Etiology of a Root Rot Disease of Sugar Beet in Texas

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ABSTRACT

A disease of sugar beet (Beta vulgaris) in Texas characterized by wilt and root rot has been shown to be caused by Fusarium oxysporum. The disease is similar to Fusarium yellows, caused by F. o. f. sp. betae, but is distinct in that a severe root rot also occurs that is not associated with Fusarium yellows. Scanning electron microscopy revealed that the pathogen inhabited the xylem, typical of Fusarium yellows. However, electrophoretic data on three enzymes (cinnamyl alcohol dehydrogenase, esterase, and glucose-6-phosphate dehydrogenase) revealed significant differences in isozymes produced between the Texas isolates of F. oxysporum and known isolates of F. o. f. sp. betae. The Texas sugar beet isolate may be a new forma specialis (or a new race of F. o. betae). However, further comparisons with additional isolates are necessary before a definite conclusion can be made.

Although not considered a major production state, Texas grows approximately 16,000 ha of sugar beets, all concentrated in a four-county area in the Panhandle. As a result, sugar beets are a significant portion of the area in that region. Several soilborne diseases occur on sugar beets in the Texas Panhandle, causing significant yield losses. Two of the most common are Rhizoctonia root and crown rot, caused by Rhizoctonia solani Kühn, and black root, caused by Aphanomyces cochlioides Drechs. In addition, a disease characterized by root rot, vascular discoloration, interveinal chlorosis of the leaves, and wilting of the foliage has increased in severity and occurrence since 1981 (S. Winter, personal communication). Fusarium spp. can be isolated readily from symptomatic root tissue, and the disease is referred to by local growers as tip rot. In the last 3 yr, the incidence of this disease has increased to the point of limiting production in many fields.

Symptomatically, the disease is similar to Fusarium yellows, caused by Fusarium oxysporum Schlecht. emend. Syd. & Hans. f. sp. beta Stewart (= F. conglutinans var. betae Stewart), first described by Stewart (20) in 1931 from Colorado. There is one major distinction, however. In Texas, the disease first is observable as a single rust-colored streak in the central stele of the young sugar beet; no external root symptoms are apparent at this time. During the hottest period of the day, infected plants may wilt slightly, but no other foliar symptoms are evident. At this stage, the disease normally would not be detected by an untrained observer. Obvious foliar symptoms, e.g., wilting and leaf chlorosis, usually are apparent first in the latter part of June, approximately 60 days after emergence. Foliar symptoms coincide with ambient day-time temperatures above 27 C. At this time, diseased roots show vascular necrosis and discoloration typical of Fusarium yellows (20), but in addition, the external portion of the root frequently is rotted at the distal end, with black streaks traveling upward. At first, only the tip of the beet is discolored. As the season progresses, however, up to one-half of the main taproot may become black and rotted. In extreme instances, the infected portion of the root rots completely, leaving only remnants of the vascular bundles. These root rot symptoms may occur in sugar beets simultaneously infected with A. cochlioides but also occur regularly in the absence of other apparent diseases. With Fusarium yellows, no external root symptoms occur (20). The root rot is easily distinguished from Erwinia soft rot, caused by Erwinia carotovora Jones subsp. betavascularorum Thomson et al (22), in that Erwinia causes a much more watery rot throughout the root and crown.

The purpose of this research was to test pathogenicity of isolates of Fusarium obtained from diseased sugar beets from Texas and to compare them with known isolates of F. o. f. sp. betae to determine if the two organisms are distinct. A portion of this work has been published (12).

MATERIALS AND METHODS
Isolation of the pathogen. Symptomatic, mature, field-grown sugar beet plants were collected from commercial fields at several locations in the Texas Panhandle. The taproots and lateral roots were washed, surface-disinfested with 10% NaClO, for 5-10 min, cut into small segments, and placed on acidified potato-dextrose agar (APDA), water agar, or Komada's (9) medium, then incubated at 22-25 C. In most cases, a Fusarium sp. grew from the vascular bundles and/or the necrotic areas of the cortex within 48-72 hr. Original isolates typically produced a salmon-colored pigment on APDA plates, but this was lost in subsequent transfers and monoclonal culture. In all cases, the isolates of Fusarium appeared similar but were not readily identifiable as F. oxysporum (16) on either PDA or carnation leaf agar because macroconidia usually were absent. Microconidia were abundant and borne on short mononidial. Chlamydo- spores formed slowly and were both terminal and intercalary.

Two monoclonal isolates were identified as F. oxysporum by P. E. Nelson (Pennsylvania State University) and C. E. Windels (University of Minnesota), although both found the isolates somewhat atypical of the species (personal communications). The monotrichous isolates have been maintained in sterile soil (14).

Pathogenicity tests. Two Texas isolates—F. oxysporum (TX) F4 and (TX) F5—were selected for further tests. Both isolates of F. oxysporum were obtained from infected plants within the same field. (TX) F4 was isolated from the distal end of a root and (TX) F5 was isolated from the crown. In addition, three other sugar beet isolates—F. o. f. sp. betae (OR) ATCC 34296, F. o. f. sp. betae (CA) 1281-1(86), and F. oxysporum (CA) 10(83)—were obtained from E. G. Ruppel (USDA-ARS, Fort Collins, Colorado) and used for comparisons. Isolate (OR) ATCC 34296 was originally isolated by MacDonald et al (11) from Oregon in 1976 and is the stalk blight pathogen reported by Gross and Leach (7). Isolate (CA) 1281-1(86) was recovered from California-grown sugar beets in 1986 by D. C. Erwin (University of California, Riverside).

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Isolate (CA) 10(83) also was obtained from California-grown sugar beets in 1983 by E. G. Ruppel. All isolates were single-spored, increased in a liquid mineral salts medium (6), and stored in sterile soil.

Two pathogenicity tests were conducted. In the first test, two sugar beet seeds (cultivar TX-9) were planted in each of 18 10 × 60 cm polyvinylchloride (PVC) tubes filled with a pasteurized sand:peat mix (4:1, v/v) (Fig. 1). The PVC tubes were placed into 15-cm-diameter pots partially filled with the same mix. Pasteurized sandy loam was backfilled around the PVC tubes to hold them in an upright position. Each PVC tube previously had been cut longitudinally and the two halves clamped together. After 6 wk, the roots had reached the bottom of the PVC tube and were growing out into the sand:peat mix in the larger pot. Each PVC tube then was lifted gently, leaving a well in the sandy loam intact, and 100 ml (1 × 10^5 microconidia per milliliter) of either F. o. f. sp. betae (OR) ATCC 34296, (TX) F4, (TX) F5, or sterile water was poured into the well. After a few minutes, the PVC tube was reinserted into the well. There were five replicated PVC tubes (two plants per tube) for each treatment. All plants were maintained in the greenhouse for an additional 3.5 mo.

In the second test, the sugar beet isolates F. o. f. sp. betae (CA) 1281-2(86) and F. oxysporum (CA) 10(83) were used instead of (TX) F5; all other treatments were the same. In this test, each isolate was grown in sterile sand:cornmeal (9:1, w/w) for 4 wk. The final population density of each isolate was adjusted to 1 × 10^8 cfu/g with sterile sand, after which 175 g was layered inside each of 15 PVC tubes, 12.5 cm below the soil line (Fig. 1). Four sugar beet seeds (cultivar TX-9) were planted in each of 60 PVC tubes, and after 10 days, plants were thinned to two per tube. There were 15 replicated PVC tubes (two plants per tube) for each treatment. All plants were maintained in the greenhouse for 10 wk.

Periodically, in both experiments, one or two PVC tubes from each treatment were selected and opened. The soil core was gently washed away with running water, and roots were observed for symptoms. At the end of each test, all plants were examined, and taproot sections were surfaced-disinfested in 10% NaClO and plated on Komada's medium for recovery of Fusarium. Additionally, a few root sections from plants inoculated with each isolate were subjected to standard fixation procedures for scanning electron microscopy. Sections (1 mm) were fixed in 2% glutaraldehyde and 0.1 M cacodylate buffer (1:1, v/v), buffer-washed twice, dehydrated in a graded alcohol series overnight, critical-point dried, sputter-coated with gold, and examined with a JOEL 25 scanning electron microscope.

### Isozyme analysis of Fusarium isolates

To determine the relatedness among Texas isolates of F. oxysporum and known cultures of F. o. f. sp. betae from Oregon and California, and to confirm the reisolation of the suspected causal agent from inoculated plants, we conducted native polyacrylamide gel electrophoresis (PAGE) of different isolates of Fusarium on 10–15% gradient gels. Three enzymes were examined: esterase (EST; EC 3.1.1.2), glucose-6-phosphate dehydrogenase (G-6-PD; EC 1.1.1.49), and cinnamyl alcohol dehydrogenase (CAD; EC 1.1.1.12).

Three separate native PAGE analyses were conducted. The first test consisted of 20 isolates and included stock cultures of each pathogen, several Fusarium spp. recovered from roots from the first inoculation test, and two isolates each of F. solani and F. graminearum as representatives of different Fusarium spp. This test was used for preliminary phenotypic differentiation of isolates. From these, eight isolates were selected and electrophoresed a second time. This test consisted of isolates (OR) ATCC 34296, (CA) 1281-2(86), (TX) F4, (TX) F5, one each of F. solani and F. graminearum, and two of F. oxysporum recovered from sugar beet roots inoculated with the Texas isolates in the first pathogenicity test. A third electrophoresis test (gel data not shown) included 10 isolates: all three isolates of F. o. f. sp. betae, both Texas isolates, three isolates of F. oxysporum recovered from plants from the second pathogenicity test, and two isolates of F. solani.

Each isolate was single-spored and increased in 50 ml of a liquid mineral salts medium (6) on a rotary shaker (110 rpm) with a 16-hr photoperiod (860 μE·m^−2·s^−1) at 23 C for 4–5 days. Cultures were filtered through Whatman No. 1 filter paper in a Büchner funnel and washed twice with sterile deionized water. The fungal mat, consisting primarily of microconidia and some mycelia, was scraped from the filter paper and placed into a sterile microcentrifuge tube, which was immediately placed in liquid nitrogen.

Protein extraction was done by placing the frozen fungal mats in cold (4 C) mortars and immediately pouring liquid nitrogen over them. Each specimen was ground with approximately 50 mg of sterile sand for 1 min. The slurry was returned to a sterile microcentrifuge tube and centrifuged at 13,000 g for 15 min, and the supernatant was retained for electrophoretic analysis. All protein samples were diluted with a concentration of 180 μg protein per milliliter by the Bradford spectrophotometric method (4).

Native PAGE was conducted with the Pharmacia PhastSystem (Piscataway, NJ) and precast gels. A 1-μl sample was applied to the cathode end, and proteins were separated with the following protocol: 400 V, 10 mA, and 2.5 W for 135 vh at 15 C. Isozymes were developed with standard staining procedures (5, 19). Each isozyme from each fungal isolate was assigned a sequential number based on descending R_1 value. Individual isozymes were then treated as binomial data and scored either as +1 if present in a given fungal isolate or as 0 if absent. No attempt was made to weight the ratings based on band density. Binomial analysis of the bands from the second and third native PAGE tests was applied to a hierarchical cluster analysis and the Simpson's coefficient with the statistical software BIOSSTAT II (18). Isozymes from all three enzymes from each fungal isolate were used in the analysis. In the first experiment, 13 bands for CAD, 10 bands for EST, and 10 bands for G-6-PD were used for analysis. In the second experiment, three bands for CAD, four bands for EST, and four bands for G-6-PD were used. Phenograms were constructed from the relatedness distances generated from the analysis.

### RESULTS

#### Pathogenicity tests

Results from both inoculation tests confirmed the pathogenicity of the Texas sugar beet isolates, (TX) F4 and (TX) F5, and each isolate of F. o. f. sp. betae (Fig. 2). (TX) F4 was isolated originally from the root tip of diseased beets, whereas (TX) F5 was isolated from the vascular tissue near the crown. Both (TX) F4 and (TX) F5 caused stunting, yellowing, and wilting of the foliage (Fig. 2A) and severe vascular necrosis (Fig. 2D and E).
addition, each isolate caused a root rot that was indistinguishable from that observed in the field (Fig. 2B–D). The root generally started at the distal end of the taproot or lateral roots and progressed to the main portion of the taproot (Fig. 2A–D). In many cases, the taproot rotted off just below the crown (Fig. 2C and D). In most cases, extensive proliferation of lateral roots occurred in response to the destruction of the taproot and was a useful symptom for discriminating Fusarium root rot and Fusarium yellows.

In contrast to the Texas isolates, none of the isolates of F. o. f. sp. betae caused root rot but did induce all the symptoms of Fusarium yellows. Aboveground symptoms induced by the Texas isolates and F. o. f. sp. betae were the same and included stunting, yellowing, necrosis, and wilt. In all cases, a F. oxysporum morphologically indistinguishable from that of the original isolates was recovered from infected root tissue.

**SEM of infected tissue.** Photomicrographs of root tissue from plants inoculated with isolate (TX) F4 or (TX) F5 revealed extensive fungal colonization of the secondary xylem tissue (Fig. 3A–D). Hyphae, as well as extensive plugging, were observed in numerous xylem and metaxylem vessels. Colonization of the xylem by (TX) F4 and (TX) F5 was similar to that of known isolates of F. o. f. sp. betae. However, extensive cellular destruction indicative of the root rot symptom was observed throughout root tissue from plants inoculated with (TX) F4 and (TX) F5 that was not seen in root tissue from plants inoculated with F. o. f. sp. betae.

**Isozyme analysis of Fusarium isolates.** In the first experiment, 20 isolates were examined (Fig. 4). On the basis of visual inspection for similarities and differences of each isolate's isozyme pattern for each of the three enzymes, eight of the 20 isolates were selected and electrophoresed a second time for each of the three enzymes (Fig. 5).

A phenogram was constructed based on matching distances (MD) (Fig. 6) generated from the analysis. The Simpson cophenetic correlation coefficient was 0.71. The smaller the MD, the more closely related the isolates are. Matching distances of 0 indicated no significant differences among isolates. Matching distances showed that the Texas isolates of F. oxysporum were distinct from F. o. f. sp. betae. Isolates 7 and 8 were stock cultures of the Texas isolates and matched with isolates 1 and 2 reisolated from inoculated sugar beet plants, indicating that the original pathogen was recovered. The Texas isolates were relatively distinct from F. o. f. sp. betae, with a matching distance of 11.9, whereas the two isolates of F. o. f. sp. betae (isolates 5 and 6) were closely related (MD = 2.36). F. solani

**Fig. 2.** Foliar and root symptoms of sugar beets inoculated with Texas isolates of Fusarium oxysporum. (A) Foliar symptoms showing death of the older leaves; (left to right) noninoculated, F. o. f. sp. betae (OR) ATCC 34296, F. oxysporum (TX) F4, and F. oxysporum (TX) F5. (B) Comparison of a control sugar beet taproot (left) with a taproot from a plant inoculated with F. oxysporum (TX) F4; note necrosis and rot at the root tip (arrow). (C) (Left) A 5-mo-old control sugar beet showing extensive root development and (right) an inoculated plant showing complete rot of the taproot; note the much smaller size of the infected beet root (arrow). (D) Crown and upper portion of a diseased beet root showing extensive rot and vascular discoloration. (E) Serial cross sections of a sugar beet root infected with isolate (TX) F5 showing vascular browning in the concentric rings of the tertiary tissue.

**Fig. 3.** Scanning electron micrographs of sugar beets inoculated with F. oxysporum (TX) F4 and (TX) F5. (A–C) Sections from the crown area of a plant inoculated with isolate (TX) F4; note hyphae in secondary xylem (arrows) and extensive plugging of the metaxylem. Scale bars = 10 μm. (D) Section from lower half of taproot from an inoculated plant infected with isolate (TX) F5; note large vascular plug in the xylem (arrow). Scale bar = 100 μm.
and *F. graminearum* were quite distinct from all isolates of *F. oxysporum*, with matching distances of 13.6 and 14.4, respectively. In the third experiment, isolates (OR) ATCC 34296 and *F. oxysporum* (CA) 10(83) were identical (MD = 0) and differed only slightly from *F. o. f. sp. betae* (CA) 1281-2(86) (MD = 2.75) (Fig. 7). Isolates 4 and 5, (TX) F4 and (TX) F5, also had matching distances of 0 and were considered identical. Isolates 6 and 7 were *F. oxysporum* recovered from infested root tissue and matched the original stock cultures of (TX) F4 and (TX) F5, indicating that they were the same isolate, thereby completing Koch's postulates. It was also evident, however, that even though the isolates of *F. o. f. sp. betae* (isolates 1, 2, and 3) were either identical or very similar to each other, they were distinct from the Texas isolates (MD = 7.88). Two isolates of *F. solani* (isolates 9 and 10) appeared to be identical (MD = 0) but quite distinct from all isolates of *F. oxysporum*.

**DISCUSSION**

Results presented here document several aspects of the sugar beet disease in Texas and raise some questions about the pathogen that cannot be answered at this time. First, we described field symptoms of wilting and root rot and confirmed that *F. oxysporum* was the causal agent. The original description of Fusarium yellows of sugar beet (20) did not include a root rot or, in fact, any external root symptom. There is ample evidence that *F. o. f. sp. betae* does not cause root rot as part of the disease syndrome (11,13,20,23). Because the Texas isolates of *F. oxysporum* do cause root rot in addition to vascular wilt, we question whether the Texas disease is different from Fusarium yellows and whether the causal agent is a distinct race or forma specialis of *F. oxysporum*.

Several possibilities must be considered when comparing the root rot disease with Fusarium yellows. First, Stewart (20) may have overlooked root rot symptoms for his original description of Fusarium yellows. This seems unlikely, however, in that root rot would be unmistakable if present in any significant amount. Second, the root rot symptom may be environmentally mediated, i.e., it may occur only in wet, heavy soil, perhaps with the involvement of a secondary organism. This is plausible, but because the Texas isolates caused root rot in pasteurized sand-peat mix and thinned isolates of *F. o. f. sp. betae* did not, we believe that root rot is a real and consistent primary symptom of the Texas disease.

Differences between the Texas isolates and those of *F. o. f. sp. betae* may warrant the designation of a new forma specialis, but we are unable to draw firm conclusions at this particular time. Morphologically, the isolates are somewhat atypical of *F. oxysporum* but very similar to that originally described as the causal agent of Fusarium yellows, *F. c. var. betae* (= *F. o. f. sp. betae*) (20). However, isozyme analyses show the Texas isolates to be distinct from isolates of *F. o. f. sp. betae*.

The teleomorph of *F. oxysporum* has not been observed, and, therefore, presumptive loci have not been confirmed by sexual compatibility of isolates. Because each protein band reflects a direct gene product, however, polymorphism between isozymes can be used to approximate genetic variance. Recent studies (2,3,15,17) have examined isozymes for distinguishing among species in several fungal genera and between formae specialis of *F. oxysporum*, with varied degrees of success.

On the basis of the phenotypic differences in isozymes and the additional root rot symptom caused by the Texas isolates, it is plausible that the Texas isolates are significantly different from *F. o. f. sp. betae* and warrant a new forma specialis designation. There is some precedence for this. Wiemer (21) distinguished Fusarium wilt of lupine caused by *F. o. f. sp. lupini* Snyder & Hans. from a wilt and root rot of lupine caused by what he named *F. o. f. sp. radicis-lupini*. Similarly, Jarvis and Shoemaker (8) used differences in temperature optima and disease symptoms (root and crown rot) to distinguish Fusarium wilt of tomato caused by *F. o. f. sp. lycopersici* (Sacc.) Snyder & Hans. from Fusarium crown and root rot of tomato caused by *F. o. f. sp. radicis-lycopersici*. Similarly, the Texas sugar beet isolate could be named *F. o. f. sp. radicis-betae* n.f. Before such a name is proposed, however, we believe that a larger pool of geographic isolates needs to be tested to determine if the differences are unique to the Texas isolates or occur within the larger geographic population of sugar beet pathogens.

Another complication in the nomenclature of this pathogen was introduced by Armstrong and Armstrong (1). On the basis of pathogenicity to common hosts within the Chenopodiales, they proposed that *F. o. f. sp. betae* should be a separate race (race 2) of *F. o. f. sp. spinaciae* (Sherb.) Snyder & Hans. It
is interesting to note that Armstrong and Armstrong (1) described *F. o. f. sp. spinaciae* as often causing a root rot on spinach. MacDonald and Leach (10) reported that *F. o. f. sp. betae* caused a vascular wilt disease in pigweed (*Amaranthus retroflexus* L.), also in the Chenopodiaceae. We have not conducted cross-inoculation studies on spinach or other species in the Chenopodiaceae with the Texas sugar beet isolates, nor have we compared our isolates with isolates of *F. o. f. sp. spinaciae*. These tests will be required before we can justify a new forma specialis. We have presented evidence that the root rot disease of sugar beet in Texas is distinct from Fusarium yellows and that there are significant differences between the two causal agents. Until other questions relating to the taxonomy are resolved, however, we will refer to the Texas pathogen as *F. o. f. sp. betae*, Texas strain.

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