Endemic and Soilborne Nature of *Erwinia carotovora* var. *atroseptica*, a Pathogen of Mature Sugar Beets

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ABSTRACT

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Investigations of the endemic and soilborne nature of *Erwinia carotovora* var. *atroseptica* (biotype KSB, hereafter called "KSB"), a pathogen of mature sugar beets, were conducted in the Sulphur Springs Valley (in Arizona) after the occurrence of root rot in July, 1975. The sugar beet field was plowed in October, 1975, fallowed until March, 1976, planted to wheat in April, harvested in August, and subsequently fallowed. Rhizosphere soil from wheat and various weed plants was collected monthly from April to August, 1976, and assayed for the presence of KSB. Soil and rhizosphere isolates were identified by serological, biochemical, physiological, and pathogenicity tests. KSB was recovered from the rhizosphere of wheat, various weed, and volunteer sugar beet plants throughout the sampling period. KSB also was recovered from the rhizosphere of a native plant, *Lupinus blumerii*, which was collected in the Chiricahua Mountains, a natural watershed of the Sulphur Springs Valley. During a 4-mo period, biotype KSB also was recovered from the rhizosphere of artificially infested sugar beets. Over time, KSB exhibited a distinct vertical distribution in field soil; it consistently survived at soil depths greater than 12 cm. The role of temperature and soil matric water potentials on survival of KSB was investigated in laboratory tests of naturally infested soils. Populations of KSB declined only slowly over a 135-day incubation period at 0 and 10 C, and persisted only briefly under dry soil conditions or fluctuating temperature and moisture regimes.

Additional key words: Erwinia spp. ecology, serology, sugar beet, root rot.

Certain varieties of *Erwinia carotovora* are associated with soft rots of a wide range of field and vegetable crops (12,16,21,22). However, views differ regarding the soilborne nature of the soft rot *Erwinia* spp. (1,3,6,12,13,14,15,22). Several factors contribute to those differences of opinion. First, many different techniques have been employed to detect populations of soft rot *Erwinia* spp. in soil (1,3,7,13) and, since the sensitivities of the techniques vary, successful recovery depends upon the method used. Second, isolates of soft rot *Erwinia* spp. recovered from the rhizosphere of cultivated (1,4,5,8,13) and noncultivated (1,2,9) plants seldom have been accurately identified as the same strain or biotype associated with a specific disease of the susceptible, cultivated plant. Third, little attention has been paid to the effects of environmental factors (soil moisture, soil temperature, and soil depth) on the survival of soft rot *Erwinia* spp.

This study was an investigation of the ecology of *Erwinia carotovora* var. *atroseptica,* the causal agent of root rot of mature sugar beets (18). The occurrence of this disease coincided with the widespread use of virus resistant sugar beet cultivars (USH 9 and 10) in California (19), Washington (17), and Arizona (18). Two distinct serological and physiological biotypes of the pathogen were found in widely separated production areas (18). Biotype KSB (hereafter called "KSB") was associated with the disease in the Sulphur Springs Valley (in Arizona); biotype CB (hereafter called "CB") was associated with root rot near Chandler, Arizona, and in California and Washington.

The source of inoculum of the sugar beet pathogen is not known and attempts to demonstrate that it is seedborne have been unsuccessful (18,20). Consequently, it has been hypothesized that the pathogen may be endemic to certain production areas (11,18,20). The pathogen could not be recovered from fallowed soil collected in fields where the disease had occurred (21); therefore, it was suggested that the sugar beet pathogen survived in the rhizosphere of nonhost plants. The occurrence of soft rot *Erwinia* spp. in the rhizosphere of nonhosts is well documented (1,2,5,8,13), but the significance of this rhizosphere population has not been determined for a specific disease caused by an identifiable member of that population. The origin of these nonhost rhizosphere populations has not been demonstrated.

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The objectives of this investigation were to determine: the survival of KSB of the sugar beet pathogen in the rhizosphere of cultivated and noncultivated plants in the Sulphur Springs Valley; whether vegetation in nonagricultural areas harbors this specific soft rot bacterium; the vertical distribution of the pathogen in the rhizosphere of mature sugar beets, and the influence of soil moisture and soil temperature on the survival of the bacterium.

MATERIALS AND METHODS

Survival potential of *E. carotovora* var. *atroseptica* in agricultural areas. Isolations were made from soil (rhizosphere and nonrhizosphere) collected from two fields (A and B) in the Sulphur Springs Valley where root rot of sugar beets had occurred in 1975. Field A was planted to sugar beets that were harvested in October, 1975, fallowed until March, 1976, planted to wheat in April and harvested in August, and subsequntly fallowed. The cropping history of field B was the same as that of field A except that corn instead of wheat was planted in April. Only corn was sampled in field B.

Each month from April to August, 1976, 10–12 weed and/or crop plants, with adhering rhizosphere soil, were collected, placed in plastic bags, and transported to the laboratory in ice boxes. Care was taken to avoid cross-contamination between plants. Identical numbers of nonrhizosphere soil samples were collected from the 0–20 cm depth about 30 cm from plants. Fallow soil, collected 1 mo after the wheat harvest, was sampled at depths of 0–20 cm and in the vicinity of original sites of rhizosphere sampling.

Isolations from rhizosphere and nonrhizosphere soil were accomplished within 1–3 days after collection as follows: approximately 5 g of roots and adhering soil (for the rhizosphere [which included the rhizoplane] determinations) or nonrhizosphere soil were placed in 50 ml of sterile distilled water (SDW) and shaken for 5 min. The suspension was serially diluted with SDW and triplicate 0.1-ml portions were spread on the surface of crystal violet pectate (CVP) (3), either directly or after enrichment (13). After 48 hr of incubation at 25 C, pectolytic colonies were subcultured on m-endo LES agar (Difco) plates, then transferred back to CVP. Cultures were transferred and maintained on nutrient agar (Difco) slants.

Soil and rhizosphere isolates were identified by serological, biochemical, physiological, and pathogenicity tests as described by Stanghellini, et al (18).

Isolations of KSB from native vegetation in nonagricultural areas. Native vegetation was collected in July, 1977, from two regions: Pinery Canyon (elevation, 1,800 m) and Fly's Peak (elevation 3,000 m), within the Chiricahua Wilderness Area of the Chiricahua Mountains, a natural watershed of the Sulphur Springs Valley (elevation 1,200 m). These regions were located 50 km from farming areas. Native plants and adhering soil were placed in plastic bags and transported to the laboratory in ice boxes. Samples were collected aseptically and processed within 24 hr. The bacterial isolates that were recovered were identified as previously described.

Vertical distribution of KSB in the rhizosphere of mature sugar beets under field conditions. This experiment was conducted in a commercial sugar beet field (loamy soil) which previously had been determined to be free of KSB, in Chandler, Arizona. Sixty milliliters of an aqueous suspension (1.4×10^{10} cells per milliliter) of biotype KSB were dispensed on 2 March in soil adjacent to the crown of 120 healthy, 6-mo-old sugar beets and in each of four sites located between the rows. One row of controls was left between infested sugar beets. After infestation, four treated sugar beets, and four control plants, were randomly sampled on 26 March, 15 May, and 23 June, and then the entire field was harvested. The plants with adhering soil were carefully extracted from soil, placed in individual plastic bags, transported to the laboratory in ice boxes, and processed within 24 hr. Rhizosphere soil was scraped from the surface of each sugar beet root at varying distances from the crown and separately assayed for the presence of KSB. All assayed sugar beets were healthy. Populations were estimated by direct plating on CVP, and pectolytic isolates were identified by physiological and serological tests (18). Additionally, nonrhizosphere soil was collected at 0–15 cm depths, processed as described for naturally infested soil, and assayed for KSB.

Influence of matric water potential and soil temperature on the survival of *E. carotovora* var. *atroseptica*. A naturally infested clay loam field soil, collected near the roots of sugar beets infected with KSB in the Sulphur Springs Valley, was used to determine the effect of various soil matric water potentials and soil temperatures on the survival of the sugar beet pathogen. Twenty-gram samples, at approximately field capacity, were either allowed to air-dry and equilibrate with ambient relative humidity (12%) and air temperature (25 C), brought to saturation by the addition of sterile distilled water (SDW), or adjusted to −0.33 bar and −15.0 bars on a pressure plate. The moisture-adjusted samples were placed inside petri dishes sealed with masking tape, and incubated at 25 C.

Over a 45-day period, total bacterial counts were estimated according to the method described by Larkin (10) and KSB counts were estimated by triplicate plating on CVP (3) with or without enrichment (13), depending on the population.

Samples (25 g) of the moist, naturally-infested soil were also placed in sealed petri dishes and incubated at constant temperatues of 0, 10, and 25 C. Soil samples also were exposed, in the greenhouse, to temperatures that fluctuated between 20 and 35 C and watered periodically to maintain a moisture level near field capacity. Populations of KSB were estimated about every 15 days over a 135-day period by direct plating on CVP (3). All of the foregoing studies of soil were repeated once.

RESULTS

Survival potential in agricultural areas. KSB consistently was recovered from the rhizosphere of wheat, various weed, and volunteer sugar beet plants over a 5-mo period in field A (Table 1). It also was recovered from the rhizosphere of corn in field B. Additionally, isolates of *E. carotovora* were obtained which were nonpathogenic to sugar beets, but were serologically reactive with antisera developed to isolates from other geographical regions in the U.S., as well as isolates identified as *E. carotovora* var. *carotovora* (18).

The sugar beet pathogen could not be recovered from fallow soil collected after the wheat harvest in field A.

Isolations from native vegetation. KSB was recovered only from the rhizosphere of *Lupinus blumerii* Greene collected from two locations in the Chiricahua Wilderness Area (Table 2). Several other unknown serotypes of *E. carotovora* also were recovered.

Vertical distribution of KSB in the rhizosphere of mature healthy sugar beets. KSB was recovered over a 4-mo period from the rhizosphere of mature sugar beets. However, it was not recovered from the rhizosphere of noninfested plants or from artificially infested soil.

Patterns of vertical distribution of KSB populations are presented in Fig. 1.

Effects of matric water potential and soil temperature on the survival of KSB. Initial KSB populations were estimated to be 6×10^3, 1.4×10^4, 2.5×10^4, and 1×10^3 colony forming units (CFU) per gram of soil that was saturated, at −0.33 bar, at −15.0 bars, and air-dried, respectively.

A sharp decline of KSB populations occurred in all moisture-

TABLE 1. Isolation of *Erwinia carotovora* var. *atroseptica* (biotype KSB) and other soft rot *Erwinia* spp. from rhizosphere soil of various weed and crop plants in agricultural areas (fields A and B)[a] in the Sulphur Springs Valley, Arizona

Date of sampling (1976)	Crop or weed	Plants sampled (no.)	Rhizospheres with *Erwinia* spp.	
			Biotype KSB[b] (no.)	Other pectolytic *Erwinia* spp. (no.)
April	*Beta vulgaris L.* (volunteer sugar beets)	7	5	3
	Triticum sp. (wheat)	6	4	3
	Sisymbrium irio (London rocket)	7	6	4
	Miscellaneous weeds	10	...	2
May	*Triticum* sp. (wheat)	5	4	3
	Amaranthus palmeri Wats (pigweed)	6	3	...
June	*Triticum* sp. (wheat)	4	3	2
	Miscellaneous weeds	8	4	3
July	*Zea mays* L. (corn)	6	5	1
	Triticum sp. (wheat)	4	3	2
August	*Triticum* sp. (wheat)	12	8	3

[a] Field A was planted to sugar beets that were harvested in October, 1975, fallowed until March, 1976, planted to wheat in April, harvested in August, and subsequently fallowed. The cropping history of field B was the same as that of A except corn was planted in April.
[b] Identification based on serological, physiological, and pathogenicity tests (18).

adjusted soils during the first 19 days of incubation at 25 C, particularly in the last 11 days when populations had declined to levels that were undetectable by direct plating (Fig. 2). After the 19th day, the enrichment technique was used prior to CVP plating. As a consequence, KSB was detected for 36 additional days, but only in soils that were saturated or at −0.33 bars.

Under fluctuating temperature and moisture regimes, KSB from naturally infested soil was recoverable by direct plating on CVP (3) for only 15 days. At a constant temperature of 25 C, KSB persisted for 30 days. However, at constant temperatures of 0 and 10 C, the KSB population, in moist, naturally infested field soil, declined only slowly throughout the 135-day incubation period (Fig. 3).

DISCUSSION

Results of these investigations provide an explanation for the occurrence of root-rot of mature sugar beets in certain geographical areas. Apparently, the sugar beet pathogen is endemic to Arizona on native *L. blumerii* in nonagricultural areas and can persist, perhaps indefinitely, in agricultural field soils as a rhizosphere inhabitant of both cultivated and noncultivated plants. In addition, the recovery of the pathogen, as well as other pectolytic *Erwinia* spp., from the rhizospheres of cultivated and noncultivated plants following a 5-mo fallow period indicates that these bacteria are soilborne and capable of persisting in fallow agricultural soils. The specific ecological habitat of soft rot *Erwinia* spp. in soil per se (fallow field soil) is not known. Although soft rot *Erwinia* spp. have been recovered from recently fallowed soils (1,13,21), they have not been recovered from soils fallowed for periods longer than 1 mo (1,3,4,12,22). Lack of recovery may be related to the soil depth sampled. Most researchers, us included, have attempted to recover soft-rot *Erwinia* spp. from soil collected from the 0–20 cm depth (3,4,14,21,22). Results of this investigation, however, indicate that KSB, even in the presence of a susceptible sugar beet, exhibits, in time, a distinct vertical pattern of distribution in rhizosphere soil, with an apparent preference for soil depths greater than 12 cm. The exact reason for this distribution is not known. Survival of soil organisms is undoubtedly governed by such environmental factors as soil moisture, temperature, aeration, and nutrient availability. These factors may become limiting to

TABLE 2. Isolation of *Erwinia carotovora* var. *atroseptica* (biotype KSB) and other pectolytic *Erwinia* spp. from rhizosphere soil of native vegetation in nonagricultural areas in Arizona

Native plant species	Plants sampled (no.)	Rhizospheres with *Erwinia* spp.	
		Biotype KSB[a] (no.)	Other pectolytic *Erwinia* spp. (no.)
Lupinus blumerii Greene	50[b]	12[c]	2
Oxytropis lambertii Pursh	10	0	0
Calliandra reticulata Gray	5	0	3
Desmodium batocaulon Gray	10	0	0
Vicia pulchella H.B.K.	6	0	2

[a] Identification of biotype KSB was based on serological, physiological, and pathogenicity tests (18).
[b] Two plants were collected at Pinery Canyon, and 48 plants at Fly's Peak, Chiricahua Wilderness Area, Chiricahua Mountains.
[c] One plant was collected at Pinery Canyon, and 11 plants at Fly's Peak, Chiricahua Wilderness Area, Chiricahua Mountains.

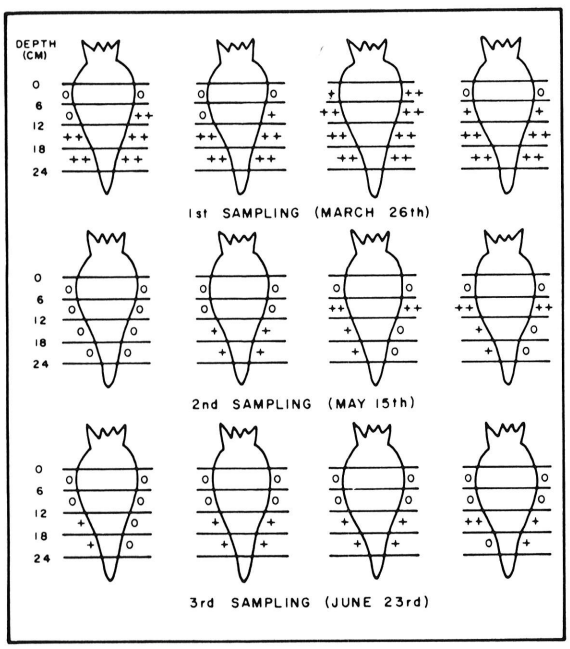

Fig. 1. Vertical distribution of *Erwinia carotovora* var. *atroseptica* (biotype KSB) in the rhizosphere of mature sugar beets. Symbols: ++ = more than 10 viable KSB cells per gram of rhizosphere soil; + = less than 10 viable KSB cells per gram of rhizosphere soil; and 0 = no KSB detected. Soil adjacent to sugar beets was artificially infested on 2 March 1976.

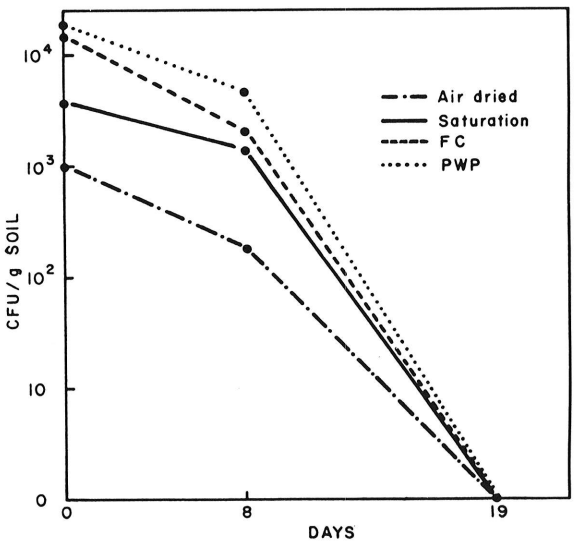

Fig. 2. Persistence of *Erwinia carotovora* var. *atroseptica* (biotype KSB) in soils adjusted to different matric water potentials. Colony forming units (CFU) obtained by direct plating on crystal violet pectate (3). FC = field capacity (−0.33 bars); PWP = permanent wilting point (−15 bars). Values represent the individual populations in 20-g samples of soil incubated at 25 C. A repeat of this experiment gave similar results.

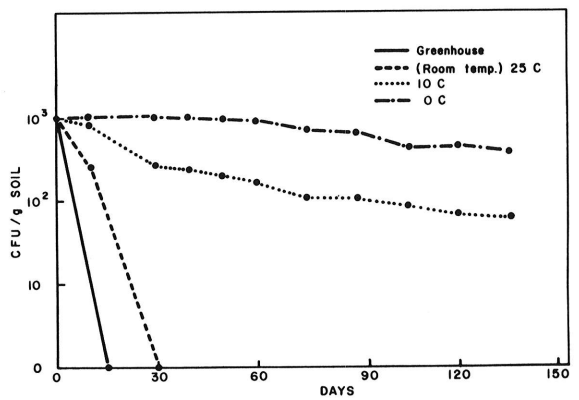

Fig. 3. Persistence of *Erwinia carotovora* var. *atroseptica* (biotype KSB) in a moist, naturally infested field soil incubated at different temperatures. Colony forming units (CFU) obtained by direct plating on crystal violet pectate (3). Soil moisture was at approximately field capacity.

survival, particularly in the surface layers of a fallowed, or even an irrigated, field soil. The surface layers of field soil are subject to extreme fluctuations in soil moisture and temperature. The inability of KSB to withstand air-dry soil conditions, as well as fluctuating temperature and moisture regimes, was demonstrated in our study. It is not surprising that soft-rot *Erwinia* spp., including the sugar beet pathogen, have not been recovered from the surface layers of soils fallowed for extended periods of time. The sugar beet pathogen was, however, capable of persisting for periods exceeding 4 mo in a moist, naturally infested field soil incubated at 0 and 10 C. Persistence of KSB in soil may occur at depths where environmental conditions (moisture and temperature) are more stable and favorable than in the surface layers and would account for the occurrence of KSB in the rhizosphere of a wide variety of cultivated and noncultivated plants following a 5-mo fallow peiod.

Further, the existence of a heterogeneous population of soft rot *Erwinia* spp., including KSB, in the rhizosphere of cultivate and noncultivated plants in agricultural areas, as well as in the rhizosphere of certain native vegetation in nonagricultural areas, supports the hypothesis of Stanghellini et al (18) that there is "regional selection of a specific biotype of the pathogen by a host from an existing heterogenous population residing naturally in soil."

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