PATHOGENICITY OF FUSARIUM OXYSPORUM CAUSING YELLOWS ON SUGARBEET IN THE RED RIVER VALLEY OF MINNESOTA AND NORTH DAKOTA

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In the late 1990s, *Fusarium oxysporum* was isolated from diseased sugarbeet plants in a field near Moorhead, MN that displayed symptoms typical of Fusarium yellows (4, 7, 10). By 2004, the disease was widespread in the Red River Valley (RRV), with about 5 to 10% of fields having some symptomatic plants (A. Cattanach, American Crystal Sugar Co., Moorhead, *personal communication*). Interveinal yellowing of older leaves typically began in mid-July and as the disease progressed, younger leaves turned yellow. Sometimes one side of the leaf was yellow or necrotic while the other half remained green. As symptomatic leaves died, they remained attached to the crown. Transverse sections of roots revealed a light gray-brown discoloration of the vascular tissue (specifically, the xylem, which conducts water), but no external rotting of roots. Fusarium yellows reduces yield and percent sucrose (10).

Fusarium yellows of sugarbeet is caused by the soilborne fungus *F. oxysporum* f. sp. *betae*. The fungus also is pathogenic to spinach and some common weeds including lamb's-quarters and pigweed (8). *F. oxysporum* f. sp. *betae* is documented as causing yellows of sugarbeet in California (3), Texas (6), Colorado, Montana, Nebraska, New Mexico, Oregon, South Dakota, and Wyoming where it causes yellows and wilt, stalk rot in seed production fields, and storage rot (2).

OBJECTIVES

The purpose of this research was to: 1) identify isolates of *Fusarium* cultured from sugarbeet roots with symptoms of Fusarium yellows and 2) compare pathogenicity of these isolates with known isolates of *F. oxysporum* f. sp. *betae*.

MATERIALS AND METHODS

Thirty-five plants collected from eight fields with symptoms of Fusarium yellows were submitted to our laboratories by agriculturists and sugarbeet industry personnel. Discolored vascular tissue was excised from roots (occasionally from stem pieces), surface-sterilized, and placed on potato dextrose agar (PDA). Isolates were purified by selection of single conidia and grown on two media (homemade PDA and carnation leaf agar) under fluorescent and black lights (16 hour photoperiod). After 10 to 14 days, isolates were identified to species following the taxonomic keys of Nelson et al. (9).

Pathogenicity was determined by dipping roots of 5-week-old sugarbeet plants ('ACH 9363') in a suspension of 10^4 conidia/ml for 8 minutes (5). Ten to 12 plants were inoculated per isolate; individual plants were a replicate. Plants then were transplanted in cone-tainers (1.5-inch diameter x 8.25-inch depth) containing sterilized soil. Three known isolates of *F. oxysporum* f. sp. *betae* (FOB, pathogenic to sugarbeet) also were included in the trials. Two isolates of FOB (13 and 216c) were from Linda Hanson (USDA-ARS, Fort Collins, CO) and the other (0-1122) was from the Fusarium Research Center, The Pennsylvania State University, University Park. The control was sterile water. Plants were placed in a greenhouse at 75 - 81 F with natural light, supplemented with illumination from high pressure sodium-vapor lamps for 16 hours daily, and lightly fertilized biweekly to avoid chlorosis from nutrient deficiency. After 6 to 7 weeks, plants were rated for disease on a 0 to 4 scale: 0 = no disease; 1 = plant stunting slight to extreme, leaves may be wilted; 2 = chlorotic leaves, some with necrosis at margins; 3 = tap root dried and brown to black in color, leaves dying; and 4 = plant dead (1). The experiment was repeated. Disease ratings were

subjected to Analysis of Variance (ANOVA) and when significant ($P \le 0.05$), means were separated by Least Significant Difference (LSD).

RESULTS

F. oxysporum was isolated from 25 plants. Of the other 10 plants, no fungi were isolated from six, *F. solani* was isolated from three, and a *F. roseum*-type was isolated from one root.

Disease severity differed between trials, but all isolates of F. oxysporum and FOB resulted in disease ratings statistically ($P \le 0.05$) greater than the water control (<u>Table 1</u>). In Trial 1, isolates of F. oxysporum averaged a disease rating of 3.3 (range: 2.7-3.7) and FOB averaged 3.1 (range: 2.7-3.4) compared to 0.2 for the water control. Eight of 12 isolates of F. oxysporum resulted in disease ratings statistically higher than the least pathogenic isolate of FOB (number 13). In Trial 2, isolates of F. oxysporum averaged a disease rating of 2.1 (range: 1.8-3.3) and FOB averaged 2.1 (range: 2.0-2.2) compared to 0.1 for the water control. One isolate of F. oxysporum had a statistically higher disease rating than cultures of FOB. Isolates of F. oxysporum and FOB recovered from inoculated plants were identical to those inoculated onto plants.

Table 1. Severity of Fusarium yellows caused by cultures of *Fusarium oxysporum* isolated from sugarbeet plants in the Red River Valley compared to known pathogenic cultures of *F. oxysporum* f. sp. *betae* (FOB) and a water control.

Treatment	Disease Severity ^{YZ}	
	Trial 1	Trial 2
Fusarium oxysporum cultures X		
36	3.7 a	2.3 bc
16	3.6 ab	1.9 bc
35	3.5 abc	1.9 bc
28	3.4 abc	3.3 a
17	3.4 abc	1.8 c
37	3.4 abc	2.1 bc
33	3.4 abc	2.4 b
20	3.4 abc	2.0 bc
18	3.0 bcd	2.1 bc
24	3.0 cd	1.9 c
13	3.0 cd	1.9 c
25	2.7 d	2.1 bc
FOB 0-1122	3.4 abc	2.0 bc
FOB 216c	3.0 bcd	2.2 bc
FOB 13	2.7 d	2.2 bc
Water Control	0.2 e	0.1 d
$LSD (P = 0.05)^{\mathbb{Z}}$	0.6	0.5

Cultures of *F. oxysporum* isolated from diseased sugarbeet plants collected in the Red River Valley of Minnesota and NorthDakota; FOB = cultures of *F. oxysporum* f. sp. *betae* known to be pathogenic to sugarbeet. Plants inoculated by root dip when 3-weeks old; 10⁴ conidia/ml.

Disease ratings on a 0 to 4 scale, 0 = no disease, 4 = death of plant. Trials 1 and 2 evaluated 6 and 7 weeks after inoculation, respectively. Each value in Trial 1 is based on 12 plants and in Trial 2 on 10 plants.

LSD = Least Significant Difference. For each column, values followed by the same letter are not significantly different, $P \le 0.05$.

DISCUSSION

Our trials confirm that isolates of *F. oxysporum* from sugarbeet with symptoms of Fusarium yellows in the RRV are *F. oxysporum* f. sp. *betae*. Although disease severity varied somewhat among isolates and between trials, all caused moderate to severe symptoms of Fusarium yellows comparable to known isolates of *F. oxysporum* f. sp. *betae*. Hanson et al. (5) found only 26 of 98 isolates of *F. oxysporum* collected from yellowed sugarbeet plants in seven states (not including the RRV) were *F. oxysporum* f. sp. *betae*; these pathogenic isolates were from Colorado, Montana, and Oregon. Analysis of isolates of *F. oxysporum* f. sp. *betae* by random-amplified polymorphic DNA markers indicates considerable genetic diversity, although there appears to be some clustering of isolates from different regions of the United States (3, 5).

Fusarium yellows is a general term to describe yellowing of sugarbeet foliage, however, this symptom does <u>not</u> always mean infection by *F. oxysporum* f. sp. *betae*. Vascular discoloration in roots (and sometimes in stems) of yellowed plants is a more reliable symptom of infection by *F. oxysporum* f. sp. *betae* than yellowing of foliage. *F. oxysporum* f. sp. *betae* infects the vascular system of sugarbeet roots (as do species of *Verticillium*). The combination of fungal mycelium; production of fungal toxins, polysaccharides and other metabolites; and production of gels as plant cell walls decompose, results in disruption of water movement upward in the plant - and typical symptoms of Fusarium yellows. In Texas, another form of Fusarium yellows of sugarbeet also occurs and is caused by F. *oxysporum* f. sp. *radicis-betae* (6). This closely related pathogen causes all symptoms typical of Fusarium yellows (e.g., vascular discoloration, yellowed and wilted foliage) but <u>also</u> produces severe tip rot on the distal end of the taproot. Other *Fusarium* species, such as *F. solani*, *F. moniliforme*, *F. avenaceum*, and *F. acuminatum* also are reported as pathogenic to sugarbeet (5). These species can cause yellowing of foliage and typically are associated with seedling disease and cortical root rot; they also infect plants as secondary invaders.

F. oxysporum f. sp. betae is a difficult pathogen to control (4, 7). It survives in soil and infected plant debris as chlamydospores, spores, and mycelium. Consequently, crop rotations can not be relied on to reduce inoculum to "safe levels" because chlamydospores survive for many years. Weed control also is important since the pathogen infects some common species including lambs' quarters and pigweed (8). Spread to non-infested fields can be reduced by cleaning equipment after use in infested fields. Plant resistance is available in other regions of the United States where the disease occurs. There appear to be differences in resistance among commercially available varieties grown in the RRV.

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