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## ***Fusarium* Species Causing Fusarium Yellows of Sugarbeet.**

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### **ABSTRACT**

**Species of *Fusarium* isolated from sugarbeet with Fusarium yellows symptoms from throughout the Western U.S.A. in 2001 were examined for pathogenicity on sugarbeet in greenhouse tests. Thirteen pathogenic isolates were obtained, of which the majority (69%) were *F. oxysporum*. However, four other species were found that caused symptoms on sugarbeet that were indistinguishable from Fusarium yellows caused by *F. oxysporum* f. sp. *betae*. These included isolates of *F. acuminatum*, *F. avenaceum*, *F. solani*, and *F. moniliforme*. Of these, only *F. acuminatum* had previously been reported to cause yellows symptoms on sugarbeet in the United States. The presence of additional species in sugarbeet has important implications for disease management.**

**Key words.** *F. verticillioides*, Fusarium wilt, *Beta vulgaris*

**F**usarium yellows causes significant reduction in root yield, as well as reduced sucrose percentage and juice purity in affected sugarbeet (*Beta vulgaris* L.) (Schneider & Whitney 1986). The disease is characterized by wilting and interveinal yellowing of the leaves, usually starting with older leaves. As leaves die, leaves generally remain attached to the crown with petioles that are tan in color. Internal symptoms consist of brown or gray-brown vascular discoloration (Schneider & Whitney 1986, Franc et al. 2001). On plants grown for seed, the seed stalk can be blighted (Schneider & Whitney 1986). The primary causal agent of Fusarium yellows in sugarbeet is *Fusarium oxysporum* Schlechtend.Fr. f. sp. *betae* (Stewart) Snyder & Hans. (FOB) (Schneider & Whitney 1986, Ruppel 1991). In addition, *F. acuminatum* Ell. & Ev. sensu Gordon has been reported to cause Fusarium yellows symptoms (Ruppel 1991). *Fusarium* species also cause seedling disease, with

isolates of *F. acuminatum* (Ruppel 1991), *F. avenaceum* (Fr.) Sacc. (Mukhodpadhyay 1987, Ruppel 1991), *F. moniliforme* Sheldon (Mukhodpadhyay 1987) and *F. oxysporum* (Ruppel 1991) causing seedling wilt or seedling yellows. In addition, *F. solani* (Mart.) Appel & Wollenw. emend Snyder & Hans. causes a seedling damping-off (Abada 1994, Ruppel 1991) or root rot of mature plants (Abada 1994, Maxon 1948). *F. oxysporum* also can cause a root rot of sugarbeet (Martyn et al. 1989, Abada 1994, Franc et al. 2001, Bürcky 2003), but a different *forma specialis* than the yellows pathogen, *F. oxysporum* f.sp. *radicis-betae* Harv. & Rush is reported to be the cause (Harveson and Rush 1998). *F. culmorum* (W.G. Smith) Sacc. has been reported to cause a crown rot of sugarbeet under drought conditions (Hull 1960).

In 2001, isolations were made from sugarbeets with foliar yellows symptoms or seed stalk blight. Fungi identified as *Fusarium* species were screened for pathogenicity on sugarbeet in a greenhouse assay. Plants were examined for yellows symptoms and root rot development. As described herein, isolates of several *Fusarium* species were found that caused yellows symptoms on sugarbeet.

## MATERIALS AND METHODS

Beets showing external symptoms of *Fusarium* yellows (yellowing, wilting, foliar necrosis) were collected from sugarbeet growing areas throughout the western U.S. *Fusarium* was isolated from these beets by cutting samples of internal tissue from the tap root or crown and surface disinfesting tissue with 0.5 % sodium hypochlorite. One sample with seed stalk blight from Oregon also was sampled by surface disinfesting seed stalk tissue showing vascular discoloration with 0.5 % sodium hypochlorite. Tissue was rinsed with sterile distilled water and plated on half-strength potato dextrose agar (PDA, Becton Dickinson and Co., Sparks, MD). Fungi that grew out were transferred to fresh PDA by hyphal-tip transfer (Windels 1992) to obtain pure cultures.

*Fusarium* isolates were identified according to the taxonomy of Nelson et al. (1983). For identification, isolates were plated on full strength PDA for color determination, and onto carnation leaf agar (CLA, Nelson et al. 1983) to examine spores and sporophores.

All *Fusarium* isolates were maintained on PDA. For long-term storage, isolates were stored desiccated on sterile filter paper at -20° C. Briefly, isolates were plated on water agar and sterile glass microfiber filter paper pieces (approximately 1 cm<sup>2</sup>) were placed equidistant from the point of transfer. When the fungus had grown through and beyond all filter paper sections, filter paper pieces were removed, placed in

sterile paper envelopes, and dried overnight over desiccant in a biocontainment hood, then stored over desiccant at  $-20^{\circ}\text{C}$ .

For inoculum preparation, a 4 mm diameter plug of fungal hyphae was transferred from the actively growing edge of a fungal colony on PDA to fresh PDA. Dishes with the fungi were incubated under 10 hr light/14 hr dark at  $22\text{-}25^{\circ}\text{C}$  for two weeks. Inoculum for pathogenicity tests was prepared by flooding individual culture plates with 5 ml of sterile water and scraping with a sterile bent glass rod to release hyphal material and spores. The contents of seven to 10 plates were poured through sterile cheesecloth into a beaker to screen out agar and large chunks of mycelium. The spore concentration was determined with a hemacytometer.

All of the *Fusarium* isolates were tested for pathogenicity on a *Fusarium*-susceptible sugarbeet released multigermline germplasm, 'FC 716' (Panella et al. 1995) in the green house. Sugarbeet seeds were broadcast in 12.7 cm<sup>2</sup> plastic trays and grown for five weeks in pasteurized potting mix (Scotts MetroMix 200, Marysville, OH). Beets were removed from soil and washed. Roots were soaked in a *Fusarium* spore suspension (macroconidia, approximately  $10^4/\text{ml}$ ) for 8 min, with the spore suspension shaken approximately every 60 sec. Control plants were soaked in sterile water for 8 min. Plants were replanted into saturated pasteurized potting mix in 3.8 cm diameter, 21 cm deep individual planting cones (Steuwe & Sons, Inc. Corvallis, OR), 10 plants per treatment. Cones were placed in cone trays in a completely randomized design with spaces between cones to prevent cross contamination. Plants were maintained for two days in an approximately  $22^{\circ}\text{C}$  greenhouse to reduce transplant shock. After two days, plants were moved to an approximately  $26\text{--}28^{\circ}\text{C}$  greenhouse. Plants were fertilized every two weeks with 15-30-15 fertilizer (Miracle-Gro, Scotts Miracle-Gro Products, Inc. Marysville, OH) and watered daily, with care taken to prevent splashing of soil or water between containers. Plants were independently examined and rated weekly for symptoms for six weeks by two individual raters using a rating scale of 0 to 4 with 0 = no disease, plants green and healthy, 1 = plants slightly stunted to extremely stunted, leaves may be wilted, 2 = leaves chlorotic, necrosis at edges of leaves, 3 = crown becoming dried and brown to black in color, leaves dying, and 4 = death of the entire plant. An average rating determined for each treatment. After six weeks, whether symptoms developed or not, plants were harvested and roots sampled to re-isolate the *Fusarium* used in the inoculation. Control plants also were sampled.

Isolates usually reported only as seedling pathogens were tested on eight-week-old beets to ensure that more mature plants also exhibited

symptoms. Only isolates that were pathogenic on five-week-old plants were tested on the older plants. Methods were similar to those for the five-week-old plants, except that beets were planted into plastic pots (25.4 cm diameter) instead of planting cones.

For isolation of *Fusarium* from greenhouse-grown plants, the tap roots were collected from at least two randomly selected plants from each treatment. Root were washed under running tap water, cut into sections of approximately 1 cm each, and surface disinfested in 0.5 % sodium hypochlorite for 30 sec. Root tissue was blotted dry on sterile filter paper and placed on PDA dishes. Dishes were incubated as described for the *Fusarium* isolates (above), and examined daily for fungal growth. Fungi isolated from inoculated plants were identified to species and compared phenotypically to the isolate used for inoculations. For more mature plants, isolations were similar to those used on the original root samples (above). All pathogenicity tests were conducted at least two times.

## RESULTS

In 2001, a total of 62 *Fusarium* isolates were collected from 69 samples. No other known fungal wilt pathogens were isolated. The *Fusarium* species isolated from symptomatic sugarbeet included: 36 *F. oxysporum*, seven *F. equiseti* (Corda) Sacc., six *F. solani*, five *F. proliferatum* (Matsushima) Nirenberg, five *F. avenaceum*, two *F. acuminatum*, and one *F. moniliforme* (= *F. verticillioides* (Sacc.) Nirenberg). Of these isolates, 13 were pathogenic on sugarbeet using our greenhouse assay, including nine *F. oxysporum*, classified as FOB. One isolate each of *F. acuminatum*, *F. avenaceum*, *F. solani* and *F. moniliforme* caused disease symptoms typical of *Fusarium* yellows, including stunting, interveinal yellowing, wilting during the heat of the day, and foliar necrosis. Some FOB isolates caused plant death. On plants inoculated at 5-weeks, four of the FOB and all isolates of other species were rated as being moderately virulent (average ratings 2.0 to 3.0) on a scale where a rating of 3.5 to 4 (complete plant death) within six weeks is highly virulent and a rating of less than 1.5 throughout the six week period following inoculation is considered to be non-pathogenic. The other five FOB isolates were highly virulent (average ratings between 3.4 and 4). No vascular discoloration was observed in the roots of control plants or plants inoculated with non-pathogenic isolates. Vascular discoloration could be observed in the roots of some beets with symptoms, but not in all roots. Some roots were too small to clearly see discoloration and vascular discoloration could not be observed in the roots of dead plants if plants

had been dead for more than a week.

All isolates could be re-isolated from beet roots at 6 weeks after inoculation. *Fusarium* isolates that matched the species used in inoculations were isolated from all beets with symptoms. *Fusarium* isolates that were non-pathogenic in this test also could be isolated from symptomless beets. No *Fusarium* species were isolated from any of the roots other than the one applied to the plant.

The same *F. acuminatum*, *F. avenaceum*, *F. solani*, and *F. moniliforme* isolates that caused disease on five-week-old beets also caused yellows symptoms on eight-week-old beets. Symptoms were similar for all isolates and similar to those caused by moderately virulent FOB isolates. Symptoms were less severe on plants inoculated at eight weeks than on plants inoculated at five weeks. Foliar symptoms included interveinal yellowing, stunting, wilting, and foliar necrosis. No plants died during the six weeks following inoculations. Vascular discoloration was found in the roots of all plants showing symptoms. Roots of control plants showed no vascular discoloration.

Pathogenic isolates were from three different states, consisting of: five *F. oxysporum*, one *F. avenaceum*, and one *F. acuminatum* from Oregon; one *F. solani* from Nebraska; and four *F. oxysporum* and one *F. moniliforme* from Colorado (Table 1). Two *F. oxysporum* isolates were obtained from the seed stalk sample.

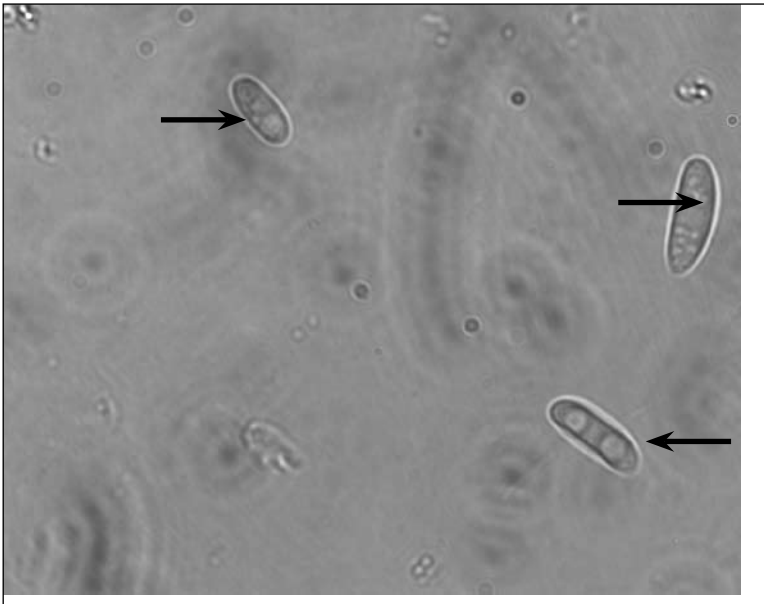
## DISCUSSION

One isolate each of *F. acuminatum*, *F. avenaceum*, *F. solani*, and *F. moniliforme* caused moderate levels of Fusarium yellows symptoms. An isolate of *F. acuminatum* from Colorado previously had been reported to cause yellows-type symptoms in sugarbeet (Ruppel 1991), but *F. avenaceum* and *F. solani* variously have been reported to cause seedling disease (Abada 1994, Ruppel 1991), root rot (Abada 1994, Maxon 1948) or postharvest rot (Bosch & Mirocha 1992) but not typical yellows symptoms. *Fusarium moniliforme* (= *F. verticillioides*) also has been reported to cause seedling damping-off (Mukhodpadhyay 1987), but not yellows.

Additional isolates of several of these species and of other species were obtained from sugarbeet but did not cause symptoms. The presence of *Fusarium* isolates from the symptomless roots of sugar beet is consistent with the work of Ruppel (1991), in which non-pathogenic isolates of several *Fusarium* species, including *F. oxysporum*, were isolated from roots. The presence *F. oxysporum* non-pathogenic on beet also is consistent with reports that other *formae speciales* of *F. oxyspo-*

*rum* can be isolated from symptomless sugarbeet roots (Gordon et al. 1989, Wickliffe 2001). Isolation from roots was unlikely to be due to residue from inoculation since *Fusarium* morphologically similar to the isolate used in inoculations could be isolated from root segments that had developed after the time of inoculation.

*Fusarium acuminatum* and *F. avenaceum* can be easily distinguished from the other pathogenic *Fusarium* species found in this study. Neither of these species produce abundant microconidia, while the other species generally produce large numbers of primarily single-celled microconidia (Fig. 1) on CLA (Nelson et al. 1983). The macroconidia of *F. acuminatum* and *F. avenaceum* also have an elongated apical cell (Fig 2A & 2B) as compared to the other pathogenic species found. A major difference among the species in this investigation was their pigmentation on PDA. Both the *F. acuminatum* and the *F. avenaceum* in this study produced a red pigmentation typical of these species (Booth 1977, Nelson et al. 1983). The undersurface of the *F. oxysporum* in this study on PDA were colorless to tan to purple, as is typical for this species (Nelson et al. 1983). For *F. solani* examined in this study, the undersurface of PDA cultures was either cream or blue, both of which have been reported for this species (Booth 1977, Nelson et al. 1983)



**Fig. 1.** Typical *Fusarium* single-celled microconidia (1000 X) (shown *F. oxysporum*). Microconidia are indicated by arrows.

**Table 1.** Characteristics of *Fusarium* isolates pathogenic on sugar beet in greenhouse tests.

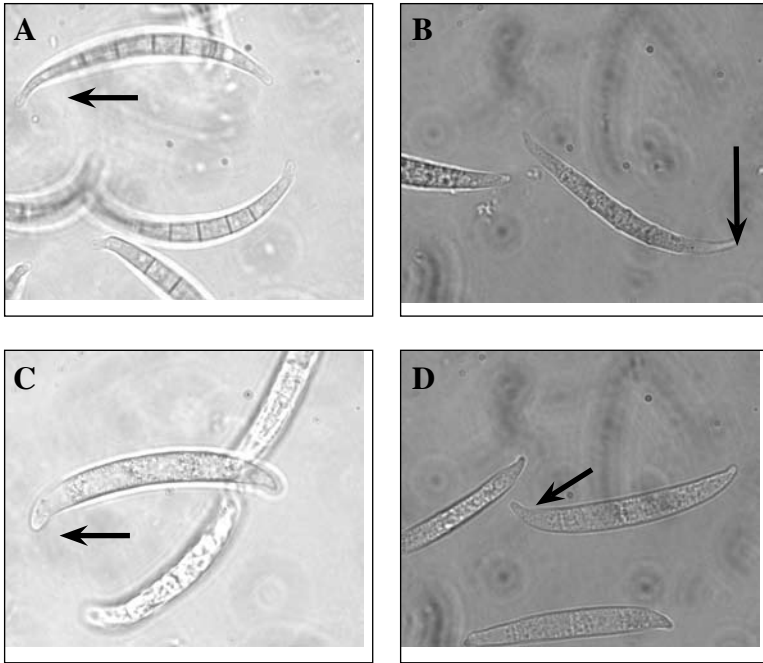
Name	Species	State <sup>1</sup>	Virulent <sup>2</sup>	Macro <sup>3</sup>	Color <sup>4</sup>	Tissue <sup>5</sup>
F0 117	<i>oxysporum</i>	OR	HV	scarce	dark purple	crown
F0 119	<i>oxysporum</i>	OR	HV	scarce	tan to purple	crown
F0 120	<i>avenaceum</i>	OR	MV	abundant	dark red	crown
F0 124	<i>acuminatum</i>	OR	MV	abundant	red	crown
F0 128	<i>oxysporum</i>	CO	MV	abundant	tan	tap root
F0 132	<i>oxysporum</i>	OR	MV	scarce	tan to orange	seed stalk
F0 137	<i>solani</i>	NE	MV	abundant	cream	tap root
F0 138	<i>oxysporum</i>	OR	HV	scarce	dark purple	crown
F0 142	<i>oxysporum</i>	OR	HV	scarce	dark purple	crown
F0 144	<i>moniliforme</i>	CO	MV	scarce	white, purple streaks	tap root
F0 146	<i>oxysporum</i>	OR	HV	scarce	violet	seed stalk
F0 148	<i>oxysporum</i>	CO	MV	present	white	tap root
F0 149	<i>oxysporum</i>	CO	MV	abundant	cream, purple flecks	tap root

<sup>1</sup>State from which sample yielding pathogenic isolate was obtained.

<sup>2</sup>Virulence of isolates on sugarbeet in the greenhouse. Highly virulent isolates had average ratings of 3.4 or higher by six weeks after inoculation.

<sup>3</sup>The relative prevalence of macroconidia on CLA cultures. Scarce means that macroconidia were difficult to find and sporodochia generally were not visible on plates. Present indicates that a few sporodochia were observed and macroconidia were easily found in sporodochia. Abundant indicates that multiple sporodochia were present and macroconidia were easily found in sporodochia and throughout the culture.

<sup>4</sup>Color of the undersurface of the culture on PDA.



**Fig. 2.** Macroconidia of *Fusarium* (all shown 1000 X). Arrows indicate apical cells. (A) *F. acuminatum* thin, curved macroconidium with elongated apical cell. (B) Slender *F. avenaceum* macroconidium with most of the curvature in the elongated apical cell. (C) *F. solani* stout, thick-walled macroconidium with blunt and rounded apical cell. (D) *F. oxysporum* thin-walled macroconidium with attenuated apical cell.

(colors not shown).

*Fusarium avenaceum* and *F. acuminatum* can be distinguished from each other particularly by the production of chlamyospores, which are thick walled resting spores (Fig. 3). Chlamyospores are produced by *F. acuminatum* while no chlamyospores are produced in the hyphae of *F. avenaceum* (Booth 1977, Nelson et al. 1983). In addition, the macroconidia of *F. acuminatum* on CLA are usually strongly curved along the whole conidium (Fig. 2A) while for *F. avenaceum* curvature, if present, is primarily in the apical cell (Fig. 2B) (Nelson et al. 1983).

We isolated one *F. moniliforme* (= *F. verticillioides*) that was pathogenic on sugarbeet. This species has not previously been reported to cause yellows symptoms. However, *F. moniliforme* and *F. oxysporum* are very similar, and *F. moniliforme* often can be confused with *F. oxysporum* (Nelson et al. 1983). Thus it may be that this species has

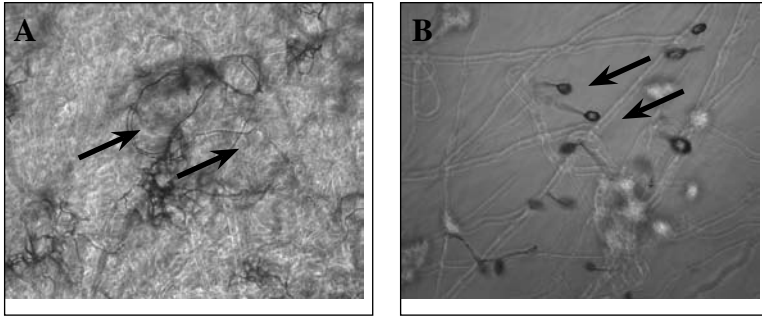


**Fig. 3.** Thick-walled chlamydospore of *Fusarium*. (1000 X). Shown is a single chlamydospore from *F. oxysporum* with thick wall.

been found on sugarbeet before but was identified as *F. oxysporum*. The most easily observed morphological difference is that on CLA *F. moniliforme* produces microconidia in chains (Fig. 4A) while in *F. oxysporum*, microconidia are formed in false heads (Fig. 4B) (Nelson et al. 1983).

*Fusarium solani* can be distinguished from the other species by several characteristics, including pigmentation (above) and the shape of the macroconidia on CLA. *Fusarium solani* macroconidia are thick-walled with a blunt and rounded apical cell (Fig. 2C) (Nelson et al. 1983). The macroconidia of *F. oxysporum* are thin-walled and have an attenuated apical cell (Fig. 2D) (Nelson et al. 1983).

While we classified the *F. oxysporum* isolates that caused yellows symptoms as FOB, these isolates have been reported to be a subgroup within *F. oxysporum* f. sp. *spinaciae* (Sherb.) Syd. & Hans. (FOS) (Armstrong & Armstrong 1976), and this taxonomy has been accepted for the American Phytopathological Society listings of Common Names of Plant Diseases (<http://www.apsnet.org/online/common/top.asp>). However, no morphological or genetic comparisons were done among the isolates in the Armstrong and Armstrong study and only a small



**Fig. 4.** Forms of production of microconidia in *Fusarium*. A - *F. moniliforme* microconidia in chains. B - *F. oxysporum* microconidia in false head. (shown 100 X) Arrows indicate spores produced.

number of isolates were tested (12 FOS from Virginia and Arkansas, and 12 FOB from Colorado and Montana). The lack of morphological or genetic comparisons and the small number of isolates used makes the combining of FOB into FOS somewhat preliminary, thus we used the classification of FOB.

The isolate of *F. acuminatum* in this study is from Oregon. The previously reported pathogenic *F. acuminatum* isolate was from Colorado (Ruppel 1991). This demonstrates that isolates of this species that are pathogenic on sugarbeet can be found in different geographic regions.

The ability of *Fusarium* species other than FOB to cause yellows symptoms on sugarbeet has implications for disease resistance breeding. Most *Fusarium* yellows resistance has been developed using FOB isolates to screen for resistance (as referenced for Great Western Sugar Co. in Ruppel 1991). However, there have been reports of loss of disease control when beets are planted in different areas (S. Godby, personal communication). While some of this lack of control may be due to variability in the FOB in different areas (Harveson & Rush 1997, Ruppel 1991), some lack of control might be due to infection by other *Fusarium* species, such as those Ruppel (1991) documented and those described here. The low number of isolates of other *Fusarium* species pathogenic on sugarbeet, compared to the number of FOB (four of the combined other species vs nine FOB in the current study) may indicate that this is a minor phenomenon. However, in some of the yellows samples received, the only pathogens found were *Fusarium* species other than FOB. It may be that these other species are the primary *Fusarium* in some fields or geographic areas.

In addition to concerns about disease resistance, the ability of dif-

ferent *Fusarium* species to cause Fusarium yellows can impact disease control recommendations. A method recommended for Fusarium yellows control is rotation with other crops (Schneider & Whitney 1986). It has been suggested that control by rotation is limited because of the wide host range for *F. oxysporum* (Schneider & Whitney 1986), but lack of control with rotation may in part be due to the activity of some of these other species. For example, small grains are included in recommended rotation crops for Fusarium yellows control (Anonymous 2000, Schwartz et al. 2001). However, *F. acuminatum* and *F. avenaceum* can be pathogenic on small grains (Hill & Blunt 1994, Mathre 1997, Wiese 1987) and other crops (Secor & Salas 2001). Similarly, *F. moniliforme* can be a pathogen of corn (Möller et al. 1999, Shim & Woloshuk 2001) and small grains (Bottalico & Perrone 2002) and *F. solani* has a broad host range (Bottalico & Perrone 2002, Li et al. 2000). Thus the impact of rotation on isolates of these species pathogenic on sugarbeet needs to be determined.

FOB has variously been reported to have poor production of macroconidia (Stewart 1931) or to have abundant macroconidia (Ruppel 1991). Ruppel (1991) suggested that some of this variability may be due to differences in culture conditions. While this may be a factor, our isolates varied in macroconidia production on both PDA and CLA, with some isolates producing abundant macroconidia on these media, as did Ruppel's (1991), while others produced only scarce macroconidia on these media. Many of the most virulent isolates produced few macroconidia (Table 1); similar to the report of Stewart (1931), while less virulent and nonpathogenic isolates frequently produced more abundant macroconidia. The *F. moniliforme* isolate also produced few macroconidia.

The low number of pathogenic isolates obtained from these samples, 13/62 (21%) of the total isolates obtained, is consistent with previous research, and is similar to the 12/48 (25%) pathogenic isolates Ruppel (1991) found from beets with yellows symptoms. Wickliffe (2001) also found a low percentage (17%) of FOB isolates from beets with symptoms, and in similar work with *F. oxysporum* from dry bean an even lower percentage, 3% (Cramer et al. 2003) was detected. Harveson & Rush (1997) found a much higher percentage of isolates pathogenic (82.5%). However, the Harveson & Rush (1997) study included *F. oxysporum* f. sp. *radicis-betae* (FORB). It may be that FORB is more competitive than FOB and thus more likely to be isolated.

Some of the yellowing or wilting in samples received may have been due to problems other than *Fusarium*. Yellowing or wilting in sugarbeet can be caused by a number of other factors, including viruses (Whitney

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and Duffus 1986, Franc et al. 2001), nutritional problems (Whitney and Duffus 1986), herbicide damage (Whitney and Duffus 1986), insects (Whitney and Duffus 1986, Hein and Johnson 2001), nematodes (Whitney and Duffus 1986, Franc et al. 2001), and other fungi (Whitney and Duffus 1986, Franc et al. 2001). No other known wilt or yellows inducing fungi were isolated from these plants, and nematodes and insects or insect feeding damage were not observed, but these could be involved in some samples. It is possible that some of the *Fusarium* isolates causing yellows were not identified. Some of the isolates found produced scarce macroconidia, and other isolates might not produce macroconidia at all, similar to the two nonsporulating isolates reported by Ruppel (1991). Such isolates might not be identified as *Fusarium*. It also may be that some pathogenic isolates are poor competitors and that saprophytic isolates out compete pathogens either in the root or on culture plates.

In order to determine the importance of different *Fusarium* species to Fusarium yellows in beets, a larger survey both of infected beets and of *Fusarium* isolates would need to be conducted. Such a study would need to be conducted using a method like hyphal-tip isolation, since some *F. oxysporum* f. sp. *betae* produce scarce macroconidia (Stewart 1931, current study), as do some other *Fusarium* species (Nelson et al. 1983, Seifert 2001). Isolation by single spore transfer may tend to favor the isolation of *Fusarium* species and *F. oxysporum* isolates that produce abundant macroconidia, and reduce the probability of isolating pathogens that do not sporulate as profusely.

### ACKNOWLEDGEMENTS

The authors would like to thank L.A. Volpe and R. Miskimins for their assistance in working with the fungi and the greenhouse assay. In addition, this work could not have been done without the assistance of sugarbeet growers, seed company representatives, and agriculturalists who provided beet samples from which to recover isolates. We would like to thank the Western Sugar Company - Grower Joint Research Committee and the Beet Sugar Development Foundation for partial funding of this research.

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