Evidence for an Expanded Host Range of Fusarium oxysporum f. sp. betae

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ABSTRACT

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Fusarium oxysporum f. sp. *betae* causes disease and premature death of susceptible sugarbeet lines grown for seed in the Willamette Valley of Oregon. In some fields, pigweed *(Amaranthus retroflexus)* plants exhibited disease symptoms characteristic of attack by a vascular pathogen. Symptoms included one-sided wilt, vascular discoloration, and death of plants. Cultures from diseased pigweed plants yielded isolates of *F. oxysporum* morphologically identical to those obtained from blighted sugarbeets. Cross-inoculations conducted in the greenhouse with single-spore isolates from sugarbeet and pigweed have demonstrated that these isolates are indistinguishable in their reaction on these hosts and both therefore are considered to be *F. oxysporum* f. sp. *betae*. The susceptibility of pigweed appears similar to that of susceptible sugarbeet lines. Field and greenhouse trials have indicated and that there also may be a relationship between the spinach and sugarbeet pathogens. The weeds *Chenopodium album, Brassica nigra,* and wild *Anethum graveolens* have been identified as symptomless carriers of the stalk blight pathogen. These susceptible and symptomless hosts could provide an explanation for the frequent occurrence of stalk blight in Oregon fields cropped with sugarbeets for the first time.

Additional key words: Beta vulgaris L., *Spinacia oleracea* L., host specificity.

Stalk blight caused by *Fusarium oxysporum* Schlecht. f. sp. *betae* (Stewart) Snyd. & Hans. in sugarbeet (*Beta vulgaris* L.) plants grown for seed in the Willamette Valley of Oregon was first reported in 1973 by Gross and Leach (9). This disease has been observed frequently in Oregon seed fields planted with the highly susceptible male-sterile lines of commercial hybrids which are grown extensively throughout California and other western states (13). Its sudden, widespread, and sometimes severe occurrence, which appeared coincidentally with the release of the newer susceptible lines, suggested that the pathogen might already have been present and established in field soils. Although the occurrence of stalk blight in fields having a history of sugarbeet production could be explained as the unnoticed build-up of the pathogen on relatively resistant older lines (13), such a hypothesis is not adequate to explain the frequent occurrence of this disease, sometimes at relatively high levels, in fields planted to sugarbeets for the first time. Studies of the association of f. sp. *betae* with sugarbeet seed (9, 12) and field observations of a frequently uneven distribution of diseased plants in such fields, do not support the hypothesis of pathogen introduction with stock seed. These facts raised the question of whether or not this pathogen was associated with other plant species that could allow it to build up and survive in soils in the absence of a sugarbeet host.

A symptomless association of wilt fusaria with hosts other than their *forma* suscepts has been amply demonstrated (3, 10, 11). Such parasitic associations are believed to serve primarily to increase the survival potential of pathogens in the absence of their *forma* suscepts. Smith and Snyder (15) provided evidence that populations of *F. oxysporum* f. sp. *vasinfectum* could build up in soils faster on nonsusceptible hosts such as barley, than under continuous cultivation of its cotton suscept.

In addition to symptomless associations with various plants, some of the wilt fusaria have been reported to have pathogenic capabilities which extend beyond their *forma* suscepts (7), resulting in controversy over the validity of some *forma specialis* designations (6). Perhaps the most notable example of nonspecificity is the complex interrelationship which Armstrong and Armstrong (4, 5, 7) described between pathogens of cotton, tobacco, sweetpotato, and *Cassia tora,* giving rise by them to a concept of primary and secondary hosts of wilt fusaria (5).

The purpose of the present investigation was to determine whether *F. oxysporum* f. sp. *betae* was associated in some way with other plant species in Oregon fields, and to identify plants which could be significant in the establishment or spread of this pathogen in field soils.

MATERIALS AND METHODS

Most of the plants collected during this study were obtained during 1973 and 1974 from a farm near Salem, Oregon, which was known to be heavily infested with f. sp. *betae* and was being used to screen sugarbeet lines for their susceptibility to this organism (13). Additional collections were made by the senior author from commercial sugarbeet fields during the 1973 and 1974 harvest periods. The plant species collected for isolation studies were pigweed (*Amaranthus retroflexus* L.), lambsquarter (*Chenopodium album* L.), black mustard [*Brassica nigra* (L.) Koch], and wild dill (*Anethum graveolens* L.). Isolations were made from root and stem

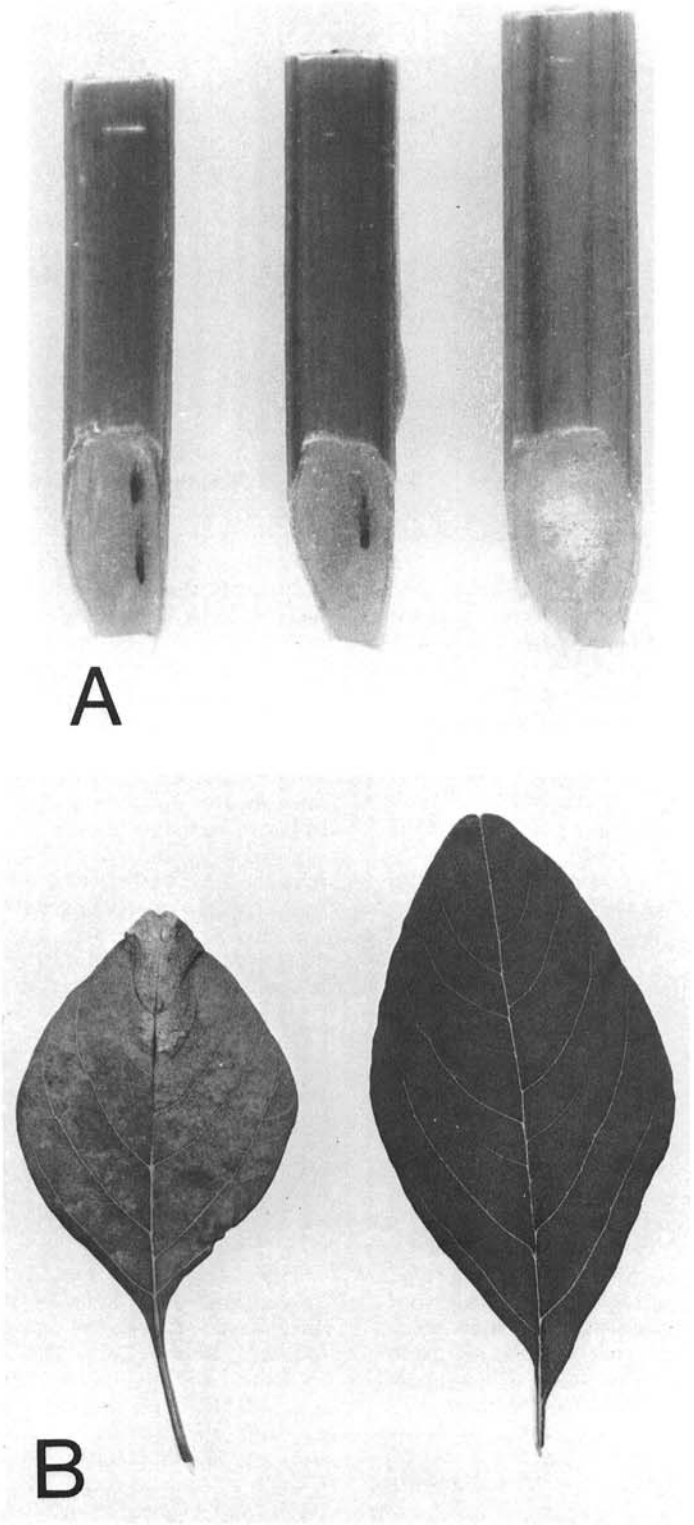

Fig. 1.-(A,B) Disease symptoms exhibited by pigweed plants infected with *Fusarium oxysporum* f. sp. *betae*. **A)** Vascular discoloration in aerial stem sections, with healthy tissue on right, and **B)** pigweed leaves, with infected leaf on left showing the characteristic necrosis and mottle associated with disease symptoms.

tissues which were washed free of adhering soil with tap water and surface-disinfested in 0.5% NaOCl for 10 minutes. Tissue pieces were cut in 0.5- to 1.5-cm lengths and plated on potato-dextrose agar (PDA), acid-potato-dextrose agar (APDA) (PDA acidified with 25% lactic acid to pH 4.5 - 5.0), a modified PCNB medium (14), or a modified Martin's rose bengal medium (RBA) (1) from which Dexon was omitted.

Pathogenicity testing.—All isolates of *F. oxysporum* obtained from infected plant parts were single-spored to PDA slants before being tested for pathogenicity. The inoculum for pathogenicity trials was prepared by growing isolates for 1-2 weeks on PDA plates under fluorescent light, and harvesting the conidia in sterile-distilled water. Conidial suspensions were freed of large mycelial fragments by filtration through cheesecloth and concentrations were determined with a hemacytometer. Unless otherwise stated, suspensions were adjusted to a concentration of 10^6 conidia/ml prior to use. Stock cultures of pathogenic isolates were stored in tubes of sterilized Yolo fine sandy loam under refrigeration to minimize alterations in cultural or pathogenic characters (17), and were routinely used in comparison trials. These isolates were activated as needed by sprinkling small quantities of infested soil on the surface of PDA plates and preparing conidial suspensions as described above.

Two methods of plant inoculation were commonly employed in tests of pathogenicity. Root-dip inoculations of 7- to 10-day-old seedlings grown in heavily seeded pots of pasteurized U.C. mix (8) were performed by removing plants from the seedling pots, rinsing their roots free of adhering soil under running tap water, and standing them upright for 15 minutes in 100-ml glass beakers containing the inoculum suspension. Control seedlings were immersed in either sterile-distilled water or autoclaved spore suspensions. After immersion in the inoculum, the seedlings were transplanted to fresh pots of U.C. mix, and approximately 5 ml of inoculum was added to the soil around each plant. Plants were maintained in the greenhouse where they were observed for symptoms of disease 10-14 days after inoculation and thereafter at intervals until the termination of the trials, which usually ran 4-8 weeks. Temperatures in the greenhouse averaged 22 C in the winter and 28 C in the summer. To test isolates on more mature plants, the 7- to 10-day-old seedlings were transplanted singly into pots of U.C. mix and allowed to reach an age of 5-6 weeks before they were inoculated. The inoculation of established plants was accomplished either by pouring a measured quantity of inoculum into the soil and leaving the plant undisturbed, or in some instances, by injuring the roots with a wooden pot label prior to the addition of the conidial suspension. These plants were observed periodically for external symptoms of disease until the trials were terminated, when the plants were harvested to check for internal symptoms and cultured for possible symptomless infections. These trials were terminated at full plant maturity for all plants except the biennial sugarbeet, which was examined after 16-20 weeks.

Unless otherwise stated, all isolates were tested for pathogenicity to sugarbeet by inoculating the susceptible male-sterile line 562HO (13). All sugarbeet seed was obtained either from the West Coast Beet Seed Co., Salem, Oregon, or the USDA Agricultural Research Station, Salinas, California. Seeds of *C. album, B. nigra,* and most other weeds were obtained from the Botany Department, University of California, Davis. Seed of *A. retroflexus* was collected from mature plants at the Oregon farm site. A high germination percentage of pigweed seed, which is mechanically resistant to germination, was obtained by a preplant treatment of seeds in concentrated H_2SO_4 for 2.5 minutes, followed by a rinse in distilled water. Seed of all other plants used in this study was obtained from commercial outlets.

RESULTS

Susceptible hosts.—Pigweed plants that exhibited disease symptoms suggestive of attack by a vascular pathogen were observed at the Oregon farm site and in several commercial fields. Symptoms included (Fig. 1) a one-sided wilt of plants, vascular discoloration extending from root tissues into the aerial systems of the plants including leaves, and ultimately the death of affected plants. A number of diseased plants were collected and isolations were made from root and aerial portions. These isolations consistently yielded a *F. oxysporum* morphologically identical to isolates cultured from blighted sugarbeets. Inoculations of seedling and juvenile pigweed plants confirmed the pathogenicity of these isolates to pigweed. Inoculated pigweed seedlings were stunted, with some leaf mottling and shriveling. This was followed by a heavy leaf drop and death of the plants. Pigweed plants inoculated in a juvenile stage showed a stunting and general chlorosis with some leaf drop and necrosis. However, the most severe symptoms of necrosis and vascular discoloration occurred when the plants entered a reproductive stage of growth. The same phenomenon has been observed on diseased sugarbeets by the authors in experimental (13) and commercial field plantings in Oregon, where the periods of most severe disease expression are during the seedling stage, and later in the bolting or reproductive stage.

Because the diseased pigweed plants had been found in fields known to be infested with the sugarbeet pathogen, cross-inoculation trials were conducted in the greenhouse to determine whether the pathogens were related. Initial tests involved the inoculation of seedlings and juvenile plants of pigweed and sugarbeet with pathogenic isolates from both hosts. The pathogens caused identical symptoms on each host and could not be differentiated either by symptom severity or by the time required to elicit symptoms. To determine whether the respective isolates could be differentiated on a larger host range, inoculations were made to seedlings of *Amaranthus retroflexus, A. palmeri* Wats., *A. graiczans* L., *A. blitoides* Wats., *Beta vulgaris* (sugarbeets 562HO, 565HO, USH9A, USH10A, USH10B, 613, 817, GWH58 and 71MSH3 and garden beet cultivars 'Crosby's Egyptian' and 'Detroit Dark Red'), *B. vulgaris* var. *cicla* (L.) Moq. (chard cultivars 'Lucullus' and 'Fordhook Giant'), *Chenopodium album, C. amaranticolor* Coste & Reyn., and *C. quinoa* Willd. The pigweed and sugarbeet isolates could not be differentiated with this host range either and caused disease only on *A. retroflexus* and sugarbeets. There were some indications of disease in *A. palmeri* and *A. blitoides,* but owing to the poor germination of available seed, these observations

involved very few plants, and are felt to be inconclusive. Isolations were made from the roots of chard, garden beet, and *Chenopodium* spp. and it was found that although no disease symptoms were expressed by these plants, their root systems had been colonized by *Fusarium*. Subsequent inoculations of sugarbeet seedlings with the reisolated cultures showed that they had retained pathogenicity to sugarbeet.

In an attempt to determine whether the susceptibility displayed by pigweed plants in the greenhouse to f. sp. *betae* was merely the result of the plants being overwhelmed with the inoculum of an aggressive pathogen or a real susceptibility, two additional tests were performed. In the first experiment, 20 seedlings each of pigweed and the sugarbeet lines 562HO, USH10A, USH10B, and 817 were inoculated with a pathogenic isolate from sugarbeet at 10^6, 10^5, and 10^4 conidia/ml. The relative susceptibility displayed by the sugarbeet lines in these inoculations (Fig. 2) closely corresponded to the results of field trials of their susceptibility to the stalk blight pathogen (817 relatively resistant, 562HO very susceptible, and USH10A&B intermediate) (13). Thus, the susceptibility displayed by pigweed, which appears to be intermediate between highly susceptible and resistant sugarbeet lines, was not merely due to excessively high inoculum dosages.

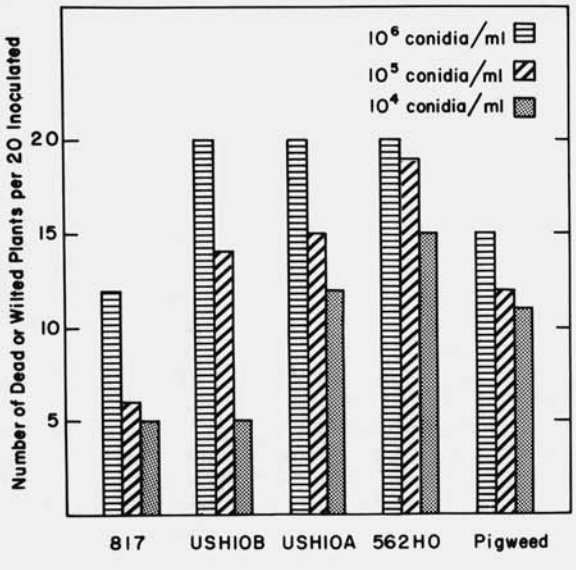

Fig. 2. The influence of inoculum level on the susceptibility of sugarbeet (817, USH10B, USH9A, and 562HO) and pigweed seedlings to *Fusarium oxysporum* f. sp. *betae*.

Since the possibility existed that in the greenhouse, pigweed might appear sensitive to any pathogenic form of *F. oxysporum*, a further test was performed in which 20 seedlings each of pigweed, sugarbeet (USH10B), spinach (Hybrid 424, Asgrow seed) and tomato (Improved Pearson) were inoculated with pathogenic isolates of f. sp. *betae*, f. sp. *spinaceae* (isolated by the senior author from diseased spinach plants provided by A. S. Greathead, Farm Advisor, Monterey Co., California) and f. sp. *lycopersici* Race 1 (obtained from K. A. Kimble, University of California, Davis). A saprophytic isolate of *F. oxysporum*, obtained from the roots of a weed plant collected at the Oregon farm site, also was included in this test as an additional check. Although disease was not induced in tomato by any isolate except the tomato pathogen, and f. sp. *lycopersici* appeared specific for tomato, this was not the case with the sugarbeet, pigweed, and spinach combinations (Table 1). The pathogens of these hosts, while showing a high level of pathogenicity to their respective suscepts, also demonstrated a degree of cross-pathogenicity. An unusual feature of this association was that f. sp. *betae* appeared to be more pathogenic on spinach than f. sp. *spinaceae* was on sugarbeet or pigweed. This relationship between these pathogens has been confirmed in repeated inoculation trials, and has also been observed by S. N. Smith *(personal communication)* working independently in Berkeley, California, with isolates she obtained from diseased sugarbeets that we sent to her in 1973.

To further test the apparent susceptibility of spinach to f. sp. *betae*, spinach seedlings were inoculated with a pathogenic isolate from sugarbeet, again using 10^6, 10^5, and 10^4 conidia/ml. The results of this test (8, 3 and 0 seedlings killed or wilted per 20 inoculated) suggested that the susceptibility shown by spinach to f. sp. *betae* could be an artifact of the greenhouse inoculation technique. As a result, a field planting was made to test this susceptibility in nature. Seeds of sugarbeet (817, 562HO, 565HO, USH10B), chard (cultivar Lucullus), garden beet (cultivar Crosby's Egyptian), and spinach (cultivar Early Hybrid No. 7) were planted at the Salem, Oregon farm site, which had never before been cropped to spinach. The planting was done in late April 1975 and preliminary

TABLE 1. Results of cross-inoculation of sugarbeet, pigweed, spinach, and tomato with isolates of *Fusarium oxysporum*

Host[a]	*Fusarium oxysporum* f. sp. *betae*	*Fusarium oxysporum* f. sp. *spinaceae*	*Fusarium oxysporum* f. sp. *lycopersici* R_1	Saprophytic *F. oxysporum*
Sugarbeet	++++[b]	+	−	−
Pigweed	++++	+	−	−
Spinach	++	++++	−	−
Tomato	−	−	++++	−

[a]Twenty seedlings of pigweed, sugarbeet (USH10B), spinach (Hybrid 424), and tomato (cultivar Improved Pearson) were inoculated with each *forma specialis*.

[b]Rating scale: (−) = no visible symptoms of disease, (+) = 1-5, (++) = 6-10, (+++) = 11-15 and (++++) = 16-20 plants dead or showing wilt symptoms.

observations were made in early June. A final evaluation of the plot was made in July 1975. The sugarbeet entries in this planting performed as expected on the basis of earlier evaluations made at this location (13). The chard and garden beet cultivars, as predicted on the basis of greenhouse tests, showed no signs of disease, although the *Fusarium* pathogen was successfully isolated from their roots. In contrast, the spinach plants showed levels of disease much more severe than had been anticipated from greenhouse tests, and were much more severely affected than any other entry in the plot. These plants, however, had developed seed stalks and entered a reproductive stage of growth, perhaps explaining the greater severity of disease. The stalk blight pathogen was isolated from throughout the root and aerial systems of these plants and its pathogenicity was confirmed in greenhouse tests, indicating that the premature death (before seed set) of the spinach plants was caused by *F. oxysporum* f. sp. *betae*.

Symptomless hosts.—Collections of *C. album, B. nigra,* and *A. graveolens* were made from experimental plots at the Oregon farm site and diseased commercial fields visited during July 1974. These weed species were chosen for collection because of their prevalence in the fields visited. Although they showed no symptoms of disease in the field, in either vegetative or reproductive stages of growth, the stalk blight pathogen was consistently isolated from their root systems. When these plants were grown in the greenhouse and inoculated as seedlings or juvenile plants with isolates of f. sp. *betae* obtained from sugarbeet, pigweed, or the symptomless weeds themselves, no evidence of disease was ever observed. *Fusarium oxysporum* f. sp. *betae* was, however, recovered from the roots of inoculated plants and shown to have retained its pathogenicity to sugarbeet. Further, the failure to elicit disease symptoms on either garden beet or chard cultivars in repeated greenhouse inoculations and in the 1975 field trials, even though the roots of these plants were colonized by the stalk blight pathogen, indicates that they, too, may serve as symptomless hosts. However, these plants would have to be allowed to overwinter in an infested field for the induction of bolting so that they could be observed in a reproductive stage, before their susceptibility, or lack of it, could be accurately determined.

DISCUSSION

Since all attempts to differentiate the pigweed *Fusarium* from the sugarbeet stalk blight pathogen were unsuccessful, it appears that the disease and death of pigweed plants observed in the Willamette Valley of Oregon is caused by *F. oxysporum* f. sp. *betae* rather than a new f. sp. This is believed to be the first report that the pathogenic capabilities of *F. oxysporum* f. sp. *betae* extend beyond sugarbeet (7). Although pigweed has been implicated in the past with other wilt *Fusaria* (2, 11), the reports describe parasitic associations rather than the pathogenic association described here.

The occurrence of diseased pigweed plants in Oregon sugarbeet fields is not an infrequent, isolated event, having been observed in several fields visited during 1973, 1974, and 1975. Diseased pigweeds were not, however, observed in any of the several Colorado sugarbeet fields visited by the senior author in September 1974. Fields were visited in the Sterling, Atwood, and Hillrose areas of northeastern Colorado, and all were infested with the Fusarium yellows pathogen (16) and had high pigweed populations. The absence of diseased pigweed in these areas, and the differences in sugarbeet cultivar susceptibility observed between Oregon and Colorado test plots (13), suggest that the Oregon stalk blight pathogen may differ somewhat from the Colorado pathogen. In addition, the pathogenicity displayed by the stalk blight pathogen toward spinach plants in the field and the cross-pathogenicity shown by isolates of f. sp. *betae* and f. sp. *spinaceae* in greenhouse tests suggests a possible relationship between these two pathogens. A detailed study should be undertaken to compare the Oregon and Colorado sugarbeet pathogens and isolates of f. sp. *spinaceae* from California and other areas to determine the precise nature of these relationships. A comparison of the two sugarbeet pathogens could provide information concerning the origin of the more recently reported Oregon pathogen (9). Although it may have been introduced originally from the Rocky Mountain area, the ability of the stalk blight pathogen to attack several plant species also suggests that it has not had a long association with a single host such as sugarbeet, and may even be indigenous to the Williamette Valley where it exists as a pathogen of pigweed and other plants. Its ability to attack sugarbeet may have become evident only after highly susceptible lines were introduced to the area. Studies of the relationship between the Oregon and Colorado pathogens could help to clarify this point.

The role played by susceptible and symptomless hosts of the stalk blight pathogen in establishment or maintenance of this organism in Oregon fields can only be speculated, although the frequent occurrence of blight in new sugarbeet plantings suggests that it may be significant. The role played by these plant species in the increase and maintenance of the small amount of inoculum which may be introduced into fields with sugarbeet seed could be particularly important in the establishment of the pathogen in new areas, since the transmission of *F. oxysporum* f. sp. *betae* to sugarbeet seedlings following the planting of naturally infested seed has not been successfully demonstrated (12).

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