BACTERIAL TOP AND STALK ROT OF CORN: INFLUENCE OF TEMPERATURE AND HUMIDITY ON DISEASE DEVELOPMENT, TOLERANCE AMONG CORN INBREDS AND HYBRIDS, AND OVERWINTER SURVIVAL OF THE INCITANT BACTERIA

by

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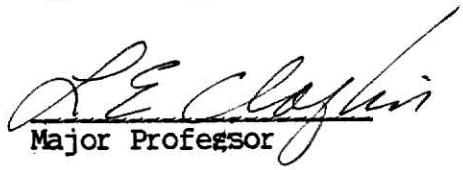

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INTRODUCTION

A destructive bacterial top and stalk rot (TSR) disease of corn (*Zea mays* L.) has been observed in Kansas (Claflin, personal communication) nearly every year and is most common in sweet corn. The causal organism was not positively identified, but similar symptoms to those described by Hoppe and Kelman (25) were observed and diagnosed as TSR. Disintegration of stalk tissue accompanied by lodging and leaf necrosis is the most typical symptom of TSR.

The causal agent of TSR was first described (58) as *Erwinia carotovora* f. sp. *zeae*. Subsequent research (23,64,84) also revealed that the primary incitant of TSR was *E. carotovora* f. sp. *zeae*. Others (54,86) reported that their corn isolates were slightly different from *E. carotovora* f. sp. *zeae* and designated them as *E. carotovora* (Jones) Holland. Dye (16) considered the *Erwinia* spp. isolates from corn to be synonomous with the "chrysanthemi" group and included *E. carotovora* var. *zeae* under *E. carotovora* var. *chrysanthemi*. Hoppe and Kelman (25) also concluded the TSR pathogen showed closer affinity to *E. chrysanthemi* than to *E. carotovora*. Later, others (4,38,82) designated the TSR causal organism as *E. chrysanthemi* (Sabet) var. *Zeae*.

TSR has been reported in Israel (83), Egypt (58,63), Southern Rhodesia (60), Union of South Africa (41,48), France (67), Italy (38), Greece (85), U.S.S.R. (56), India (23,54), Australia (37), and Columbia (82). Recently TSR was reported in Brazil (55). In the U.S., the disease has been reported in Wisconsin (25) and North Carolina (27).

TSR is most prevalent and destructive in areas with high rainfall, on land subject to flooding and/or when the crop is irrigated with

overhead sprinklers. The disease is also favored by high temperature (30-35C) and poor air circulation (73). Reifschneider and Lopes (55) reported that 37% of the sweet corn plants growing in fields furrow-irrigated were infected with TSR and 24% of these plants subsequently died. In North Carolina (27) and Wisconsin (25), TSR has been observed following overhead irrigation with water obtained from a nearby stream and that natural infection is accomplished by entry of the bacteria into the whorl of the corn plant. TSR has also been observed in fields that have not been irrigated in which case it could be insect transmitted (79). Under experimental conditions (79), 25% of maize borer (Chilo partellus Swinhoe) larvae were capable of vectoring the causal agent.

In the U.S., TSR incidence was reduced by adding chlorine to irrigation water (80). Antibiotics has been successful in a few cases but their use is restricted in use (52,59,77). The use of resistant cultivars, aside from good cultural practices, is the only logical control of TSR disease of corn.

Considerable progress has been made in understanding the ecology of soft rot Erwinia spp., in particular, Erwinia carotovora pv. carotovora and Erwinia carotovora pv. atroseptica causing backleg of potato. This was achieved by various methods developed which are highly sensitive in detecting and enumerating limited bacterial populations. One method is by various serological procedures (50). Little is known on the overwintering survival of the bacteria causing TSR. In common with many other plant pathogenic bacteria and Erwinia spp., the bacteria are thought to overwinter in plant residues.

The primary objective of this study was to screen corn inbreds and hybrids for resistance to the TSR bacterial organisms by using a whorl

inoculation method at three different growth stages. The pseudostem injection technique (83) of inoculation was also utilized on corn entries during the 1983 growing season.

The second major objective was to study the overwintering survival of the causal incitants using Fluorescent Antibody Staining (FAS) procedures.

The final objective was to study the influence of different temperatures and relative humidities on the development of TSR under controlled environments.

LITERATURE REVIEW

A. Methods of inoculation

Numerous inoculation procedures have been developed to differentiate various levels of resistance in corn germplasm to TSR, including the use of a hypodermic syringe (53), puncturing the stalk with a screwdriver (65), whorl inoculation (22), cutting the stalks (78), and pseudostem injection (83). Of these, the whorl inoculation and the pseudostem injection techniques provided uniform and reproducible results.

In the whorl inoculation method, inoculum was prepared by suspending the bacteria in 0.7% Tween 40 and then pipetting 1-5 ml of the suspension into the whorl of corn plants. The effect of Tween 40 appears to reduce surface tension and facilitate movement of inoculum downward between leaf blades into the whorl. This technique demonstrates the probable means of bacterial entry into plants under field conditions.

With the pseudostem injection, Victoria (83) was able to detect, with different inoculum concentrations, differences in tolerance between corn lines. The pseudostem technique requires a great amount of time and effort in injecting plants and the technique does not demonstrate the probable means of bacterial entry into the corn plants under natural field conditions.

B. Etiology

1. Factors that influence disease development

Temperature, rainfall and humidity have a marked influence on infection and development of TSR in corn. *E. carotovora* and *E.*

chrysanthemi differ with respect to optimal temperature for growth resulting in an affect on their geographical distribution. Temperature is a major factor in the ability of different Erwinia spp. to infect their hosts (50). Strains of E. carotovora grow at 37 C but most do not grow at 39 C; growth of E. chrysanthemi is relatively inhibited at 39 C. The optimum temperature for growth of E. carotovora f. sp. zeae is 30 C to 35 C, minimum at 5 C and the maximum, at 40 C (23).

Moisture-temperature requirements for infection of corn seedlings with E. carotovora f. sp. zeae varied with the soil texture (61). Optimum conditions for infection were 35C and 70% moisture in light loam soil. In lighter soils, infection occurred at lower temperature and higher moisture levels.

2. Symptoms

Bacterial infections generally result in maceration and rotting of parenchymatous tissue of the affected organ. Tips of the uppermost leaves wilt, followed by a slimy soft rot at the base of the whorl. The decay spreads rapidly downward until the plants collapse. Pith tissue is often reduced to a slimy mass and frequently emits an unpleasant odor (8). Ludbrook (37) observed that ear and shank tissue were also infected. The apical growth was arrested and no tassel or grain development occurred when the disease appeared a month before tasseling.

C. Resistance of corn to top and stalk rot bacteria

Corn germplasm resistance to TSR involves many physiological, morphological, and perhaps functional characteristics, which are in turn influenced by numerous factors. There is no known inbred or hybrid of corn immune to TSR (8). Other research (83) showed no homogenous host

response or immune reaction among 35 corn cultivars.

Very few reports have been published concerning sources of resistance to TSR. Sabet (61) reported that Early American and K60 genotypes were selected as resistant under field conditions; however, they were found to be susceptible when inoculated under greenhouse conditions. Numerous local varieties were considered resistant to TSR in Egypt (62). Thind and Payak (78) reported highly resistant cultivars using the cut stalk method, although the same cultivars showed TSR symptoms under field evaluations. No resistant germplasm was detected in 20 cultivars tested in India (53). Different levels of resistance was detected in corn cultivars evaluated using the pseudostem injection with different levels of inoculum concentration and analyzed using the Weibull system (83).

D. Ecology of Erwinia soft rot spp.

Erwinia spp. have been reported to overwinter in infested plant debris in the soil after harvest and can persist as long as the plant material is not completely decomposed (24). They are capable of surviving in the rhizosphere or in association with plant material of cultivated and non-cultivated crops (11,19,30,51) and in some agricultural soils (40). In contrast, other researchers have claimed that E. carotovora does not survive in field soils for an extended period of time (21,42,49). These differences were attributed to the inefficiency of techniques for detecting limited numbers of bacteria in soil (40). Erwinia soft-rot spp. were isolated from rhizosphere soils of many weeds and crop plants, and from some field soils containing plant residues, but no Erwinia soft-rot spp. were detected in fallow

soils devoid of vegetation or recognizable plant residues (7). Lim (34) reported that E. chrysanthemi could survive in mineral soil for 18 days but was undetectable in nonsterile peat moss 1 day after infestation. E. chrysanthemi cells were detected 14 days after pineapple leaves were infested in the field but the pathogen could not be detected on detached leaves by the fifth day (35).

E. Serology

1. Use of serology for identifying bacteria

Routine diagnosis of plant bacterial diseases and ecological and epidemiological studies have been hampered by the difficulty in identifying strains of the organisms. Biochemical and physiological tests which are routinely used to identify pathogenic bacteria are not entirely satisfactory. The tests are often complicated, difficult to interpret and require weeks to months to complete. Recently, serology has been accepted as a reliable tool for identifying plant pathogenic bacteria. In medical microbiology, its application is widespread and the ability of medical bacteriologists to provide quick and accurate serological identification of bacteria indicates a similar potential for identification of plant pathogenic bacteria (71).

The greatest importance in serology is the choice of serological test and its proper use. Such tests as agglutination, immunofluorescent (IF) and Ouchterlony Double Diffusion (ODD) are well adapted for identifying bacteria when they are properly used (2,17,85). Of these three tests, IF staining is perhaps the best test available. It is easily performed, very sensitive, and rapid but requires expensive equipment and is moderately specific. The high sensitivity of IF makes the test useful for rapid diagnosis (71).

IF staining was first suggested for identifying bacteria in plants in 1943 (47). Later, Morton (43) reported the superiority of direct IF over agglutination tests for rapid identification of X. vesicatoria in extracts of diseased tissue. IF has been used to identify E. aroidae in extracts of host tissues and soil (28), P. phaseolicola in preparations of bean seeds (9,81) and leaves (76), P. solanacearum in soil (26), P. tabaci in leaves and agar culture (46), E. atroseptica in culture (2,46) and in preparations of tubers, leaves, insects and soil (2), X. albilineans in host tissue (33), X campestris in culture (70) and in soil (14), C. michiganense in seeds (1,81) and the phony peach bacterium extracted from peach roots (18).

2. Serotypes and relationship between E. carotovora and E. chrysanthemi and among strains

Serological studies on the relationships of Erwinia soft-rot spp. indicate that E. carotovora, E. atroseptica and E. chrysanthemi are related but there is a greater degree of similarity between E. carotovora and E. atroseptica than E. chrysanthemi (20,31,66). Strains of E. atroseptica tend to form a more homogenous group than E. carotovora strains, which shows considerable heterogeneity. DeBoer et al., (13) classified E. atroseptica strains into 1 sero-group and E. carotovora strains into 18 sero-groups. E. chrysanthemi was reported (68) to form three distinct sero-groups which were apparently related to pathotypes and the susceptible host. Yakrus and Schaad (85) grouped the 37 strains of E. chrysanthemi from 18 different hosts into four serovars and they found no correlation between host of origin and serovar in their investigation.

Other reports (31,44,57,69) of E. chrysanthemi and closely related species indicated monospecific relationships except when either a single purified immunogen, e.g., pectic lyases (39), or 0 antigen was used (66). Brenner et al. (5) concluded that E. chrysanthemi stains isolated from corn and grasses were closely related but another report (85) suggests that those capable of causing corn stalk rot do not have identical cell surface antigens. The use of IF with antiserum prepared to gluteraldehyde-fixed E. chrysanthemi cells exhibited no cross reactions with E. atroseptica or E. carotovora (2). Other studies to identify E. carotovora from potato and sugar beet (75), and to identify E. sepedonicum from potato (74) have shown antiserum to gluteraldehyde-fixed cells to be specific at the species level.

MATERIALS AND METHODS

A. General

1. Bacterial cultures

E. chrysanthemi pv. *zeae* (ATCC 27386) and *E. carotovora* pv. *zeae* (ATCC 29274) were used in this experiment they were obtained from the American Type Culture Collections (12301 Parklawn Dr., Rockville, MD 20852).

The bacteria isolates were stored as a stock suspension at 4C in sterile distilled water for regular use and lyophilized for long term storage. The cultures were grown on Yeast Extract-Dextrose-Calcium Carbonate Agar (YDCA) (15).

2. Preparation of inocula

Inocula were prepared by streaking YDCA plates with a loopful of the stock suspension. Plates were incubated for 48 hours at 28C and bacteria suspensions were prepared by harvesting the colonies with a sterile rubber spatula and suspending the cells in 0.7% Tween 40 solution or sterile distilled water. The resulting suspensions were adjusted to the desired turibidity: 150, 200 or 250 Klett Unit (O.D. = 0.3, 0.4, 0.5) at 600 nm on the Klett Submersion Photoelectric Colorimeter (Model 800-3). The corresponding colony forming units (CFU) were $2x10^8$, 3.5×10^8, 5×10^8, respectively. The number of viable cells present in each suspension was determined by the plate-count technique adopted by Kiraly et. al. (29). The inocula were used immediately after preparation. During the process of inoculation in the field, the suspensions were placed in an ice chest to avoid lysis of bacteria.

B. Influence of temperature and relative humidity on disease development

Two corn cultivars, Gold Cup, (Sweet corn hybrid, Joseph Harris Co., Inc., Rochester, N.Y.) and O's Gold Exp 6485 (a yellow, dent corn, O's Gold Seed Co., Farmer City, IL 61842) were planted in 7 cm pots containing Baccto potting soil (Michigan Peat Co., Houston, Texas) and grown under greenhouse conditions at 28 (+5)C and relative humidities of 50% (+10) during the day and 70% (+10) during the night. The plants were grown in the greenhouse for 21 days, then transferred to the growth chamber for the maximum-minimum temperature regimes and relative humidities after inoculation.

Twenty plants of each entry were inoculated by pipetting one ml of the bacterial suspension (3.4×10^8 CFU/ml) into the whorl. Care was taken not to jostle the plants after inoculation to prevent loss of inoculum. The inoculated plants were incubated in a growth chamber (Model E 15, Controlled Environments Ltd., Winnipeg, Canada) with the following temperature-relative humidity combinations: 1) 92% relative humidity and day-night temperature regimes of 21-10 C, 24-13 C, 27-17 C, 29-18 C, 32-21 C, 35-24 C, and 2) at a maximum-minimum temperature regime of 29-18 C and relative humidity levels of 92, 70, and 55%. A 14-hour light period was utilized and the intensity was 12,917 lux (1200 ft. candles).

The plants were rated seven days after inoculation. Disease ratings were based on percentage of plants showing stalk rot in relation to total inoculated plants. Plants with stalk rot could be identified by lodging and by easy removal of inner whorl leaves with the stalk being soft-rotted and brown. Occasionally, plants which had not collapsed and from which inner whorl leaves could not be easily removed

not included in the percentage of plants having stalk rot.

C. Screening procedures utilized in evaluating corn cultivars to E. carotovora and E. chrysanthemi:

1. Field evaluations

Hybrids were obtained from BoJac Hybrid Corn Co., Mt. Pulaski, IL; Dekalb Ag Research, Inc., Dekalb, IL; Fontanelle Hybrids, Nickerson, NE; Funk Seeds, Bloomington, IL; Growers Seed Association, Lubbock, TX; Horizon Seeds, Inc., Lincoln, NE; Hoegemeyer Hybrids, Hooper, NE; Joseph Harris, Rochester, NY; Migro, Tekamah, NE; NC+ Hybrids, Lincoln, NE; O's Gold, Farmer City, IL; Pioneer Seeds, Johnston, IA; Prairie Valley Hybrids, Inc., Phillips, NE; Golden Harvest (Rob-See-Co), Waterloo, NE; Taylor-Evans Seed Co., Tulia, TX; and Wilson Seeds, Harlan, IA. Twenty inbred and hybrid lines were obtained from Holden's Foundation Seed, Inc., Williamsburg, IA.

The plots were established at the Rocky Ford Farm near Manhattan, KS. Grain sorghum (Sorghum bicolor L.) and wheat (Triticum aestivum L.) were grown previously in the plots. Fertilizer was broadcasted at rates of 150 kg/ha of N and 50.0 kg/ha of P prior to planting. Seeds were planted on June 19, 1982 and May 27, 1983 with a modified White Plant-Aire Planter in rows 76.2 cm apart with the seed spaced 17.8 cm apart within the row. Furadan 15G (FMC Corporation, Agric. Chem. Group, Philadelphia, PA) was applied at a rate of 1.12 kg (a.i.)/ha for insect control. A split plot experimental design was utilized with four replications per experiment. The plots were furrow irrigated and other cultural operations were conducted as needed.

Four plants of each hybrid were inoculated in the whorl at 21, 42

and 63 days after planting in 1982 and 28, 49 and 70 days after planting in 1983. This approximated early whorl, late whorl, and silking stages of growth, respectively.

Five plants of each corn inbred and hybrid were inoculated in the whorl and the pseudostem 40 days after planting (mid-whorl stage) at the same time to compare inoculation techniques.

2. Inoculation procedures

a) Whorl method:

Five ml of inoculum (2.0×10^8 CFU per ml suspended in 0.7% Tween 40) was placed in the whorl of each plant with a Cornwall pipetting syringe. Disease ratings were taken 17 days after inoculation and were based on a scale of 0 = no symptoms, 1 = small necrotic areas at the base of the whorl, 2 = 25-49, 3 = 50-74, 4 = 75-100% of the tissue rotted at the base of the whorl, and, 5 = lodging accompanied by extensive necrosis of leaf tissue. Stalks of inoculated plants were split lengthwise to ascertain disease severity.

b) Pseudostem injection method:

A bacterial suspension (0.2 ml) containing 5×10^8 CFU/ml in sterile distilled water was injected with a hypodermic syringe in the center of the pseudostem just above the apical meristem. Prior to injecting the plants, a small quantity of sterile petrolatum was placed on the inoculation site. The needle (26-guage) was inserted through the petrolatum and into the center of the stalk. After the inoculum was injected and the needle removed, the petrolatum was immediately smeared over the injection site to alleviate leakage of the inoculum. Inoculated plants were evaluated 7 days after injection by the following

scale: 0 = no visible symptom, 1 = slight discoloration and necrosis at the injection site, 2 = slight internal necrosis, 3 = marked rotting, including death of the growing point but no visible external symptoms, 4 = extensive necrosis with visible external symptoms, and 5 = death of the tissue above the injection site. Stalks of inoculated plants were split lengthwise to determine the rating.

D. Fluorescent Antibody Staining (FAS)

1. Antisera preparation:

Cultures of E. carotovora pv. zeae (ATCC #29274) and E. chrysanthemi pv. zeae (ATCC #27386) were streaked onto YDCA and placed in an incubator for 48 hours at 28 C. The cells were removed from the petri plates with a rubber spatula, washed in sterile 0.01 M potassium phosphate buffered (pH 7.2) saline (0.85% NaCl) (PBS) and then centrifuged at 12,000 rpm for 3 minutes. The pellet was suspended in sterile PBS and centrifuged as previously described. This procedure was repeated 3 times. After the last centrifugation, the cells were resuspended in PBS, and dialyzed in a 2% gluteraldehyde (Sigma Chemical, St. Louis, MO) solution for 3 hours. The cells were then dialyzed in PBS at 4 C for 24 hours with 5 changes of PBS to remove the gluteraldehyde.

New Zealand white rabbits were injected subcutaneously with an equal volume of bacterial suspension (0.5 ml) and Freund's incomplete adjuvant (Difco, Detroit, MI). The solution was emulsified with the aid of a Spex mixer-mill (Spex Industries, Inc., Metuchen, NJ) for 2 minutes at high speed. The injection schedule was at weekly intervals, and after the fourth injection, the rabbits were bled from the marginal ear vein. The injections continued until the 2-fold agglutination titer

after the fourth injection, the rabbits were bled from the marginal ear vein. The injections continued until the 2-fold agglutination titer exceeded 1:2560. The serial 2-fold dilutions were determined in 10 x 75 mm glass tubes containing 0.85% NaCl and after the transfers had been completed, the rack containing the test tubes was placed in a water bath and incubated at 54 C for 2 hours. The tubes were left overnight at room temperature and then the final titer recorded. In most cases, each rabbit received 7-10 injections. The serum was collected from the blood after 3-4 hours at room temperature, pipetted into serum bottles and stored at 4 C without preservatives.

2. Fractination of antisera

The globulin component of the antisera was removed by adding 6.7 ml of saturated $(NH_4)_2\ SO_4$ dropwise over a period of ca. 20 minutes to 10 ml of antisera. The solution was stirred at room temperature for 3-4 hours and then centrifuged at 13,000 rpm for 20 minutes. After the procedure was repeated twice, the pellet was dissolved in 10 ml of sterile PBS and dialyzed against 100-fold volumes of PBS for 48 hours at 4 C with frequent changes of buffer to remove $(NH_4)_2\ SO_4$. The globulin fraction was centrifuged at 10,000 rpm for 20 minutes to remove any precipitate and then stored in serum bottles at 4 C without preservatives.

3. Conjugation of antisera

The conjugation procedure was similar to that reported by Allen and Kelman (2). Prior to conjugation of antisera with fluorescein isothiocyanate (FITC), the protein concentration was adjusted to 1% (10 mg/ml) by diluting (1:10 or 1:20) the sample with 0.1 M $Na_2\ CO_3$-$NaHCO_3$

Tokyo, Japan) at 280 m and the protein concentration was calculated using the following formula:

$\frac{O.D. \times D.F.}{1.3}$ = X mg/ml protein, where D.F. = dilution factor (10 or 20). To dilute the serum to 1% protein concentration, 10 ml of serum was made up to X ml with 0.1 M CO_3 buffer (pH 9.5).

A solution of 5 mg FITC in 8 ml 0.1 M CO_3 buffer (pH 9.5) was added dropwise with stirring to 10 ml of 1% protein antiserum. Sterile saline (0.85% NaCl) was added to make up the volume to 20 ml and a pH of 9.5 was obtained by adding 1 M NaOH. Conjugation of FITC-antiserum continued for 6 hours at room temperature without stirring under darkened conditions. Following conjugation, unreacted FITC was separated from conjugated serum by centrifugation at 10,000 rpm for 20 minutes and by passing the supernatant through a Sephadex G-25M column (Pharmacia Fine Chemicals AB, Sweden). Conjugated serum was then dialyzed against PBS at 4C in the dark for 72 hours with frequent changes of PBS until the pH reached 7.2. The serum was filter-sterilized with a 0.2 μm Millipore filter (Gelman Science, Inc., Ann Arbor, Michigan) and stored in serum bottles at 4 C without preservatives.

The highest dilution of labeled antiserum that would still provide acceptable brightness was determined using the block titration procedure (2). Labeled antiserum was diluted 2-fold with 0.01 M PBS (1:4, 1:8, 1:16 and 1:32 = volume antiserum: total volume). Test cultures were stained with each dilution of antiserum and fluorescence was examined under the microscope.

Specificity of FAS was tested against different related and non-

related strains. Eighteen E. chrysanthemi strains from various locations were tested against antiserum to E. chrysanthemi pv. zeae (ATCC #27386). Ten strains of E. carotovora subsp. carotovora and 4 strains of E. carotovora subsp. atroseptica representing different serogroups were tested against antiserum to E. carotovora pv. zeae (ATCC #29274). These strains were obtained from Dr. A. K. Chatterjee. Several unrelated bacterial plant pathogens were also tested against both antisera.

4. Isolation of bacteria from debris and soil

Soil and overwintered TSR infested corn debris (leaves, stem, seeds and roots) samples were collected from the field plot in April, 1983. Leaves, stems, seeds, and roots were handled separately and were cut into small (2-3 mm^2) pieces and suspended overnight in sterile double-distilled water (1:9 w/v). The suspension was filtered through a double layer of cheesecloth and a loopful was smeared on a slide. Ten g of soil was added to 100 ml of double-distilled water and stirred for 45 minutes at full speed with a magnetic stirrer. Then 0.7 gm of a flocculating agent (crystalline $Ca(OH)_2$ and $MgCO_3$, 2:5, v/v) was added and the mixture was shaken for about 30 sec. After the material had settled for 1 hour, 40 ml of the clear supernatant was centrifuged at 12,000 rpm for 20 minutes and the pellet was resuspended in 1 ml sterile double-distilled water. A loopful of the suspension was smeared on a slide.

Suspensions of corn debris and soil were also plated onto crystal violet pectate (CVP) medium (10).

5. Staining procedure

The direct FAS procedure was utilized in this study. A loopful (3 mm) of bacterial cells were placed on slides, air dried and then heat-fixed. A drop of diluted (1:8) FITC-labeled AS was placed over the smear and then incubated in a moist chamber at 37 C for 30 min. Following incubation, the excess AS was removed by rinsing in 0.01 M PBS for 10 minutes. The slides were quickly rinsed in deionized water, blot-dried, and coverslips mounted with buffered glycerin - 0.01 M PBS (9:1, v/v) (pH 9.5). Stained preparations were examined under a 40x objective using an Olympus (Model BH) photomicroscope (Olympus, Tokyo, Japan) equipped with an HBO/2 super pressure mercury lamp (Osram, Germany), FITC exciter filter, UV dichroic mirror and No. 0-530 barrier filter (transmission range of 380-420 μ).

6. Ouchterlony Double Diffusion Test

Ouchterlony plates were prepared with 1.0% Bacto-agar (Difco Laboratories, Detroit, MI) in 0.01 M PBS (pH 7.2) and 0.02% sodium azide (3). A template was used to cut seven (5 mm dia.) wells with a distance of 6 mm between wells. The antigen was prepared by harvesting 48-hr old bacterial cells from culture plates and then grinding 2 ml of the bacterial suspension (10^8-10^9 CFU/ml) in a test tube containing 1 cc of glass beads (0.17-0.18 mm diameter) at top speed of a Vortex-Genie mixer (Scientific Industries, Inc., Bohemia, NY) for 2 minutes. The AS was diluted 1:8 and placed in the center wells. Disrupted cells of the homologous antigen (10 μl) were placed in each of the remaining three alternate outer wells. Plates were incubated in a moist chamber at 26 C for 48 hours.

RESULTS

A. Influence of temperature and relative humidity on disease development

The effect of temperature and relative humidity (RH) on TSR disease development was determined by injecting each bacterial isolate into the whorl of 10 corn plants of O's Gold Exp. 6485 and Gold Cup hybrids three weeks after planting. The quantal response system (i.e., all or none response) was used to score inoculated plants. Initial results indicated no difference in tolerance between the two corn lines tested. Subsequently, experiments were continued using O's Gold Exp. 6485 and each experiment was repeated twice.

The percentage (number of plants infected/total number of plants inoculated) of plants exhibiting TSR symptoms as influenced by temperature and relative humidity is presented in Table 1. Highest disease incidence occurred with both E. chrysanthemi and E. carotovora under the high temperature (32 or 35 C) or at 29 C with high (92%) RH. Disease incidence was less at lower temperatures. In all cases, RH had a more pronounced effect on E. carotovora. TSR symptoms on plants grown under high temperatures were pronounced as all of the leaves were wilted, chlorotic, and the number of lodged plants increased. Disease development was significantly reduced at low temperatures with moderate symptoms; only the inner-most leaves wilted and soft rot was confined at the base with no lodging.

B. Germplasm evaluation:

Differential responses of the various hybrid genotypes to E. chrysanthemi and E. carotovora could only be detected when inoculated at

Table 1. Influence of temperature and relative humidity on bacterial top and stalk rot incidence in corn (O's Gold Exp. 6485)

Temperature Light-dark	Relative humidity (%)	Disease incidence (%)	
		E. chrysanthemi	E. carotovora
35-24[x]	92	100 a[y]	100 a
32-21	92	100 a	100 a
29-18	92	100 a	98 a
29-18	70	53 c	35 d
29-18	55	53 c	25 d
27-16	92	90 ab	78 b
24-14	92	83 b	75 b
21-10	92	68 c	50 c

[x]temperature cycles = 14 hours light, 10 hours dark

[y]numbers with the same letters are not significantly different at 0.05 (LSD comparison test)

late whorf stage. None of the hybrids would be considered tolerant to E. chrysanthemi and 75% were rated at 4.0 or above (Table 2). Only 2 hybrids had ratings of 2.9 or below and plants of these hybrids would likely be barren at maturity. Sixty percent of the hybrids inoculated with E. carotovora had ratings of 3.0 or above. With one exception (Growers Seed NS 212), the ratings were less on hybrids inoculated with E. carotovora although this probably is not of significance as none of the yellow dent hybrids could be classified as resistant. Ratings in the range of 1 to 2 at the time of evaluation (17 days after inoculation) could conceivably be in a 4 to 5 category at a later evaluation. The lowest rating (1.4) of the hybrids (Golden Harvest H-2515) evaluated is probably not sufficient to prevent yield loss. Stressing of corn plants by biotic or abiotic diseases prior to tasseling inevitably results in yield losses.

Ratings of the sweet corn hybrids are misleading as the maturity dates are considerably earlier than the yellow dent hybrids. The plants were whorl-inoculated 42 and 49 days after planting in 1982 and 1983, respectively. Spring Gold has a maturity date of 67 days and, consequently, would be silking at the time of inoculation and be less vulnerable to disease development. Plants in the whorl stage of growth provide an ideal environment with optimum humidity, succulent growth and movement of the incitant bacteria into the leaf sheath by rain or condensate moisture is less restrictive. Plants in this growth stage are smaller and dispersal of the overwintering bacteria by rain or wind would enhance disease incidence.

Twenty inbreds and hybrids were evaluated for tolerance to E. chrysanthemi and E. carotovora under field conditions (Table 3). The

Table 2. Response of corn hybrids to E. chrysanthemi and E. carotovora when inoculated at late-whorl stage of growth.

Entry	E. chrysanthemi	E. carotovora
Dekalb XL 394	5.0[x] a[y]	4.6 a
NC + 59	4.8 ab	3.9 abcd
Bo-Jac 923	4.7 abc	4.2 abc
Pioneer 3186	4.7 abcd	3.6 bcdef
Funk G-4507	4.6 abcde	3.7 abcdef
Pioneer 519	4.6 abcde	4.3 ab
Migro SPX 36	4.5 abcde	3.3 cdefg
Fontanelle 590	4.5 abcde	2.8 fghij
O's Gold TX 311	4.5 abcde	3.8 abcdef
O's Gold Exp 6485	4.5 abcde	2.9 efghi
Bo-Jac 56	4.5 abcde	3.5 bcdef
Golden Harvest XR434	4.5 abcde	2.8 efghij
Horizon 841	4.4 abcde	3.2 defgh
Dekalb XL 63	4.4 abcde	1.9 jk
O's Gold SX 5500 A	4.4 abcde	3.8 abcde
Dekalb Exp 1001	4.3 abcde	3.6 bcdef
Growers Seed NS 212	4.2 abcde	4.2 abc
Golden Harvest XR 432	4.2 abcde	3.2 defgh
Pioneer 3195	4.1 bcde	3.4 bcdef
Hoegemeyer SX 2649	4.1 bcde	2.9 efghi
NC + 5288	4.1 bcde	2.9 efghi
Golden Acres T-E 6975	4.1 bcde	3.5 bcdef
Growers Seed Exp 246	4.0 bcdef	3.1 defghi
Wilson 2317	4.0 bcdef	2.3 hijk
Prairie Valley X 1078	3.9 cdef	3.0 defghi
Horizon 215	3.8 def	3.4 bcdefg
Wilson 1900	3.8 ef	2.5 ghij
Wilson 2317-B150	3.3 fg	2.2 ijk
Funk G-4323	2.8 g	2.0 jk
Golden Harvest H-2515	2.5 g	1.4 k
Gold Cup	0.5 h	0.3 l
Spring Gold	0.0 h	0.0 l

[x]Rating scale of 0-5, where 0 = no symptoms, 1 = small necrotic areas at base of whorl, 2 = 25-49, 3=50-74, 4 = 75-100% of tissue necrotic at base of whorl, 5 = lodging accompanied by extensive necrosis of leaf tissue. Average of 2-years data.

[y]Numbers with the same letter in a column are not significantly different at the 0.01 level.

plants were inoculated at the mid-whorl (8-10 leaf) stage of growth. As with the commercial hybrid test, all but one entry (B68Ht) had lower ratings in the E. carotovora evaluation. The inbred, H60 had the lowest rating (1.0) in the E. carotovora evaluation whereas Hi 33 was the lowest in the E. chrysanthemi test. The inbreds, H107, A671, SD34, H60, and H93Ht are worthy of future use in developing tolerant inbreds. None of the genotypes examined in the E. chrysanthemi test are acceptable for future evaluations. Victoria (83) ranked A632 Ht as moderately resistant, A632 as susceptible and, A619 as highly susceptible to E. chrysanthemi. A632 and A619 were not evaluated in our test and A632 Ht was rated at 3.1.

In most cases, crosses between resistant X susceptible parents and highly susceptible X resistant parents will result in resistant progeny and is irrelevant whether the resistant parent was male or female. When crosses were made between highly susceptible X moderately resistant, the progeny were susceptible (83). It was impossible to correlate these findings with our materials as most of the inbreds possessed the Helminthosporium turcicum gene (Ht) whereas, in most of the single cross hybrids, the Ht gene was not present in the parents. In addition, our materials would all be classified as susceptible to E. chrysanthemi. Assuming the inbreds were isogenic except for the presence of the Ht gene, our results may differ to those reported by Victoria (83). Hi 33 was the most tolerant (2.0) inbred in the E. chrysanthemi test. When crossed with B73 (3.0), the resulting hybrid was rated at 2.7. According to Victoria, on the basis of resistant levels in the different hybrid populations, resistant genes are transmitted genetically and are considered to be dominant. On the other hand, moderately resistant X

Table 3. Reaction of corn inbreds and hybrids inoculated with Erwinia chrysanthemi and E. carotovora at the mid-whorl stage of growth

Germplasm	E. chrysanthemi	E. carotovora
H107	3.8[x]a[y]	1.4 ghij
H100	3.7 ab	1.7 defghij
B84 x Mo17	3.4 abc	2.2 abcdefg
Va 26 Ht	3.3 abcd	1.8 cdefghij
B 84	3.3 abcd	1.7 defghij
Mo 17 x N 28	3.3 abcd	2.6 abc
Mo 17 Ht	3.2 abcd	2.0 bcdefgh
A619 Ht	3.2 abcd	2.1 abcdefgh
A632 X H 107	3.2 abcd	2.1 abcdefgh
B 73 X Mo17	3.2 abcd	2.4 abcdef
B 73 X Pa 91	3.2 abcd	2.1 abcdefgh
B 73 X H 107	3.2 abcd	2.9 a
B 73 X B 84	3.1 abcde	2.6 abc
B 73 X A 671	3.1 abcde	2.3 abcdef
H 108	3.1 abcde	1.9 bcdefghij
A 632 Ht	3.1 abcde	2.3 abcdef
B 73 X Va 26	3.1 abcde	2.45abcde
Pa 91	3.0 abcde	1.6 efghij
SDP 310	3.0 abcde	1.5 fghij
B 73 Ht	3.0 abcde	2.0 bcdefgh
Mo 17 X A 634	3.0 abcde	2.55abcd
H 93 X H 108	3.0 abcde	2.55abcd
Mo 17 X B 79	2.9 bcdef	2.3 abcdef
A 619 Ht X A 632 Ht	2.9 bcdef	2.45abcde
Mo 17 X B 68	2.9 bcdef	2.2 abcdefg
Mo 17 X H 100	2.9 bcdef	2.8 ab
B 73 x H 60	2.8 cdef	2.2 abcdefg
A 671	2.8 cdef	1.3 hij
B 73 X SDP 310	2.8 cdef	2.4 abcdef
A 632 X SD 34	2.8 cdef	1.95 bcdefghi
A 634 Ht	2.7 cdefg	1.9 bcdefghij
SD 34	2.7 cdefg	1.1 ij
B 73 X Hi 33	2.7 cdefg	2.6 abc
B 73 X Va 22	2.7 cdefg	1.9 bcdefghij
H 60	2.6 defg	1.0 j
Va 22	2.6 defg	1.6 efghij
B 79	2.6 defg	1.6 efghij
H 93 Ht	2.3 efg	1.1 ij
B 68 Ht	2.1 fg	2.3 abcdef

Table 3. (Continued)

Hi 33	2.0	g	1.7	defghij

[x]Rating scale of 0.5 where 0 = no symptoms, 1 = small necrotic areas at base of whorl, 2 = 25-49, 3 = 50-74, 4 = 75-100% of tissue rotted at base of whorl, 5 = lodging accompanied by extensive necrosis of leaf tissue.

[y]Numbers with the same letter in a column are not significantly different at the 0.05 level.

resistant inbreds produces susceptible progeny.

The whorl and pseudostem inoculation techniques were evaluated on 40 inbred and hybred lines (Table 4). The plants were inoculated at the mid-whorl stage of growth. The readings on the whorl inoculated plants were almost identical for both E. chrysanthemi and E. carotovora. Differences were noted between the two incitants of TSR on the pseudostem technique and significant differences were also observed between the two techniques. Although neither technique is ideal, the whorl technique is more comparable to bacterial dissemination under natural conditions and, for mass screenings, would take considerably less time and effort to perform.

The differences between the two techniques may be partially explained by the differences in bacterial numbers and the time interval between inoculation and evaluation. Approximately 1.0×10^9 CFU/ml were placed in the whorl whereas 1.0×10^8 CFU/ml were inoculated into the pseudostem. The ratings were taken 17 days after the whorl inoculation and 7 days after the pseudostem inoculation and the rating scale was slightly different due to the varied techniques.

Inoculating corn plants at the 6-leaf stage of growth with E. chrysanthemi and E. carotovora always resulted in a loss (Table 4). A delay of several weeks or until the plants had reached the 12-leaf stage of growth before inoculation lessened the disease development although most of the plants would be barren at maturity. The results of Table 4 are similar to those reported in Table 2 in which no entry exhibited tolerance to the bacterial incitants. The 1983 growing season was plagued by extremely hot and droughty climatic conditions. These conditions may account for the relatively low rating of 2.3 for those

Table 4. Comparison of inoculation techniques and stages of plant growth for ascertaining genetic variability to top and stalk rot disease of corn under field conditions

Inocula	Inoculation technique[w]		Growth stage			
	Whorl	Pseudostem	6-leaf		12-leaf	
			1982	1983	1982	1983
E. chrysanthemi	4.9[x]a[y]	2.9 b	5.0[z]a	5.0 a	3.7 b	4.2 b
E. carotovora	4.8 a	2.0 c	5.0 a	5.0 a	3.7 b	2.3 c

[w]Plants inoculated at 8-10 leaf stage of growth.

[x]Rating scale of 0-5 where 0 = no symptoms, 1 = small neurotic areas at base of whorl, 2 = 25-49, 3 + 50-74, 4 = 75-100% of tissue rotted at base of whorl, 5 = lodging accompanied by extensive neurosis of leaf tissue. Each number represents an average of inoculating 20 plants from each of 40 inbreds and hybrids.

[y]Numbers with the same letter are not significantly different at the 0.05 level.

[z]Rating scale of 0-5 and each number represents an average 16 whorl-inoculated plants of each of 32 hybrids.

plants inoculated with E. carotovora. In addition, other experiments (Tables 2 and 3) have shown that genotypes tested have more tolerance to E. carotovora. Conversely, E. chrysanthemi may be a more aggressive pathogen in corn than E. carotovora.

C. Fluorescent Antibody Staining (FAS)

Block titration tests showed that the staining titer for FITC conjugated AS to E. chrysanthemi and E. carotovora was 1:8, however, a titer of 1:4 was also used to test the homologous antigens and to detect the TSR incitants in the overwintering study. Results of the FAS and ODD tests of E. chrysanthemi and E. carotovora antisera are presented in Table 5.

Specificity of the FAS was initially evaluated by testing closely related and non-related strains. FAS was highly specific to both E. chrysanthemi and E. carotovora homologous antigens and no fluorescence was observed with 18 strains of E. chrysanthemi tested against E. chrysanthemi (ATCC #27386) antiserum. The 10 E. carotovora strains and 4 strains of E. atroseptica were negative against E. carotovora (ATCC #29274) antiserum. The non-related strains were negative. No cross reaction was observed between E. chrysanthemi and E. carotovora.

A modified Ouchterlony double diffusion (ODD) was used to confirm the results obtained with FAS. Both antisera with the homologous antigen resulted in a well defined precipitin band. There was no evidence that any of the test strains had a common immunogen with either antisera as no precipitin lines were observed. The five corn strains of E. chrysanthemi (D22, D23, D24, D25, D26) also failed to react with either antisera. These results are in agreement with those of R. Dickey (personal communication). No cross reaction between E. chrysanthemi pv.

Table 5. Reaction of various bacterial species and strains against antisera prepared to *Erwinia chrysanthemi* and *E. carotovora*

Bacterial species and strain number	Host	Sero-group	Donor-location	*E. chrysanthemi* FAS	*E. chrysanthemi* ODD	*E. carotovora* FAS	*E. carotovora* ODD
E. chrysanthemi:							
ATCC 27386	*Zea mays*	X[y]	ATCC U.S.A.	+[2]	+	-	-
D4	*Euphorbia pulcherrima*	X	Dickey, Conneticut	-	-	-	-
D5	*Dieffenbachia amoena*	X	Dickey, Florida	-	-	-	o
D6	*Musa paradisiaca*	X	Dickey, Columbia	-	-	-	o
D7	*Syngonium podophyllum*	X	Dickey, Florida	-	-	-	o
D9	*Porthenium argentatum*	X	Dickey, U.S.A.	-	-	-	o
D10	*Chrysanthemum morifolium*	X	Dickey, U.K.	-	-	-	o
D11	*Chrysanthemum morifolium*	X	Dickey, U.K.	-	o	-	o
D12	*Dieffenbachia picta*	X	Dickey, Italy	-	-	-	o
D13	*Parthenium argentatum*	X	Dickey, U.S.A.	-	-	-	o
D14	*Chrysanthemum* spp.	X	Dickey, New York	-	o	o	o
D16	*Chrysanthemum* spp.	X	Dickey, Penn.	-	o	o	o
D17	*Chrysanthemum* spp.	X	Dickey, U.S.A.	-	-	-	o
D21	*Chrysanthemum* spp.	X	Dickey, unknown	-	o	o	o
D22	*Zea mays*	X	Kelman, Wisconsin	-	-	-	-
D23	*Zea mays*	X	Dickey, Egypt	-	o	-	o
D24	*Zea mays*	X	Dickey, India	-	-	-	-
D25	*Zea mays*	X	Dickey, Columbia	-	-	-	-
D26	*Zea mays*	X	Dickey, France	-	-	-	-
E. stewartii	*Zea mays*	X	Claflin, Kansas	-	o	-	o
E. carnegieana	*Echinocatcus* spp.	X	Starr, Arizona	-	o	-	o
P. andropogonis	*Zea mays*	X	Starr, Florida	-	-	-	-
X. holcicola	*Sorghum bicolor*	X	Starr, Australia	-	-	-	-
E. carotovora subsp. *carotovora*							
Ecc 21	*Solanum tuberosum*	2	DeBoer, Canada	-	-	-	-
Ecc 26	*Solanum tuberosum*	5	DeBoer, Canada	-	-	-	-
Ecc 61	*Solanum tuberosum*	10	DeBoer, Canada	-	-	-	-
Ecc 63	*Solanum tuberosum*	9	DeBoer, Canada	-	-	-	-

Table 5. (Continued)

Bacterial species and strain number	Host	Sero-group	Donor-location	E. chrysanthemi FAS	E. chrysanthemi ODD	E. carotovora FAS	E. carotovora ODD
Ecc 71	Solanum tuberosum	3	DeBoer, Canada	-	-	-	-
Ecc 166	Solanum tuberosum	3	DeBoer, Canada	-	o	-	-
Ecc 189	Solanum tuberosum	6	DeBoer, Canada	-	-	-	-
Ecc 190	Solanum tuberosum	4	DeBoer, Canada	-	-	-	-
Ecc 192	Solanum tuberosum	18	DeBoer, Canada	-	-	-	-
Ecc 193	Solanum tuberosum	6	DeBoer, Canada	-	-	-	-
ATCC 29274	Zea mays	X	ATCC	-	-	+	+
E. carotovora subsp. atroseptica							
Eca 3	Solanum tuberosum	1	DeBoer, Canada	-	-	-	-
Eca 6	Solanum tuberosum	18	DeBoer, Canada	-	-	-	-
Eca 196	Solanum tuberosum	20	DeBoer, Canada	-	-	-	-
Eca 198	Solanum tuberosum	22	DeBoer, Canada	-	-	-	-

[y] X represents unknown serogroups

[z] + = positive, - = negative, 0 = test not performed

zeae and E. carotovora pv. zeae was observed.

D. Overwintering study:

Samples were taken from the hybrid evaluation plot at the Rocky Ford Farm in April 1983. The plants were left standing in the field without tillage. Stalk, leaf, root, seed and soil samples were obtained. E. chrysanthemi and E. carotovora cells were detected by FAS in stalk tissue only. The overwintering populations were very low and were undetectable on crystal violet-pectate agar. The low populations were attributable to the time interval from inoculation until sampling and that the stalk tissue was nearly macerated by the incitant bacteria. As a result, the bacteria were not as protected in the inoculated plants through the winter as would be other incitants (e.g., Corynebacterium nebraskense) which do not commonly cause soft rot symptoms.

E. chrysanthemi and E. carotovora isolated from overwintered stalk debris were tested for pathogenicity by inoculating 3-week-old corn plants. The plants were placed in the growth chamber at 28C day, 21C night for 7 days. All inoculated plants developed symptoms characteristic to TSR.

The possible role of the causal organisms of TSR being disseminated by seed was examined by collecting seeds from plants that were inoculated at silking. The seeds were planted in 7 cm pots containing Baccto potting soil and grown under greenhouse conditions. Germination of the seeds was 100% and no symptoms of TSR was observed 42 days after planting.

DISCUSSION

TSR disease of corn in Kansas occurs primarily during hot and wet climatic conditions and has been observed mostly on late planted sweet corn or just after emergence of early maturing hybrids that were in a continuous double crop sequence of sweet corn. These observations coincide with our temperature-relative humidity studies under growth chamber conditions. The influence of high temperature on disease incidence is primarily related to the optimum growth temperatures of 34-37 C and 28-30C for E. chrysanthemi and E. carotovora, respectively. Most bacterial diseases and particularly those that cause soft-rots, are enhanced under high humidity or rainfall conditions. This requirement may explain the relative resistance of plants in the reproductive stage as the pith tissue contains less moisture than those plants that are in the vegetative stage of growth. On the other hand, it is not known if the vascular bundles are actively invaded as new infection sites in the stalk tissue do not appear to follow the vascular bundles. Infection in other internodes is a progression of disease development through the node. In addition, symptoms were rarely detected in those internodes below the site of inoculation.

Only a few inbreds (H107, A671, SD34, H60 and H93 Ht) possessed sufficient tolerance to E. carotovora to be used in future commercial breeding programs. No inbreds were satisfactory for E. chrysanthemi. Although our screening program was artificial, no reliable technique has been developed for TSR to approximate natural conditions and, therefore, the genotypes may have expressed more tolerance if the inoculum levels were lowered. Our inoculum levels of 10^8 CFU/ml were excessive and additional research is needed to ascertain levels that would more likely

approximate field conditions.

Natural openings in the plants such as stomates and hydathodes are assumed to be the portal of entry for the bacteria. The role of insects in vectoring the causal agents has been basically ignored. Potential insect vectors in Kansas include corn root worms (Diabrotica spp.), flea beetles (Chaetocnema spp.) seed-corn maggot (Hylemya platura) and possibly chinch bugs (Blissus leucopterus). The relationship of flea beetles and Stewart's wilt (Erwinia stewartii) of corn is well-documented and research involving insect vectors may provide additional insight in understanding the etiology of TSR.

The effect of TSR on stand and yield losses in Kansas is unknown. TSR symptoms may closely resemble other diseases of corn including Stewart's wilt, Goss's wilt, and stalk rot caused by Pythium spp. TSR is probably more prevalent than has been reported. The effect on yield of yellow dent hybrids is probably negligible except in isolated areas within a field where drainage problems exist. It is assumed that most field corn in Kansas has reached a stage of growth that is less vulnerable to the TSR incitants prior to hot temperatures. In spite of the low relative humidities in the Great Plains, most of the corn in Kansas is irrigated with overhead sprinklers. In the case of center pivots, most are programmed to complete the circle in 72-84 hours. This should provide sufficient humidity in the whorls of corn plants for disease development.

The Ouchtenlony double diffusion (ODD) test is often utilized to establish antigen relationships and placement of bacterial strains into serogroups. None of the strains of E. chrysanthemi or E. carotovora, including corn strains D22, D23, D24, D25 and D26, reacted with antisera

produced to our isolates of E. chrysanthemi and E. carotovora. It appears that those strains of E. chrysanthemi and E. carotovora causing stalk rot in corn do not have identical cell surface antigens (85). Because of the limited number of test strains and with unknown serogroups (as in the case of E. chrysanthemi strains), it was impossible to determine the serogroups of E. chrysanthemi and E. carotovora corn pathovars. The strains of E. carotovora tested only represent nine serogroups according to DeBoer (13). E. carotovora was not related to E. chrysanthemi.

Antigen specificity is an important factor that must be considered when using an immunological procedure as a diagnostic tool. Gluteraldehyde-fixed whole cells as antigens have been shown to be highly specific at species level (2). Our results show that specificity is perhaps extended to various strains as E. chrysanthemi corn strains exhibited no reaction with conjugated E. chrysanthemi antisera in both FAS and ODD tests and the FAS was also specific to E. carotovora. This has also been confirmed in another laboratory (R. Dickey-personal communication).

Used in combination with other methods of determining the inoculum levels in debris (72), FAS proved to be an ideal technique in the ecological investigations of TSR pathogens. The technique proved useful because of its high specificity. The possibility of identifying contaminated or foreign strains is unlikely. On the other hand, if used as a diagnostic tool, antigens should consist of several strains from the geographical location where the testing is to be conducted. The technique was relatively simple and in all cases, it permitted detection of the bacteria at a lower population level than those recovered on

selective media.

In order to recover overwintered E. chrysanthemi and E. carotovora cells, it was necessary to suspend host debris in sterile double distilled water overnight as the tissue was partially desicated and isolation would not be possible using standard procedures (as with fresh tissues). Both causal agents were recovered from infested host debris but only from those stalks above ground. Cultures obtained from overwintered debris were virulent when tested on corn seedlings in the greenhouse. Leben (32) has referred to plant pathogenic bacterial cells in a state of reduced metabolism as hypobiotic cells. He suggested that hypobiotic cells survive in dry leaf, stem and root lesions of annual plants and that they could live longer without added nutrients and would be more likely to survive the physical and chemical stresses that cause the death of actively metabolizing cells.

No attempt was made to determine the overwinter survival of the pathogens in debris that was incorporated in the soil. In the case of other soft rot Erwinia spp., they overwintered in plant residues remaining in the soil after harvest and were detectable as long as the plant material is not completely decomposed (7,12,45).

Neither E. chrysanthemi or E. carotovora were recovered from seeds. Survival of the pathogens in or on seeds mostly have been speculative (73). Soft rot Erwinia spp. usually are absent in true or botanical seeds. Seeds from diseased plants could be contaminated externally, but the bacteria tend to die rapidly as the moisture content in the seeds is reduced (50). Rangarajan and Chakravarti (53) also found no evidence that the TSR incitants were being carried along with corn seeds.

TSR incitants were not recovered from rhizosphore soil obtained

from adjacent inoculated plants. This suggests that inocula used in the screening procedure were not sufficient to infest the soil or the bacterial cells were not capable of survival in the absence of host tissue. The decline of Erwinia spp. in soil may be caused by antagonists among the autochthonous resident populations of bacteria, actinomycetes, and fungi and the antibiotics they produce (36). Nutrient availability may also be a key factor affecting both population numbers and survival in soil (50). Failure to isolate soft-rot Erwinia spp. from the soil does not necessarily mean the organism is entirely absent from the field. The organisms could be located in sparsely distributed pockets throughout the field and the chance of detecting those sites is minimal (40).

From our results, several practices may be exercised by the grower to reduce or eliminate TSR incidence. Shredding the plant and incorporating the debris in the soil after harvest would appear promising in reducing the overwintering populations. Allowing animals to graze on the plant debris may also serve a useful role, paticularly if the area is vulnerable to soil erosion. Early planting in the spring may also be beneficial as TSR is favored by high temperatures and humidities and has little effect on plants in the silking stage. By early planting, the corn would be at a stage of growth that would be less likely to be damaged before the onset of hot temperatures. Corn in Kansas is usually planted in late april and most of the plants will be silking at the onset of high temperatures (July 1) and this may account for the reduced incidence of TSR. Rotating with crops other than corn and sorghum would appear to greatly reduce TSR incidence, particularly since the bacterial incitants are not detectable in seed.

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BACTERIAL TOP AND STALK ROT OF CORN: INFLUENCE OF TEMPERATURE AND HUMIDITY ON DISEASE DEVELOPMENT, TOLERANCE AMONG CORN INBREDS AND HYBRIDS, AND OVERWINTER SURVIVAL OF THE INCITANT BACTERIA

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ABSTRACT

Bacterial top and stalk rot (TSR) of maize is incited either by Erwinia chrysanthemi pv. zeae or E. carotovora pv. carotovora. The disease has been reported from most continents and in North Carolina and Wisconsin. Disintegration of stalk tissue accompanied by lodging and necrosis of leaf sheath tissue are principal symptoms.

Thirty-two commercial hybrids and forty known genotypes of inbreds and hybrids were screened for tolerance to E. chrysanthemi and E. carotovora. No hybrids were scored as tolerant to either organism. Five inbreds (H 197, A 671, SD 34, H 60, and H 93 Ht) exhibited tolerance to E. carotovora, but none were tolerant to E. chrysanthemi. Disease development was enhanced when plants were inoculated at the 6-leaf stage of growth. Those plants inoculated at silking exhibited leaf chlorosis without stalk disintegration. The whorl inoculation technique was easier to perform and more reliable than the pseudostem technique.

TSR development was greatly influenced by warm temperatures and high relative humidity. Disease incidence was greatest (98-100%) at temperatures of 35, 32, and 29 C with 92% RH. Reducing the RH from 92 to 70% decreased the disease incidence of E. chrysanthemi 47% and E. carotovora by 64%.

E. chrysanthemi and E. carotovora overwintered in stalk tissue; no cells of these causal organisms were detected in leaves, roots or soil. E. chrysanthemi and E. carotovora do not appear to be disseminated on seed.

In Ouchterlony double diffusion tests, no cross reactions were observed with antiserum prepared to E. chrysanthemi (ATCC 27386) and other E. chrysanthemi isolates including 5 corn pathovars.