The Association of Fusarium oxysporum f. sp. betae with Nonprocessed and Processed Sugarbeet Seed

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ABSTRACT

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A blight that is caused by *Fusarium oxysporum* f. sp. *betae* in sugarbeet (*Beta vulgaris*) seed plants occurs in the Willamette Valley of Oregon. Seeds collected from severely diseased plants were infested with the pathogen at a level of 0.45% and 0.23% of the seed from a heavily diseased commercial planting carried the pathogen. *Fusarium* was eliminated from seed by surface disinfestation in NaOCl or repeated washing with sterile distilled water, which indicated that its presence on sugarbeet seed was as an external contaminant. Commercial processing of sugarbeet seed, in which superficial tissues are milled away and discarded, significantly reduced the percentage of infested seed. Although the pathogen was found on seed, seedlings produced from naturally infested seed planted in pasteurized greenhouse soil were not diseased. Although highly susceptible seed-bearing parents may be severely diseased, the low level of seed-borne inoculum and relatively greater resistance of hybrid cultivars appears to make the transmission of this pathogen less efficient than that of other seed-borne wilt fusaria.

Additional key words: seed transmission, seed-borne fungus.

A blight caused by *Fusarium oxysporum* Schlecht. f. sp. *betae* (Stewart) Snyd. and Hans. in sugarbeet (*Beta vulgaris* L.) seed crops in the Willamette Valley of Oregon was reported in 1973 by Gross and Leach (9). Although the pathogen can attack sugarbeet seed crops shortly after planting in August-September when it causes damping-off of young seedlings, the most noticeable symptoms occur in the spring and summer after the crops have been overwintered in the field to induce bolting. Developing seed stalks of infected plants may exhibit leaf wilting, vascular necrosis, and premature death. In fields where the blighting of seed stalks is severe, yield losses may result.

Gross and Leach (9) reported that 1-3% of the seed harvested from severely diseased fields carried the pathogen. This is significant because most of the seed used in commercial sugarbeet production in the western United States is grown in the Willamette Valley. Prior to the report of *F. oxysporum* f. sp. *betae* in Oregon (9), it had not been reported outside of the Rocky Mountain area where it was first described by Stewart (19) in 1931. There is now concern that it may be introduced into new areas with contaminated seed.

Several vascular fusaria have been reported to be seed-borne. Snyder (18) found *F. oxysporum* f. sp. *pisi* to be associated with pea seed, but the level of infestation was so low that he could not determine how the pathogen was carried. Other reports (5, 6, 7, 8, 11) provide evidence that pathogens are carried both internally and externally, whereas some workers (2, 10) reported the presence of fusaria only as external contaminants of seed. Singh et al. (17) found that the cumin wilt pathogen (*F. oxysporum* f. sp. *cumini*) contaminates cumin seed externally during threshing. They felt, however, that the pathogen under favorable conditions was able to penetrate the seed coat and be carried internally as well. In a study of *Plantago* Russell (16) reported a seed-borne *F. oxysporum* pathogen which apparently is present in superficial seed tissues, since the level of infestation was reduced following the abrasive removal of external seed layers for the extraction of mucilage.

Sugarbeet seed differs from that of many crops, because its corky, superficial seed tissues are milled away and discarded before sale. This processing results in hard, compact seed of uniform size for use in mechanical planters, and has been found to decrease the amount of *Phoma betae* carried on sugarbeet seed (Leach and MacDonald, *unpublished*).

The study reported here was undertaken to determine how *F. oxysporum* f. sp. *betae* is carried on sugarbeet seed and whether processing reduces the level of seed-borne inoculum.

MATERIALS AND METHODS

Seed samples were obtained from two commercial plantings following the harvest in August, 1973. One seed sample (Hybrid cultivar USH10B-1, lot #3274) was obtained from a field in which seed stalks did not die until late in the growing season and seed yield was normal. The other (GWH61-73R, lot #3179) was obtained from a heavily diseased crop in which seed yield was reduced to one-half that obtained from nearby unaffected fields of the same sugarbeet lines. Whole seed (before cleaning or processing) was obtained from both lots. A large sample of partially processed seed (cleaned and reduced in bulk about 20% prior to shipment to a contracting sugar company) was obtained from lot 3179. Approximately one-half of this sample was sent to the Great Western Sugar Co., Longmont, Colo., where it was processed and sized as commercial seed and returned. This gave a complete series of processing steps from a single seed lot

that was presumed to carry a high level of *Fusarium*. To obtain seed with a maximum level of contamination, individual severely diseased plants were collected during the August 1974 harvest period from several fields producing USH10B seed. The seed from these plants (designated USH10B-H) was harvested by hand, and separated from seed stalk debris by screening with a 2-mm (No. 10) sieve.

A modified peptone-PCNB medium (15) and a modified Martin's rose bengal medium (RBA) as described by Abawi and Lorbeer (1), but without Dexon®, were used in assays of sugarbeet seed for *F. oxysporum* f. sp. *betae*. Both media were equally effective for seed assays when made up to a concentration of 50 μg 2,4-dichlorophenoxyacetic acid (2,4-D) per ml medium in cooled agar to inhibit seed germination and seedling development. Preliminary tests indicated that this concentration of 2,4-D had no noticeable effect on the growth in culture of *F. oxysporum* f. sp. *betae*. Potato-dextrose agar (PDA) was used throughout this study as a general culture medium.

Intact seeds were assayed in lots of 500 by pressing five seeds into the agar medium in each of 100 petri plates. Several lots were assayed from each sample. Seeds were plated with and without disinfestation treatments, which consisted of immersion in 0.5% NaOCl for 10 minutes in partial vacuum (400-600 mm Hg) with constant stirring. This ensured penetration of the NaOCl into the tiny air pockets resulting from the rough texture of nonprocessed sugarbeet seed (Fig. 1). Disinfested seeds were rinsed in sterile distilled water to remove residues of NaOCl and blotted dry with cheesecloth before being plated on agar medium. Plates were incubated at room temperature, with RBA plates stored in a dark cabinet or under sheets of aluminum foil (14). Seeds were observed for colonies of *Fusarium* 5 days after plating and thereafter at 4-day intervals for 3-4 weeks. All colonies distinguishable as *Fusarium* on the assay plates were mass-transferred to PDA plates for identification.

Isolates identified as *F. oxysporum* were single-spored to PDA slants, and grown for 2 weeks under daylight-type fluorescent lights providing a 14-hour light period. The pathogenicity of wild-type isolates was then determined by culturing on PDA, harvesting conidia in sterile distilled water, filtering the suspension through four layers of cheesecloth, adjusting it to a concentration of 10^6 conidia/ml with a hemacytometer, and inoculating sugarbeet seedlings of the susceptible male-sterile line 562HO (13) by dipping the roots in the inoculum suspension. The seedlings were transplanted to pots of U.C. mix (3) and maintained in the glasshouse where they were observed for symptoms 7-10 days after inoculation. Isolates that had not caused symptoms on seedlings after 28 days were considered to be nonpathogenic.

The ability of seed-borne *Fusarium oxysporum* f. sp. *betae* to cause disease in sugarbeet seedlings was studied in a series of greenhouse planting trials. Whole seed from the commercial samples 3179 and 3274 were planted in large flats of soil mix prepared by uniformly blending pasteurized Yolo fine sandy loam (YFSL), river sand, and U.C. mix (5:1:1, v/v). Seeds were evenly spaced in flats and covered to a uniform depth of 2.5 cm. Seeds which failed to germinate by the 7th day after planting were recovered, surface-disinfested in 0.5% NaOCl, and plated on 1.5% water agar to check for pathogenic organisms. Emerged seedlings were periodically observed for

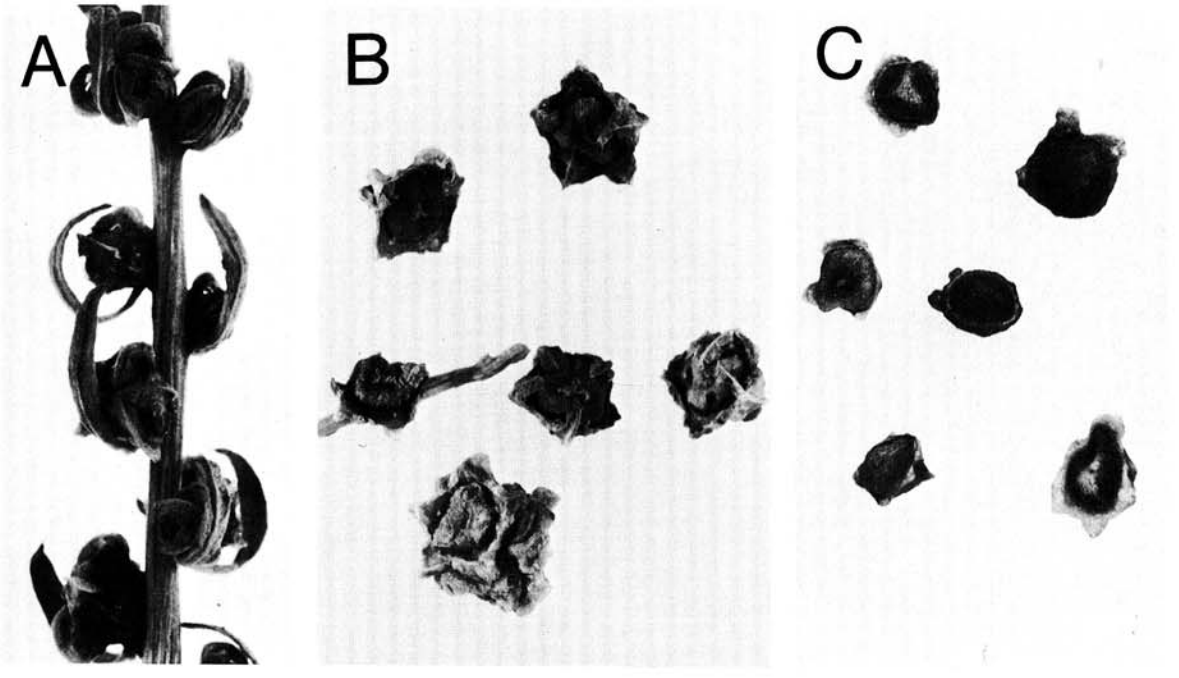

Fig. 1-(A to C). **A)** Minor seed-bearing branch of sugarbeet inflorescence showing seed attachment and the small leaves which comprised much of the debris associated with USH10B-H seed. **B)** Whole seed of lot 3179 showing rough, corky nature of superficial seed tissues. **C)** Processed seed of lot 3179 showing reduction of superficial tissues following commercial processing.

symptoms of wilt during the 4- to 6-week duration of each trial. All suspect seedlings were removed, surface-disinfested, and plated on water agar.

RESULTS

Seed assays.—The highest level of contamination by *F. oxysporum* f. sp. *betae* was on hand-harvested USH10B-H seed (Table 1). This was expected owing to the severe disease symptoms exhibited in the seed-bearing plants from which this seed had been harvested. To confirm the presence of the pathogen in the seed-bearing parents, isolations were made from root and seed stalk tissues, including the minor seed-bearing branches of the seed stalk (Fig. 1), on PCNB medium following surface disinfestation in NaOCl. The presence of the pathogen was confirmed in all these tissues. However, although the seed stalks were found to be extensively invaded, the pathogen apparently had not entered the seed produced on these plants. Surface disinfestation of the hand-harvested seed resulted in elimination of the *Fusarium* pathogen (Table 1), which suggested that it had been present only as an external contaminant.

Since *Fusarium* was eliminated by surface disinfestation, a test was run to determine whether the partial-vacuum method of disinfestation was too severe a technique. Two lots of 500 USH10B-H seeds were washed 15 times in sterile distilled water, blotted dry with cheesecloth, and plated on PCNB medium. This method also resulted in the removal of the pathogen (Table 1). The wash water from the first three rinses of the seed was assayed for *Fusarium* by pipetting 1-ml portions of the residue suspension onto PCNB agar plates. Pathogenic isolates were obtained in the residue assay, indicating that the pathogen had been present on some seeds as an external contaminant and that it was removed by the water wash.

Although the USH10B-H seed carried the highest level of *Fusarium* of any of the samples, it was lower than expected from an earlier report (9). Because of this, an additional method was utilized to assay 100 USH10B-H seed to see if differences would occur. Nondisinfested seeds were ground in 1 ml of sterile distilled water with a mortar and pestle and the slurry from each seed was spread over the surface of a single PCNB agar plate. One pathogenic isolate was obtained by this method (Table 1), which apparently is not a significant departure from the more convenient method of plating intact seed.

Assays of the two commercial seed lots are summarized in Table 2. Whole seed from lot 3179, in which severe blight had been observed in the seed crop, had the highest level of *F. oxysporum* f. sp. *betae*. That level, however, was lower than that found in seed harvested by hand from severely diseased plants (Table 1), but this would be expected since a commercial field would contain plants in many different stages of infection. The pathogen was not isolated from seed lot 3274. Stalk blight symptoms during July and August 1973, prior to harvest, were not so severe on this seed crop as on the crop from which lot 3179 was obtained. Again, surface disinfestation of seeds with NaOCl resulted in the complete elimination of *F. oxysporum,* which further suggested that its presence on seed was as an external contaminant.

The assay results of whole, partially processed, and processed seed are indicated in Table 2. The removal of superficial seed tissues by the milling process (Fig. 1) significantly reduced the level of seed infestation, based upon a Poisson Distribution analysis ($P = 0.05$) (12).

Because *F. oxysporum* f. sp. *betae* apparently was present on seeds only as a surface contaminant, assays were conducted of the debris normally accompanying seed lots. Debris from the hand-harvested USH10B-H seed, which consisted primarily of fragments of the small

TABLE 1. Comparison of different methods of surface disinfestation and seed assay for *Fusarium oxysporum* f. sp. *betae* on hand-harvested USH10B-H seed from severely diseased sugarbeet plants

Number of seeds plated	Treatment[a]	Seeds yielding *F. oxysporum* f. sp. *betae* (No.)	(%)
2,000	none	9	0.45
1,000	NaOCl	0	0.0
1,000	water	0	0.0
100	ground	1	1.0

[a]Seed treatment prior to plating. None = seed plated without disinfestation, NaOCl = seed disinfested with 0.5% NaOCl under partial vacuum, water = seed washed in sterile distilled water, and ground = individual nondisinfested seeds assayed by grinding in sterile distilled water and spreading seed coat fragments over the surface of agar plates.

TABLE 2. Levels of *Fusarium oxysporum* f. sp. *betae* present on commercial sugarbeet seed as affected by seed processing and laboratory disinfestation

Seed sample	Processing grade[a]	Number of seed plated	Disinfested	Seeds yielding *F. oxysporum* f. sp. *betae* (No.)	(%)
GWH61-3179	Whole	3,000	...	7	0.23
GWH61-3179	Part.	2,000	...	1	0.05
GWH61-3179	Proc.	3,000	...	1	0.03
GWH61-3179	Whole	2,000	NaOCl[b]	0	0.0
GWH61-3179	Proc.	1,000	NaOCl	0	0.0
USH10B-3274	Whole	2,000	...	0	0.0

[a]Whole = whole seed, Part. = partially processed seed, and Proc. = fully processed seed.
[b]Disinfested in 0.5% NaOCl using partial vacuum treatment.

leaves associated with the seed stalk (Fig. 1), was lightly sprinkled over the surface of 50 plates of PCNB medium (0.25 g/50 plates). The *Fusarium* pathogen was consistently isolated from this debris. The fine dust-like debris from processed seed (lot #3179) was collected by vacuum-cleaning a portion of the seed sample with a small hollow probe, which was connected to a larger diameter glass tube into which a cotton plug had been inserted to act as an impaction filter. The dust collected in this manner was also sprinkled over the surface of 50 PCNB agar plates at a rate of 0.25 g/50 plates. All isolates of *F. oxysporum* obtained from this debris were nonpathogenic.

Planting trials.—No seedlings were infected with *F. oxysporum* f. sp. *betae,* although several died as a result of infection by *Phoma betae* or *Rhizoctonia solani.* In an effort to detect low-grade infections, 10-20 seedlings were randomly selected from each flat at the end of the trials and plated on PCNB medium. These results also were negative, and no fungi consistently were associated with nongerminated seeds, although *Phoma betae* and *Alternaria* spp. were isolated from a few seeds. Additional planting trials were conducted using the naturally infested USH10B-H seed as well as surface-disinfested and inoculated USH10B seed (lot #1070) and 563HO (lot #7432), the latter a susceptible male-sterile line (13). Each seed sample was planted in separate flats of pasteurized YFSL, and three generations of seedlings were grown in the soil of each flat. The USH10B-H seed was planted in noninfested soil and in soil into which approximately 10 cc of the *Fusarium*-infested seed stalk debris was incorporated at each planting. Seedling disease caused by *F. oxysporum* f. sp. *betae* was only observed in flats planted with the inoculated seed. A bioassay for the pathogen, using seedlings of the highly susceptible CMS line 565HO (13), also was positive only in soils planted with inoculated seed.

DISCUSSION

The levels of seed-borne *Fusarium* observed in this study were lower than those reported in 1973 by Gross and Leach (9). Our study further indicates the presence of *F. oxysporum* f. sp. *betae* on sugarbeet seed as an external contaminant, although Gross (D. C. Gross, *personal communication*) observed a slight amount of internal carriage, as indicated by the failure of NaOCl treatments to effect complete elimination of the pathogen. These apparent inconsistencies probably can be explained by the fact that the two studies involved seed harvested in different years. Those utilized in the present study were collected from the normally dry 1973 and 1974 harvest periods, whereas the samples tested by Gross were obtained from the 1972 harvest. A rain occurred during the 1972 harvest period, and this could have provided a more favorable environment for the pathogen to penetrate superficial tissues of the seed and to be carried internally. Such a situation was hypothesized by Singh et al. (17) in their studies on cumin wilt. Whether commercial processing would effectively reduce internally borne inoculum is not known, but it has been found to greatly reduce the amount of *Phoma betae* carried within sugarbeet seed, except in the most severe (> 80% infected) cases (Leach and MacDonald, *unpublished*).

The contamination of sugarbeet seed with *F. oxysporum* f. sp. *betae* could occur as the result of cultural practices during harvest, when cut, mature seed stalks are windrowed in the field for a 2-week curing prior to threshing. During this contact with the soil there would be ample opportunity for surface contamination of seed. However, since the seed stalks from which the USH10B-H seed were harvested were collected while standing erect in the field, other factors, such as wind-blown contaminated soil or infected plant debris, may be involved in contamination of seed.

Since virtually all of the seed used in commercial sugarbeet production in California is grown in the Willamette Valley of Oregon, it is probable that large amounts of contaminated seed have entered California for many years. Yet, despite a high level of susceptibility observed (13) in the seed-bearing parents of the hybrid cultivars USH9 and USH10 (which are grown extensively throughout California) this pathogen has not yet been reported in this state. Surveys of sugarbeet fields in Yolo, Solano, and Sacramento counties by the senior author have failed to detect the disease. This suggests that transmission of the *Fusarium* pathogen does not commonly occur in the hybrid seed plantings. The failure to detect transmission of *F. oxysporum* f. sp. *betae* in either commercial fields or experimental greenhouse plantings in pasteurized soil, which provides less competition to the pathogen from soil microflora than natural field soil (4), is interesting because many of the reports to date either provide direct evidence for (2, 10, 11, 18) or infer (7, 8, 17) infection of progeny plants by seed-borne wilt fusaria. In each of these cases, however, it appears that the authors were discussing systems in which the genotype of the progeny plants, at least with respect to susceptibility to *Fusarium,* is essentially the same as the genotype of the parent plants. This would allow the seed-to-progeny (SP) transfer to occur fairly efficiently under the mechanisms described by Baker and Smith (4), provided environmental factors are not limiting. Sugarbeets, on the other hand, appear to represent a unique situation among the reports of seed-borne wilt fusaria, in that although the seed-bearing parents are highly susceptible to infection, the hybrids produced on them appear to gain some resistance from the pollinator lines (13). This relative decrease in susceptibility may prevent the SP transfer and repress the introduction of this pathogen into commercial sugarbeet fields.

A further inhibition of transfer may result from fungicidal seed treatments which are commonly applied to sugarbeet seed after processing to protect seedlings against damping-off organisms. However, it was not possible to test this hypothesis because of the low levels of seed-borne inoculum and our inability to detect transmission. From the results of this study it is felt that the association of *F. oxysporum* f. sp. *betae* with hybrid sugarbeet seed does not present a serious, immediate threat to California's sugar industry. Protection against the future expression of disease lies with the incorporation of high levels of resistance in the hybrid cultivars.

LITERATURE CITED

1. ABAWI, G. S., and J. W. LORBEER. 1971. Population of Fusarium oxysporum f. sp. cepae in organic soils in New York. Phytopathology 61:1042-1048.
2. BAKER, K. F. 1953. Fusarium wilt of China Aster. U.S. Dep. Agric. Yearb. 1953:572-577.
3. BAKER, K. F. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agric. Exp. Stn., Ext. Serv. Man. 23. 332 p.
4. BAKER, K. F., and S. H. SMITH. 1966. Dynamics of seed transmission of plant pathogens. Annu. Rev. Phytopathol. 4:311-334.
5. BOLLEY, H. L. 1903. Flax and flax seed selection. N. D. Agric. Exp. Stn. Bull. 55:171-198.
6. BOLLEY, H. L., and T. F. MANNS. 1932. Fungi of flax seed and flax-sick soil. N.D. Agric. Exp. Stn. Bull. 259.57 p.
7. ELLIOTT, J. A. 1923. Cotton-wilt, a seed-borne disease. J. Agric. Res. 23:387-393.
8. ELLIOTT, J. A., and R. F. CRAWFORD. 1922. The spread of tomato wilt by infected seed. Phytopathology 12:428-434.
9. GROSS, D. C., and L. D. LEACH. 1973. Stalk blight of sugarbeet seed crops caused by Fusarium oxysporum f. sp. betae. Phytopathology 63:1216. (Abstr.).
10. KENDRICK, J. B. 1934. Seed transmission of Fusarium yellows of beans. Phytopathology 24:1139 (Abstr.).
11. KLISIEWICZ, J. M. 1963. Wilt-incitant Fusarium oxysporum f. carthami present in seed from infected safflower. Phytopathology 53:1046-1049.
12. LITTLE, T. M., and F. J. HILLS. 1972. Statistical methods in agricultural research. Calif. Agric. Ext. Publ. ATX-377. 242 p.
13. MAC DONALD, J. D., L. D. LEACH, and J. S. MC FARLANE. 1976. Susceptibility of sugarbeet lines to the stalk blight pathogen Fusarium oxysporum f. sp. betae. Plant Dis. Rep. 60:192-196.
14. PADY, S. M., C. L. KRAMER, and V. K. PATHAK. 1960. Suppression of fungi by light on media containing rose bengal. Mycologia 52:347-349.
15. PAPAVIZAS, G. C. 1967. Evaluation of various media and antimicrobial agents for the isolation of Fusarium from soil. Phytopathology 57:848-852.
16. RUSSELL, T. E. 1975. Plantago wilt. Phytopathology 65:359-360.
17. SINGH, R. D., S. L. CHOUDHARY, and K. G. PATEL. 1972. Seed transmission and control of Fusarium wilt of cumin. Phytopathol. Mediterr. 11:19-24.
18. SNYDER, W. C. 1932. Seed dissemination in Fusarium wilt of pea. Phytopathology 22:253-257.
19. STEWART, D. 1931. Sugarbeet yellows caused by Fusarium conglutinans var. betae. Phytopathology 21:59-70.