaves; plants infected with Stewart's wilt also indicate the presence of *C. pulicaria* in the field (Adams and Los, 1986; Hoffman et al., 1995; Esker, 2001; Cook, 2003). The presence of beetles on plants is affected by environmental conditions such as temperature, rainfall, and wind; these factors also influence the results of all available sampling methods.

CONTROL

Cultural Control

Chaetocnema pulicaria overwinters in the soil along fencerows and waterways. They are able to develop and reproduce on secondary hosts. Dill (1979) suggested burning grass near areas where corn would be planted the following year in order to control flea beetles. Adjustment of planting dates may reduce the severity of flea beetle feeding and/or incidence of Stewart's wilt. In most regions of the United States, corn planted earliest in the spring is most severely damaged by flea beetles and Stewart's wilt. By moving the planting date, corn seedlings, which are most susceptible, may escape periods when flea beetle populations are high.

Host Plant Resistance

Plants are not resistant to the flea beetle or its feeding. However, some corn hybrids are resistant to Stewart's wilt. Systemic infection is affected by host reaction and growth stage at the time of infection. The bacterium may move systemically through susceptible plants (Braun 1982), but in resistant plants, movement of *P. stewartii* in the vascular system is restricted to within a few centimeters of feeding wounds. However, the exact growth stage at which resistance begins to restrict movement sufficiently to control Stewart's wilt is not known. Resistant hybrids may not prevent systemic infection or main stalk death when flea beetles feed on the leaf tissue close to the growing point before the V2 or V3 stage (Pataky et al., 1995). Michener et al. (2003) hypothesized that natural systemic infection of moderately resistant to resistant hybrids in field trials was caused by flea beetle feeding prior to the V2 stage or during the first two weeks after planting. At later growth stages, Stewart's wilt ratings were lower and resistance was thought to be more effective because infection sites were farther from the growing point and movement of the bacterium was limited. Host plant resistance to Stewart's wilt is summarized more completely in the CABI Crop Protection Compendium worksheet for *Pantoea stewartii*.

Biological Control

No biological control agents have been reared and released, although natural enemies have been found in field conditions.

Chemical Control

Chemical control of *Chaetocnema pulicaria* can be accomplished by seed treatment insecticides, in-furrow or banded insecticide application, or foliar insecticide applications. Control of *C. pulicaria* before *P. stewartii* is transmitted to plants reduces disease incidence and plant death. Populations of *C. pulicaria* were reduced and disease incidence decreased more effectively by in-furrow and banded applications of carbofuran than by foliar insecticide treatments (Heichel et al., 1977). Imidacloprid seed treatment reduced flea beetle densities and reduced the number of feeding scars and disease symptoms per corn leaf in the greenhouse (Munkvold et al., 1996). Imidacloprid, thiamethoxam, and clothianidin seed treatments reduced disease incidence in the field (Pataky et al., 2000). Foliar insecticides have also been shown to reduce Stewart's wilt severity (Ayers et al., 1979)

Disease Forecasting

Winter survival of *C. pulicaria* is dependent on temperatures in December, January, and February. Greater survival is expected after a mild winter as compared to a winter with colder temperatures. Consequently, the potential for Stewart's wilt also is higher. A disease forecasting system is used to predict flea beetle survival and subsequently, the risk of Stewart's wilt for the following crop season (Stevens, 1934; Boewe, 1949; Eastburn, 1996; Ries and Pataky, 1997; Esker, 2001; Cook, 2003)

Field Monitoring/Economic Threshold Levels

Field monitoring of *C. pulicaria* has been done with sticky traps, sweep nets, aspirators, and visual counts on plants. An action threshold used in the northeastern United States is 6 adults per 100 corn plants (Adams and Los, 1986; Hoffman et al., 1995). In Illinois, a threshold of 5 beetles per yellow sticky trap per day has been proposed (Cook, 2003).

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APPENDIX 3: EPPO PEST RISK ASSESSMENT SCHEME

EPPO Pest Risk Assessment Scheme
(Decision-making scheme)

STAGE 1: INITIATION

IDENTIFY PEST

This section examines the identity of the pest to ensure that the assessment is being performed on a real identifiable organism and that the biological and other information used in the assessment is relevant to the organism in question.

1. Is the organism clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank? If yes - **go to 3**.

   YES. *Erwinia stewartii* (Syn. *Pantoea stewartii*)

2. Attempt to redefine the taxonomic entity so that the criteria under 1 are satisfied. Is this possible? If yes - go to 3 If no - go to 22

THE PRA AREA

The PRA area can be a complete country, several countries or part(s) of one or several countries.

3. Clearly define the PRA area. **Go to 4**.

   This PRA is a global PRA and is relevant for any country other than where *E. stewartii* is present. The PRA process is that considered relevant by the countries of EPPO.

EARLIER ANALYSIS

The pest, or a very similar pest, may have been subjected to the PRA process before, nationally or internationally. This may partly or entirely replace the need for a new PRA.

4. Does a relevant earlier PRA exist? If yes - **go to 5**. If no - go to 7.

   A previous version of this PRA is posted on the International Seed Federation seed health web site (www.worldseed.org/seed_health.htm). The previous PRA was prepared in December 2000 using the FAO / IPPC "Guidelines for Pest Risk Analysis" (Publication No. 2, February 1996). This revision follows the FAO / IPPC guidelines for "Pest risk analysis for quarantine pests" adopted in 2001 (ISPM No 11).

5. Is the earlier PRA still entirely valid, or only partly valid (out of date, applied in different circumstances, for a similar but distinct pest)? If partly valid - **go to 6** If not valid - go to 7

This PRA utilizes the more recent international standard ISPM No 11 "Pest risk analysis for quarantine pests".

6. Proceed with the assessment, but compare as much as possible with the earlier assessment.

Go to 7

STAGE 2: PEST RISK ASSESSMENT

SECTION A: PEST CATEGORIZATION (QUALITATIVE CRITERIA OF A QUARANTINE PEST)

GEOGRAPHICAL CRITERIA

This section considers the geographic distribution of the pest in the PRA area.

7. Does the pest occur in the PRA area? If yes - go to 8. If no - **go to 9**.

Several instances of isolated occurrences of Stewart's wilt have been reported throughout the world, including Europe; however, *E. stewartii* has never become established outside of the area to which Stewart's wilt is endemic in the United States.

8. Is the pest of limited distribution in the PRA area?

Note: 'of limited distribution' means that the pest has not reached the limits of its potential range either in the field or in protected conditions; it is not limited to its present distribution by climatic conditions or host-plant distribution. There should be evidence that, without phytosanitary measures, the pest would be capable of additional spread. If yes - go to 18 If no - go to 22

POTENTIAL FOR ESTABLISHMENT

For the pest to establish, it must find a widely distributed host plant in the PRA area (do not consider plants which are accidental/very occasional hosts or recorded only under experimental conditions). If it requires a vector, a suitable species must be present or its native vector must be introduced. The pest must also find environmental conditions suitable for survival, multiplication and spread, either in the field or in protected conditions.

9. Does at least one host plant grow to a substantial extent in the PRA area, in the open, in protected conditions or both? If yes - **go to 10**. If no - go to 22.

Yes. Maize is grown widely in many countries with temperate to tropical environments.

10. Does the pest have to pass part of its life cycle on a host plant other than its major host (i.e. obligate alternate host plant)? If yes - go to 11. If no - **go to 12**.

No. *Erwinia stewartii* appears to be a pathogen that exists predominately in two hosts, *Zea mays* plants and an insect vector, *Chaetocnema pulicaria*.

11. Does the alternate host plant also occur in the same part of the PRA area as the major host plant? If yes - **go to 12**. If no - go to 22.

12. Does the pest require a vector (i.e. is vector transmission the only means of dispersal)? If yes - **go to 13**. If no - go to 14.

Yes. *Erwinia stewartii* is disseminated nearly exclusively by its vector, *Chaetocnema pulicaria* – the corn flea beetle. The vector also serves as the overwintering host of *E. stewartii*.

13. Is the vector (or a similar species which is known or suspected to be a vector) present in the PRA area or likely to be introduced. If in doubt, a separate assessment of the probability of introduction of the vector (in section B1) may be needed. If yes - **go to 14** If no - go to 22.

The vector, *Chaetocnema pulicaria*, and a related species, *C. denticulata* have not been reported in Europe or in the Palearctic region. *Chatecnema pulicaria* occurs only in the Nearctic region. It is unlikely that the vector could be introduced in maize seed.

14. Does the known geographical distribution of the pest include ecoclimatic zones comparable with those of the PRA area? If yes - **go to 18**. If no - go to 15.

Yes. Conditions that are favorable for the growth and development of maize are suitable for *E. stewartii* and Stewart's wilt. Weather affects the survival of the insect vector but does not appear to have substantial impact on *E. stewartii*.

15. Is it probable, nevertheless, that the pest could survive and thrive in a wider ecoclimatic zone that could include the PRA area? If yes - **go to 18**. If no - go to 16.

16. Could the ecoclimatic requirements of the pest be found in protected conditions in the PRA area? If yes - go to 17. If no - go to 22.

17. Is a host plant grown in protected conditions in the PRA area?
If yes - go to 18. If no - go to 22

POTENTIAL ECONOMIC IMPORTANCE

Economic impact principally concerns direct damage to plants but may be considered very broadly, to include also social and environmental aspects. The effect of the presence of the pest on exports from the PRA area should also be allowed for. In deciding whether economically important damage or loss to plants may occur, it is necessary to consider whether climatic and cultural conditions in the PRA area are conducive to damage expression, which is not always the case even if both host and pest survive under these conditions.

Note: when performing a PRA on a pest that is transmitted by a vector, consider also any possible damage that the vector may cause.

18. With specific reference to the host plant(s) which occur(s) in the PRA area, and the parts of those plants which are damaged, does the pest in its present range cause significant damage or loss? If yes - go to 21. If no - **go to 19**.

No. Except for a few, small sporadic outbreaks, economic losses in maize due to *E. stewartii* wilt have been inconsequential in North America for the past 50 years because of adequate levels of host resistance in maize hybrids grown where Stewart's wilt occurs. Economic losses in sweet corn can be significant when susceptible or moderately susceptible hybrids are grown in these areas.

19. Could the pest, nevertheless, cause significant damage or loss in the PRA area, considering ecoclimatic and other factors for damage expression? If yes - **go to 21**. If no - go to 20.

Yes, but minimal. The economic impact of introducing *E. stewartii* into the PRA area will depend on establishment of the bacterium as a result of the presence of the insect vector in the PRA area, the geographic distribution and prevalence of Stewart's wilt within the PRA area, and the level of resistance or susceptibility of the maize cultivars being grown in the PRA area. Losses could occur for a limited time in a limited area where the bacterium and its vector are established if susceptible or moderately susceptible cultivars are grown in that area. Introduction and establishment of *E. stewartii* in the PRA area also could result in phytosanitary regulations being imposed by trading partners. Also, resources would be necessary to screen germplasm and breed maize for resistance in order to control Stewart's wilt.

20. Would the presence of the pest cause other negative economic impacts (social, environmental, loss of export markets)? If yes - **go to 21**. If no - go to 22.

21. This pest could present a risk to the PRA area. **Go to section B**.

Yes, but minimal risk.

22. This pest does not qualify as a quarantine pest for the PRA area and the assessment can stop. However, if this is the first time that the decision-making scheme has directed you to this point, it may be worth returning to the question that led you here and continuing through the scheme in case the remaining questions strongly indicate categorization as a possible quarantine pest. In this latter case, seek a second opinion to decide whether the answers which led you to this point could be given a different reply.

SECTION B: QUANTITATIVE EVALUATION

The second part of the risk assessment process firstly estimates the probability of the pest being introduced into the PRA area (its entry and establishment) and secondly makes an assessment of the likely economic impact if that should happen. From

these two aspects, it should be possible to consider the level of 'pest risk' presented by the pest; this can then be used in the pest risk management phase to decide whether it is necessary to take phytosanitary measures to prevent the introduction of the pest, or if the measures chosen are appropriate for the level of risk. The questions in this section require an evaluation from minimum probability or impact (1) to maximum probability or impact (9). This must be done by an expert who can make an estimate according to the information provided (following the format of the check-list of EPPO and also according to comparison with other pests.

Answer as many of the following questions as possible, insofar as they are relevant to the pest concerned. If you cannot answer a particular question, do not give any score. Note whether this is because of lack of information or because the question is irrelevant to the pest concerned.

1. Probability of introduction

Introduction, as defined by the FAO Glossary of Phytosanitary Terms, is the entry of a pest resulting in its establishment.

ENTRY

List the pathways that the pest could be carried on.

1. **Maize seed. (This primarily relates to seed for sowing not seed for processing that is considered as grain.)**
2. ***Chaetocnema pulicaria,* adult corn flea beetles.**

Note: a pathway can be any form of human activity that could transport the pest from a particular origin, e.g. plants and plant products moving in trade, any other traded commodity, containers and packing, ships, planes, trains, road transport, passengers, mail, etc. Note that similar means of pest transport from different origins can present greatly different probabilities of introduction, depending on the concentration of the pest in the area of origin. The pathways given should be only those already in operation, or proposed.

1.1 How many pathways could the pest be carried on? (few = 1; many =9)

1 - few (maize seed and infected adult insect vectors)

1.2 For each pathway, starting with the most important pathway identified above (i.e. that which carries the greatest trade or which is most likely to act as a means of introduction) and then in descending order of importance, answer questions 1.3 – 1.13. If one of the questions 1.3a, 1.5a, 1.7a or 1.12a is answered by 'no', the pathway could not act as a means of entry for the pest, and the scheme will return directly to this point, omitting later questions. Use expert judgement to decide how many pathways to consider. **Go to 1.3**

For maize seed.

*****1.3a Could the pest be associated with the pathway at origin?

Note: does the pest occur in the area of origin? Is the pest in a life stage which would be associated with commodities, containers, or conveyances? If yes - **go to 1.3b**.

Yes. The seed-borne nature of *Erwinia stewartii* is unequivocal, however, based on rates of plant-to-seed and seed-to-seedling transmission, the probability of transmitting *E. stewartii* is extremely remote when seed is produced on resistant or moderately resistant seed parent plants. Seed transmission is possible at extremely low levels when seed is produced on systemically-infected plants and kernel infection exceeds 10%.

*****1.3b How likely is the pest to be associated with the pathway at origin? (**not likely = 1;** very likely = 9)

1 – Not likely. Plant-to-seed transmission has not been observed when seed parent plants are infected non-systemically. Kernel infection was below 0.025% when seed parent plants were rated as resistant and below 0.2% when seed parent plants were rated as moderately resistant. Plant-to-seed transmission ranged from about 0.2 to 10% for seed produced on highly susceptible, systemically-infected plants.

1.4 Is the concentration of the pest on the pathway at origin likely to be high? (**not likely = 1**; very likely = 9)

1 – Not likely. (See answer to 1.3b)

1.5a Could the pest survive existing cultivation or commercial practices?

Note: these are practices mainly in the country of origin, such as pesticide application, removal of substandard produce, kiln-drying of wood. **If yes - go to 1.5b**. If no - go to 1.2 .

Yes.

1.5b How likely is the pest to survive existing cultivation or commercial practices? **(not likely = 1; very likely = 9)**

5– likely. The pest cannot be eliminated from plantings but current management practices in the field ensure that the overall level of the pathogen in the field crop is very low.

*****1.6 How likely is the pest to survive or remain undetected during existing phytosanitary procedures?

Note: existing phytosanitary measures (e.g. inspection, testing or treatments) are most probably being applied as a protection against other (quarantine) pests; the assessor should bear in mind that such measures could be removed in the future if the other pests were to be re-evaluated. The likelihood of detecting the pest during inspection or testing will depend on a number of factors including: ease of detection of the life stages which are likely to be present. Some stages are more readily detected than others, for example insect adults may be more obvious than eggs; location of the pest on the commodity - surface feeders are more readily detected than internal feeders; symptom expression - many diseases may be latent for long periods, at certain times of the year, or may be without symptoms in some hosts or

cultivars and virulent in others; distinctiveness of symptoms - the symptoms might resemble those of other pests or sources of damage such as mechanical or cold injury; the intensity of the sampling and inspection regimes;distinguishing the pest from similar organisms. (**not likely = 1**; very likely = 9).

1 – Not very likely. The probability of seed harboring *E. stewartii* can be assessed from visual inspections of seed production fields and/or an ELISA-based seed health test.

1.7a Could the pest survive in transit?

Note: consideration should be given to: speed and conditions of transport; vulnerability of the life-stages likely to be transported; whether the life cycle is of sufficient duration to extend beyond time in transit; the number of individuals likely to be associated with a consignment. Interception data can be used to estimate the ability of a pest to survive in transit. If yes - **go to 1.7b** If no - go to 1.2

Yes. *Erwinia stewartii* can be detected from seed for up to 3 years. The bacterium has been recovered from seed for up to 5 months after harvest. Thus, it is highly probable that seed-borne *E. stewartii* would survive during storage and transport from the North America to the PRA area even though the number of viable bacteria per seed probably will decrease during this period.

1.7b How likely is the pest to survive in transit? (**not likely = 1; very likely = 9**)

6 – Likely.

1.8 Is the pest likely to multiply during transit? (**not likely = 1**; very likely = 9)

1 – Not likely. The number of viable bacteria per seed probably will decrease during transit.

1.9 How large is movement along the pathway?

Note: the volume of material being moved. (**not large = 1**; very large = 9)

1 - Not large – Shipments of seed for planting are smaller than shipments of seed (grain) for processing.

1.10 How widely is the commodity to be distributed throughout the PRA area?

Note: the more scattered the destinations, the more likely it is that the pest might find suitable habitats. (not widely = 1; **very widely = 9**)

9 – Very widely.

1.11 How widely spread in time is the arrival of different consignments?

Note: introduction at many different times of the year will increase the probability that entry of the pest will occur at a life stage of the pest or the host suitable for establishment. (**not widely = 1**; very widely = 9)

1 – Not widely. Seed is planting during a month or two. Seed for planting is imported at times when the seed has maximum viability.

1.12a Could the pest transfer from the pathway to a suitable host?

Note: consider innate dispersal mechanisms or the need for vectors, and how close the pathway on arrival is to suitable hosts. If yes **go to 1.12b**. If no go to 1.2.

Yes, the bacterium can transfer from seed to seedlings at an extremely low level (i.e., about 0.02% seed-to-seedling transmission). However, the bacterium can be transferred from infected seedlings to other suitable hosts only if a vector is present.

*****1.12b How likely is the pest to be able to transfer from the pathway to a suitable host? (**not likely = 1**; very likely = 9)

1 – Not likely. Rates of seed-to-seedling transmission are about 0.02%. If less than 1% of seed harbor *E. stewartii*, less than 1 in 500,000 seedlings would be infected. In order for *E. stewartii* to be transferred from infected seedlings to other maize plants, the insect vector, *C. pulicaria*, must feed on the infected seedling, acquire the bacterium and successfully transmit it to another plant. The vector is not known to occur in the PRA area.

1.13 Is the intended use of the commodity (e.g. processing, consumption, planting, disposal of waste) likely to aid introduction?

Note: consider whether the intended use of the commodity would destroy the pest or whether the processing, planting or disposal might be done in the vicinity of suitable hosts. (**not likely = 1; very likely = 9**)

5 Possibly – depending on the rate of seed transmission that is very low and the presence of the vector that is absent from all areas other than the USA.

Note: Questions marked with an asterisk (*) are to be considered as more important than the others in the same section.

For the vector, *Chaetocnema pulicaria*.

*****1.3a Could the pest be associated with the pathway at origin?

Note: does the pest occur in the area of origin? Is the pest in a life stage which would be associated with commodities, containers, or conveyances? If yes - **go to 1.3b**.

Yes, *C. pulicaria,* the vector of *E. stewartii*, occurs in the area of origin; however, the insect is in a life stage that is unlikely to be associated with the commodity as seed for planting, the containers used to transport the

seed or other methods of transport (hitchickers). As many as 80% of flea beetles sampled in August in Iowa harbored *Erwinia stewartii*. At the end of the growing season, *E. stewartii*-infected beetles comprised 4% to 77% of various samples collected from seven locations in Iowa in 1998, 1999, and 2000. However, by the time corn is harvested for seed, flea beetles have migrated to and are collected from other grasses rather than in corn fields. Thus, it is unlikely that the insect is associated with seed. The insect does not feed on seed.

*****1.3b How likely is the pest to be associated with the pathway at origin? (**not likely = 1**; very likely = 9)

1 – Not likely. The number of *Erwinia stewartii*-infected flea beetles varies among years and locations depending on the size of the population of the second summer generation of *C. pulicaria*. During the growing season, the proportion of the population vectoring the bacterium also varies (e.g. from 0.04 to 0.77 in recent samples). About a month prior to harvest as corn leaves senesce, the insect vector migrates from corn fields to feed on and overwinter near grasses with green leaf tissue. The vector is not associated with pathways that involve corn seed.

1.4 Is the concentration of the pest on the pathway at origin likely to be high? (**not likely = 1**; very likely = 9)

1 – Not likely. (See answer to 1.3b)

1.5a Could the pest survive existing cultivation or commercial practices?

Note: these are practices mainly in the country of origin, such as pesticide application, removal of substandard produce, kiln-drying of wood. If yes **- go to 1.5b**. If no - go to 1.2 .

Yes, but not likely.

1.5b How likely is the pest to survive existing cultivation or commercial practices? (**not likely = 1;** very likely = 9)

1 – not likely. *Erwinia stewartii* –infected *C. pulicaria* cannot be eliminated from other grasses near maize fields but current management practices (time of harvest, seed processing.) ensure that it is unlikely to find flea beetles associated with maize seed.

*****1.6 How likely is the pest to survive or remain undetected during existing phytosanitary procedures?

Note: existing phytosanitary measures (e.g. inspection, testing or treatments) are most probably being applied as a protection against other (quarantine) pests; the assessor should bear in mind that such measures could be removed in the future if the other pests were to be re-evaluated. The likelihood of detecting the pest during inspection or testing will depend on a number of factors including: ease of detection of the life stages which are likely to be present. Some stages are more readily

detected than others, for example insect adults may be more obvious than eggs; location of the pest on the commodity - surface feeders are more readily detected than internal feeders; symptom expression - many diseases may be latent for long periods, at certain times of the year, or may be without symptoms in some hosts or cultivars and virulent in others; distinctiveness of symptoms - the symptoms might resemble those of other pests or sources of damage such as mechanical or cold injury; the intensity of the sampling and inspection regimes;distinguishing the pest from similar organisms. (**not likely = 1**; very likely = 9).

1 – Not very likely.

1.7a Could the pest survive in transit?

Note: consideration should be given to: speed and conditions of transport; vulnerability of the life-stages likely to be transported; whether the life cycle is of sufficient duration to extend beyond time in transit; the number of individuals likely to be associated with a consignment. Interception data can be used to estimate the ability of a pest to survive in transit. If yes - **go to 1.7b** If no - go to 1.2

Yes. *Erwinia stewartii* can be recovered from flea beetles the following spring, and could survive in flea beetles during transit, but they are unlikely to be with the commodity at or after harvest.

1.7b How likely is the pest to survive in transit? (**not likely = 1; very likely = 9**)

6 – Likely.

1.8 Is the pest likely to multiply during transit? (**not likely = 1**; very likely = 9)

1 – Not likely. The number of viable bacteria per beetle and the number of beetles probably will decrease during transit.

1.9 How large is movement along the pathway?

Note: the volume of material being moved. (**not large = 1**; very large = 9)

1 - Not large – Shipments of seed for planting are smaller than shipments of seed for processing (i.e., grain).

1.10 How widely is the commodity to be distributed throughout the PRA area?

Note: the more scattered the destinations, the more likely it is that the pest might find suitable habitats. (not widely = 1; **very widely = 9**)

9 – Very widely.

1.11 How widely spread in time is the arrival of different consignments?

Note: introduction at many different times of the year will increase the probability that entry of the pest will occur at a life stage of the pest or the host suitable for establishment. (**not widely = 1**; very widely = 9)

1 – Not widely. Seed is planting during a month or two. Seed for planting is imported at times when the seed has maximum viability.

1.12a Could the pest transfer from the pathway to a suitable host?

Note: consider innate dispersal mechanisms or the need for vectors, and how close the pathway on arrival is to suitable hosts. If yes **go to 1.12b**. If no go to 1.2.

Yes, the bacterium could be transmitted from infected flea beetles to suitable host plants.

*****1.12b How likely is the pest to be able to transfer from the pathway to a suitable host? (**not likely = 1; very likely = 9**)

3 – Possible. The insect vector, *C. pulicaria*, must feed on and transmit the bacterium to seedlings after infected vectors are introduced.

1.13 Is the intended use of the commodity (e.g. processing, consumption, planting, disposal of waste) likely to aid introduction?

Note: consider whether the intended use of the commodity would destroy the pest or whether the processing, planting or disposal might be done in the vicinity of suitable hosts. (**not likely = 1**; very likely = 9)

1 – not likely. Depends the presence of the vector with the seed in consignments, which is highly unlikely.

Note: Questions marked with an asterisk (*) are to be considered as more important than the others in the same section.

ESTABLISHMENT

1.14 How many host-plant species are present in the PRA area? (**one or very few = 1**; many = 9)

1 - Very few. All types of maize (*Zea mays)* are hosts of *E. stewartii*. The bacterium also has been isolated infrequently from teosinte (*Euchlaena mexicana*) and eastern gama grass (*Tripsacum dactyloides*). Plants of many other genera have been inoculated successfully with *E. stewartii* in the greenhouse or may serve as weak secondary hosts, but few are host under natural conditions.

1.15 How extensive are the host plants in the PRA area? (rare = 1; **widespread = 9**)

9 – Widespread. More than half of the world's maize crop (about 600 million metric tons in 1997) is produced outside of the US, Canada and Mexico. Approximately 18% of the crop is produced China. About 15% is produced in central and South America. About 13% is produced in the Paelarctic region, of which nearly half is produced in France, Romania, and Italy.

1.16 If an alternate host is needed to complete the life cycle, how extensive are such host plants in the PRA area? (rare = 1; widespread = 9) .

Not needed.

*****1.17 If a vector is needed for dispersal, how likely is the pest to become associated with a suitable vector?

Note: is the vector present in the PRA area, could it be introduced or could another vector be found? (**not likely = 1**; very likely = 9)

1. Not likely. The vector, C*. pulicaria*, is required for dispersal and winter survival. The vector is not present in the PRA area. Other efficient vectors have not been identified. Circumstantial evidence suggests that other vectors are not present outside of the US, i.e., infrequent occurrences of Stewart's wilt have been reported throughout the world but *E. stewartii* has never become established and persistent epidemics of Stewart's wilt have not occurred in those areas probably due to the absence of *C. pulicaria*. It is unlikely for the vector to be introduced.

1.18 (Answer this question only if protected cultivation is important in the PRA area.) Has the pest been recorded on crops in protected conditions elsewhere? (no = 1; often = 9)

1.19 How likely are wild plants (i.e. plants not under cultivation, including weeds, volunteer plants, feral plants) to be significant in dispersal or maintenance of populations? (**not likely = 1**; very likely = 9)

1 – Not likely. The insect vector, *C. pulicaria* is the primary overwintering host for *E. stewartii*. The vector is currently absent from the PRA area.

1.20 How similar are the climatic conditions that would affect pest establishment in the PRA area and in the area of origin?

Note: the climatic conditions in the PRA area to be considered may include those in protected cultivation. (not similar = 1; **very similar = 9**)

9 – Can be very similar. Conditions that are favorable for the growth and development of maize are suitable for *E. stewartii*. However, Stewart's wilt is rarely epidemic in the southern-most portions of the United States which may indicate that prolonged periods of warm temperatures adversely affect the insect vector, *C. pulicaria*, or the bacterium. Winter weather also affects the survival of the insect vector. The vector is unable to survive prolonged periods with temperatures below freezing.

1.21 How similar are other abiotic factors in the PRA area and in the area of origin?

Note: the major abiotic factor to be considered is soil type; others are, for example, environmental pollution, topography/orography. (not similar = 1; very similar = 9)

Not applicable.

1.22 How likely is the pest to have competition from existing species in the PRA area for its ecological niche? (very likely = 1; not likely = 9)

Not applicable.

1.23 How likely is establishment to be prevented by natural enemies already present in the PRA area? (very likely = 1; not likely = 9)

Not applicable.

1.24 If there are differences in the crop environment in the PRA area from that in the area of origin, are they likely to aid establishment?

Note: factors that should be considered include time of year that the crop is grown, soil preparation, method of planting, irrigation, whether grown under protected conditions, surrounding crops, management during the growing season, time of harvest, method of harvest, etc. (**not likely = 1**; very likely = 9)

1 – Not likely.

1.25 Are the control measures which are already used against other pests during the growing of the crop likely to prevent establishment of the pest? (**very likely = 1; not likely = 9**)

3 – Somewhat likely. If maize cultivars grown in the PRA are derived largely from US Corn Belt field corn pedigrees (i.e., Iowa Stiff Stalk Synthetic backgrounds), resistance to Stewart's wilt will be present in the crop. Soil, seed and foliar applied insecticides also will lower the probability of an insect vector acquiring *E. stewartii*.

*****1.26 Is the reproductive strategy of the pest and duration of life cycle likely to aid establishment?

Note: consider characteristics which would enable the pest to reproduce effectively in a new environment, such as parthenogenesis/self-crossing, duration of the life cycle, number of generations per year, resting stage, etc. (**not likely = 1**; very likely = 9)

1 – Not likely. *Erwinia stewartii* is a non-motile, non-spore-forming, facultative anaerobic bacterium that is disseminated within the growing season only by an insect vector, *C. pulicaria.* It survives from season to season in the insect vector. If the vector is not present, the bacterium will not be spread within the growing season or survive between growing seasons.

*****1.27 How likely are relatively low populations of the pest to become established? (**not likely = 1**; very likely = 9)

1- Not very likely. In a seed lot with less than 1% kernel infection, *E. stewartii* probably will not be transmitted successfully to seedlings, but

if it is, it would be reasonable to find less than 2 infected seedlings per 1 million plants. *Erwinia stewartii* will not become established or spread in the PRA area unless a vector feeds on these infected seedlings, acquires the bacterium, and transmits *E. stewartii* to other maize plants. There is no evidence that any insect other than *C. pulicaria* is capable of efficiently vectoring *E. stewartii*. *Chaetocnema pulicaria* has not been reported from the PRA area.

*****1.28 How probable is it that the pest could be eradicated from the PRA area ? (**very likely = 1**; **not likely = 9**)

1 – Very likely. If the insect vector is not present, *E. stewartii* introduced to the PRA area would be eradicated naturally following one growing season. Apparently, this has occurred previously based on infrequent reports of Stewart's wilt from various regions of the world.

or

9 – Not likely. If the vector is present, eradication is unlikely unless the vector is eradicated.

1.29 How genetically adaptable is the pest?

Note: is the species polymorphic, with, for example, subspecies, pathotypes? Is it known to have a high mutation rate? This genotypic (and phenotypic) variability facilitates the pest's ability to withstand environmental fluctuations, to adapt to a wider range of habitats, to develop pesticide resistance and to overcome host resistance. (**not adaptable = 1**; very adaptable = 9)

1 – Not adaptable. Unlike many other species of *Erwinia* (e.g., *E. herbicola*), *E. stewartii* appears to be a relatively homogeneous organism. The pathogen appears to have been streamlined to exist in two specific hosts (i.e., *Zea mays* and *Chaetocnema pulicaria*).

1.30 How often has the pest been introduced into new areas outside its original range?

Note: if this has happened even once before, it is important proof that the pest has the ability to pass through most of the steps in this section (i.e. association with the pathway at origin, survival in transit, transfer to the host at arrival and successful establishment). If it has occurred often, it suggests an aptitude for transfer and establishment. (**never = 1; often = 9**)

3 – Infrequently. Stewart's wilt has been reported infrequently from various regions of the world, presumably due to transmission of *E. stewartii* through seed. Seed transmission has never been confirmed in these occurrences and it is not known if phytosanitary regulations to prevent the introduction of *E. stewartii* were followed in these instances. In each case of documented reports of Stewart's wilt, *E. stewartii* failed to become established. The disease has not become endemic to any region of the world other than North America where the vector and pathogen occur simultaneously.

2. ECONOMIC IMPACT ASSESSMENT

Identify the potential hosts in the PRA area, noting whether wild or cultivated, field or glasshouse. Consider these in answering the following questions. When performing a PRA on a pest that is transmitted by a vector, consider also any possible damage that the vector may cause.

According to the pest and host(s) concerned, it may be appropriate to consider all hosts together in answering the questions once, or else to answer the questions separately for specific hosts.

Note that, for most pest/crop/area combinations, precise economic evaluations are lacking. In this section, therefore, expert judgement is asked to provide an evaluation of the likely scale of impact. Both long-term and short-term effects should be considered for all aspects of economic impact.

2.1 * How important is economic loss caused by the pest within its existing geographic range? (little importance = 1; very important = 9)

2 – Very little. Economic losses in maize due to Stewart's wilt have been inconsequential in North America for the past 50 years except for a few, small sporadic outbreaks. In sweet corn, economic losses can be significant when susceptible or moderately susceptible hybrids are grown.

2.2 How important is environmental damage caused by the pest within its existing geographic range?

Note: environmental damage may be impact on ecosystem health, such as effects on endangered/threatened species, keystone species or biodiversity. (**little importance = 1**; very important = 9)

1- Little importance. No known environmental damage.

2.3 How important is social damage caused by the pest within its existing geographic range?

Note: social effects could be, for example, damaging the livelihood of a proportion of the human population, or changing the habits of a proportion of the population (e.g. limiting the supply of a socially important food). (**little importance = 1**; very important = 9)

1- Little importance. No known social damage.

2.4 How extensive is the part of the PRA area likely to suffer damage from the pest?

Note: the part of the PRA area likely to suffer damage is the endangered area, which can be defined ecoclimatically, geographically, by crop or by production system (e.g. protected cultivation).(very limited = 1; whole PRA area = 9)

6 – PRA area with climatic conditions similar to the Corn Belt of the United States. Some PRA areas where the maize may be introduced for planting

may be hotter than the US Corn Belt and there is no information on the disease under these circumstances, although Stewart's wilt does not occur frequently in the southern portion of the US where temperatures are warmer than the Corn Belt.

Spread potential is an important element in determining how fast economic impact is expressed and how readily a pest can be contained.

2.5 How rapidly is the pest liable to spread in the PRA area by natural means? (very slowly = 1; very rapidly = 9)

3 – Moderately slowly. Introduction and establishment of *E. stewartii* is most likely to occur as an isolated event rather than at many points. Spread of the disease from a focus of introduction would coincide with the dispersal of *C. pulicaria*. Although the geographic distribution of *C. pulicaria* may increase about 600 km each season, the range of occurrence of Stewart's wilt could recede if the vector is unable to survive between growing seasons due to winter temperatures below 0 C. Damage due to Stewart's wilt is associated nearly entirely with infection that results from the overwintering generation of the vector.

2.6 How rapidly is the pest liable to spread in the PRA area by human assistance? (very slowly = 1; very rapidly = 9)

Not applicable. Human assistance is not pertinent.

2.7 How likely is it that the spread of the pest could be contained within the PRA area?

Note: consider the biological characteristics of the pest that might allow it to be contained in part of the PRA area; consider the practicality and costs of possible containment measures. (very likely = 1; not likely = 9)

5 – Moderately possible. Seed treatment insecticides (imidacloprid, thiomethoxam, and clothianidin) control corn flea beetles and reduce the incidence of Stewart's wilt infected seedlings by about 75%. Seed, soil, and foliar applications of insecticides also could limit the spread of Stewart's wilt by controlling the vector.

*****2.8 Considering the ecological conditions in the PRA area, how serious is the direct effect of the pest on crop yield and/or quality likely to be?

Note: the ecological conditions in the PRA area may be adequate for pest survival but may not be suitable for significant damage on the host plant(s). Consider also effects on non-commercial crops, e.g. private gardens, amenity plantings. (not serious = 1; very serious = 9)

3 – Minor seriousness. Stewart's wilt has little economic impact on maize grown in the United States where the disease is endemic except for situations in which extremely susceptible hybrids are grown for specialty purposes (e.g., processing sweet corn). The disease probably would have

a similar minimal impact on maize in the PRA area. During the initial growing season of introduction and in the subsequent two or three growing seasons, the disease probably would occur in a limited area. The economic impact may be significant to producers in that area if the cultivars being grown are susceptible to Stewart's wilt, but the overall impact in the PRA area would be minimal because the disease would not be widespread. By the time Stewart's wilt becomes widespread, cultivars with moderate to high levels of resistance should have been identified and could be grown in the PRA area.

2.9 How likely is the pest to have a significant effect on producer profits due to changes in production costs, yields, etc., in the PRA area? (not likely = 1; very likely = 9)

2 – Not very likely. Stewart's wilt has minimal impact on producer profits and production costs in the US where it is endemic. For most producers, the costs of control are minimal or non-existent since the costs of seed of resistant and susceptible hybrids do not differ. Therefore, there is no increase in production costs when producers employ the most effective and efficient method to control Stewart's wilt. When producers choose to grow susceptible hybrids (usually sweet corn producers), an additional production cost of about US$3 per hectare results from the use of seed treatment insecticides to control corn flea beetles.

2.10 How likely is the pest to have a significant effect on consumer demand in the PRA area?

Note: consumer demand could be affected by loss in quality and/or increased prices. (**not likely = 1**; very likely = 9)

1- Not likely. Stewart's wilt has never impacted the demand or prices paid for maize grain in the United States where the disease is endemic.

2.11 How likely is the presence of the pest in the PRA area to affect export markets?

Note: consider the extent of any phytosanitary measures likely to be imposed by trading partners.(not likely = 1; very likely = 9)

6 – Moderately likely. Introduction and establishment of *E. stewartii* could result in phytosanitary regulations imposed by trading partners. Seed produced in the PRA area, and in some instances grain produced in the PRA, area would need to meet phytosanitary requirements.

2.12 How important would other costs resulting from introduction be?

Note: costs to the government, such as research, advice, publicity, certification schemes; costs (or benefits) to the crop protection industry. (little importance = 1; very important = 9)

3 - Minor importance. Additional resources would be necessary to screen germplasm and breed maize for resistance in order to control Stewart's

wilt. Since highly adapted sources of resistance have been identified and the inheritance of resistance is known (using both classical and molecular genetics), identifying and developing resistant cultivars should be a relatively easy process. In the United States, these costs have been borne primarily by the seed industry without increasing the cost of seed for maize hybrids with high levels of Stewart's wilt resistance. This research has been incorporated in maize breeding programs as a routine part of developing new hybrids.

2.13 How important is the environmental damage likely to be in the PRA area? (**little importance = 1**; very important = 9)

1 – Little importance. No environmental damage.

2.14 How important is the social damage likely to be in the PRA area? (**little importance = 1**; very important = 9)

1 – Little importance. No social damage.

2.15 How probable is it that natural enemies, already present in the PRA area, will affect populations of the pest if introduced? (very likely = 1; not likely = 9)

Not applicable.

*****2.16 How easily can the pest be controlled?

Note: difficulty of control can result from such factors as lack of effective plant protection products against this pest, occurrence of the pest in natural habitats or amenity land, simultaneous presence of more than one stage in the life cycle, absence of resistant cultivars).(**easily = 1**; with difficulty = 9)

1 – Easily. Adequate levels of simply-inherited, dominant resistance to Stewart's wilt are prevalent in field maize hybrids grown in the Corn Belt of the United States. Stewart's wilt has no economic impact on resistant hybrids. Seed treatment insecticides, and to a lesser extent, soil and foliar applied insecticides also provide relatively good levels of control of this disease.

2.17 How likely are control measures to disrupt existing biological or integrated systems for control of other pests? (not likely = 1; very likely = 9)

2 - Not very likely. Maize cultivars with Stewart's wilt resistance must also have resistance to other pathogens and pests that are prevalent in the PRA area. Resistance is highly compatible with IPM systems.

2.18 How likely are control measures to have other undesirable side-effects (for example on human health or the environment)? (**not likely = 1**; very likely = 9)

1 - Not likely.

2.19 Is the pest likely to develop resistance to plant protection products? (**not likely = 1**; very likely = 9)

1 – Not very likely. In the past 70 years since resistance to Stewart's wilt was first reported, there have been no known instances of isolates of *E. stewartii* that are capable of overcoming that resistance, i.e., there appears to be no race specificity among populations of *E. stewartii*.

3. FINAL EVALUATION

At the end of the procedure, the assessor will have at his disposal:

(1) one or several sets of replies (1-to-9 scores) to questions 1.1-1.13, for one or several pathways (if no pathways have been retained, the probability of introduction will be zero); (2) one set of replies (1-to-9 scores) to questions 1.14-1.30; (3) one or several sets of replies (1-to-9 scores) to questions 2.1-2.19, for single, grouped or separate hosts (according to the manner of answering which has been chosen).

The assessor should first consider the quality and quantity of the information used to answer the questions, and give an overall judgement of how reliable the pest risk assessment can be considered. If other relevant information is available that has not been considered, this should be noted. By the means of his choice, the assessor should attempt to make a separate estimate of the probability of introduction of the pest and its probable level of economic impact. As explained in the introduction, these estimates cannot, on the basis of the procedure used in the scheme, be expressed in absolute units. The numerical scores may be combined, weighted and averagedin appropriate ways that may enable the assessor who uses them consistently to make useful comparisons between pests, pathways and hosts. No particular mode of calculation is specifically recommended by EPPO. Certain questions have been identified as more important than others, and the assessor should take due account of this. The assessor may then combine his estimates of probability of introduction and probable economic impact to formulate a single estimate of pest risk. This may usefully be compared with one or several reference levels of risk to decide whether the pest should be considered to be a quarantine pest, so that phytosanitary measures should be taken against it. Finally, the scores given in answer to the different sections (particularly that on pathways) may be used again in pest risk management.

APPENDIX 4. ELISA-BASED SEED HEALTH TEST FOR *ERWINIA STEWARTII* DEVELOPED AT IOWA STATE UNIVERSITY

SEED HEALTH TEST FOR *ERWINIA STEWARTII*

ELISA KIT PROCEDURE WITH AGDIA PATHOSCREEN-ES

Extraction of the bacterium

1. Place 4 replicates of 100 corn kernels, per sample, into 100 ml. of General Sample Extraction Buffer (described below), and soak overnight at room temperature.
2. Grind seeds to fine particles in a homogenizer and pour slurry through two layers of cheesecloth.
3. Disinfect the homogenizer between replicates and samples by rinsing with distilled water, followed by a rinse with a laboratory or hospital standard detergent, then a final rinse with deionized water.

Preparation of Buffers

A. PBS-TWEEN Buffer/Wash

1. Empty contents of the PBS-TWEEN satchel into a 1 liter container. Add distilled water to make one liter PBS-TWEEN (buffer/wash).

B. General Extraction Buffer

1. Add to 1 liter distilled water provided dry EXTRACTION Buffer.

   Mix the buffer powder with a small amount of distilled water to make a slurry, then gradually add more distilled water to the final volume. Also add 20 ml TWEEN-20 per liter of EXTRACTION Buffer.
2. Mix well.
3. Store at 4°C.

Preparation of Erwinia stewartii controls

1. Add 2 ml of the general sample extraction buffer to each of the provided vials, positive (+) ES and negative (-) ES controls, and mix well. Freeze remaining controls in smaller single use containers for later use.

Loading ELISA Plates

1. Add 100 µl of the sample filtrate of each replicate to one well on a pre-coated ELISA plate for *Erwinia stewartii*. Change micropipette tips between each replicate.
2. Add 100 µl of positive (+) ES and negative (-) ES controls to 3 wells each.
3. Incubate for 2 hr at room temperature or overnight at 4°C. Always incubate in a humid container.
4. Wash the plates 6 times with PBS-TWEEN (buffer/wash). Tap plate gently after final rinse to remove remaining wash buffer.

Addition of enzyme conjugate

1. Dispense 100 µl of prepared enzyme conjugate (as prepared below) into each well and into two blank control wells.

*Preparation of enzyme conjugate**

A. To make enzyme conjugate diluent mix 4 parts of PBS-TWEEN (buffer/wash) to one part of MRS component (provided).
B. Add enzyme conjugates A and B at the dilution of 1/100.

*Note:The volume of enzyme conjugate prepared is dependent on the number of test wells that are being used. On the basis of 100 µl needed for each test well, a helpful way of estimating the amount of diluent needed is to prepare 1 ml for each 8-well column of test wells. 10 µl of enzyme conjugates A and B each, at a dilution of 1/100, would be added to 1 ml of enzyme conjugate diluent.

2. Incubate 2 hr at room temperature.
3. Wash 6 times with PBS -TWEEN.

<u>Addition of substrate</u>

1. Dispense 100 µl of substrate (as prepared below) per well.

Substrate preparation

A. Add 1 OPD stick to 10 ml of OPD buffer solution. The paper end of the stick is submerged into room temperature OPD buffer solution for 2 min.
B. Swirl the stick slightly before removing from the solution. Always prepare OPD buffer solution immediately before use.

2. Incubate for 20 minutes at room temperature in the dark, or until the positive control is showing significant color.
3. Add 50 µl (one drop) 3M Sulfuric Acid.

<u>Evaluating ELISA Plates</u>

1. Evaluate visually, or measure on an ELISA plate reader at 490 nm. The threshold for a positive reaction on the plate reader should be > 3X that of the negative control.

APPENDIX 5. SAMPLING SEED FOR *ERWINIA STEWARTII* USING ELISA

Probability of sampling infected kernels and estimates of the proportion of infected kernels.

An ELISA-based seed health test for *Erwinia stewartii* was developed at Iowa State University (Appendix 4). Using this assay, the probability of detecting *E. stewartii* can be estimated from the binomial distribution based on the following:

$$P_{(S = 0)} = 1 - q^n$$

where, S = total number of infected kernels sampled, q = proportion of non-infected kernels, n = number of kernels assayed, and $P_{(S = 0)}$ = the probability of not sampling an infected kernel,. This probability assumes that the assay detects all infected kernels and that kernels are selected randomly.

This test was accepted by the National Seed Health System. The ELISA-based seed health test assays four 100-kernel samples to determine if seed are infected with the *E. stewartii*. If 1% of the kernels in a seed lot are infected (i.e., p = 0.01 and q = 0.99), there is a 98.2% chance that an infected kernel will be sampled in the assay:

$$P_{(S = 0)} = 1 - .99^{400} = 0.982$$

If eight 100-kernel samples are assayed, there is a 99.97% chance that an infected kernel will be sampled if 1% of the seed are infected:

$$P_{(S = 0)} = 1 - .99^{800} = 0.9997$$

The proportion of seed infected with *Erwinia stewartii* can be estimated from an equation for group samples:

$$p = 1 - (1 - Q)1/n$$

where, *p* = the estimated proportion of infected seed, Q = proportion of positive samples, and n = number of kernels per sample.

Using the ELISA-based seed health test with four 100-kernel samples, we would estimate that 0.29% of the kernels are infected if one of four 100-kernel samples is positive:

$$p = 1 - (1 – 0.25)1/100 = 0.00287$$

The estimate is 0.7% infected kernels if two 100-kernel samples are positive and 1.4% infected kernels if three 100-kernel samples are positive:

$$p = 1 - (1 – 0.50)1/100 = 0.00691$$

$$p = 1 - (1 – 0.75)1/100 = 0.01377$$

Sampling procedures using a 0.5% threshold of estimated *E. stewartii*-infected kernels from the ELISA-based seed health test.

An 0.5% threshold for estimates of kernel infection from the ELISA-based seed health test ensures that seed lots have less than 1% *E. stewartii*-infected kernels which represents a reasonable risk for seed-borne *E. stewartii* considering rates of seed-to-seedling transmission

and the absence of an insect vector outside of North America. Seed-to-seedling transmission has not been observed in seed lots with less than 10% kernel infection except for a single instance in which the estimate of kernel infection was 9% ± 3.3%. Rates of seed-to-seedling transmission of *E. stewartii* are about 0.02% for seed produced on naturally-infected plants (i.e., plants that were not mechanically inoculated with *E. stewartii*). Thus, a threshold of 0.5% would produce less than 1 in 1,000,000 infected seedlings. Since the insect vector, *Chaetocnema pulicaria* has not been reported outside of the Nearctic region, there is little likelihood that *E. stewartii* could become established.

Using the procedure approved by the National Seed Health System, there is a 98.2% probability of sampling infected kernels in four 100-kernel samples if 1% of the seed is infected. Thus, if zero or one of the four samples is positive, there is a reasonable probability that the level of kernel infection is below 1%, and seed should be accepted. Any seed with two or more positive samples could be re-tested and accepted if the estimated percentage of infected kernels is below 0.5% (see Table 1). Seed with fewer than 39.5% positive 100-kernel samples (i.e., $Q \leq 0.394$) meet the 0.5% threshold (Fig 1).

Table 1. Percentage of *Erwinia stewartii*-infected kernels estimated from for four, eight, twelve, and sixteen 100-kernel samples of maize seed assayed with an ELISA–based seed health test

Number of 100-kernel samples	Number of positive samples	Proportion of positive samples (Q)	Estimated % of infected kernels	Recommendation
4	0	0	<0.29	Accept
4	1	0.25	0.29	Accept
4	2	0.5	0.69	Retest or Reject
4	3	0.75	1.38	Retest or Reject
4	4	1.0	>1.38	Retest or Reject
8	0	0	<0/13	Accept
8	1	0.125	0.13	Accept
8	2	0.25	0.29	Accept
8	3	0.375	0.47	Accept
8	4	0.5	0.69	Retest or Reject[a]
8	5	0.625	0.98	Retest or Reject[a]
8	6	0.75	1.38	Retest or Reject[a]
8	7	0.875	2.06	Retest or Reject[a]
8	8	1.0	>2.06	Retest or Reject[a]
12	0	0	<0.09	Accept
12	1	0.083	0/09	Accept
12	2	0.167	0/18	Accept
12	3	0.25	0.29	Accept
12	4	0.333	0.4	Accept
12	5	0.417	0.54	Retest or Reject[a]
12	6	0.5	0.69	Retest or Reject[a]
12	7	0.583	0.87	Retest or Reject[a]
12	8	0.667	1.09	Retest or Reject[a]
12	9	0.75	1.38	Retest or Reject[a]
12	10	0.833	1.77%	Retest or Reject[a]
12	11	0.917	2.46%	Retest or Reject[a]
12	12	1.0	> 2.46%	Retest or Reject[a]
16	0	0	< 0.06%	Accept
16	1	0.0625	0.06%	Accept
16	2	0.125	0.13%	Accept
16	3	0.1875	0.21%	Accept
16	4	0.25	0.29%	Accept
16	5	0.3125	0.37%	Accept
16	6	0.375	0.47%	Accept
16	7	0.4375	0.57%	Retest or Reject[a]
16	8	0.5	0.69%	Retest or Reject[a]

16	9	0.5625	0.82%	Retest or Reject[a]
16	10	0.625	0.98%	Retest or Reject[a]
16	11	0.6875	1.16%	Retest or Reject[a]
16	12	0.75	1.38%	Retest or Reject[a]
16	13	0.8125	1.66%	Retest or Reject[a]
16	14	0.875	2.06%	Retest or Reject[a]
16	15	0.9375	2.73%	Retest or Reject[a]
16	16	1.0	> 2.73%	Retest or Reject[a]

[a] An unlimited number of 100-kernel samples can be tested. Based on all samples tested, seed lots with an estimated percentage of infected kernels below 0.5% should be accepted.

Fig. 1. Relationship between proportion of positive 100-kernel samples and estimated percentage of kernels infected with *Erwinia stewartii*. If the percentage of positive 100-kernel samples is less than 39.5% (i.e., proportion, $Q \leq 0.394$) the estimated percentage of *E. stewartii*-infected kernels is below 0.5%.