Dicotyledonous Weeds as a Source of *Fusarium oxysporum* Pathogenic on Soybean

J. B. HELBIG, Former Graduate Student, and R. B. CARROLL, Associate Professor, Department of Plant Science, University of Delaware, Newark 19711

ABSTRACT

Helbig, J. B., and Carroll, R. B. 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. Plant Disease 68:694-696.

Twenty-one dicotyledonous weeds were studied for their potential role in Fusarium blight of soybean, a disease of increasing importance in the Delmarva region. Isolations were made from root and stem tissue of 800 weed plants collected throughout the growing season from conventional tillage and no-tillage soybean fields at 20 locations. *Fusarium oxysporum* was obtained from 16 weed species. No direct relationship to tillage practice was noted. Sixteen of 17 isolates of *F. oxysporum* from weeds were pathogenic on the soybean cultivar Essex under greenhouse conditions. No symptoms of *F. oxysporum* infection were observed in weed plants collected from the field or when inoculated in the greenhouse with a soybean isolate. Common dicotyledonous weeds in soybean fields can serve as symptomless hosts of the blight pathogen, which retains pathogenicity for soybean.

Abundant literature exists on weeds that serve as alternate hosts for plant viruses and prokaryotic parasites and the details of the parasitic life cycles have been determined in many cases. Weeds as alternate or expanded hosts for fungal pathogens have received much less attention.

Karunakaran et al (9) showed that the clove spot pathogen, *Colletotrichum gleosporioides*, survived on the weed *Clerodendron infortunatum*, retained pathogenicity, and served as an important source of inoculum. Wooliams (16) was able to isolate *Verticillium dahliae* from the common weeds pennycress, common purslane, and lambsquarter and demonstrated that these isolates were pathogenic on tomato. McKeen and Thorpe (14) isolated *V. dahliae* from common ragweed, giant ragweed, cocklebur, and velvetleaf. This pathogen was obtained from velvetleaf in three of the five years included in the study.

McDonald and Leach (12) found that the sugar beet stalk blight pathogen, *Fusarium oxysporum* f. sp. *betae*, was harbored by lambsquarter, black mustard, and wild dill, which remained symptomless. Isolates from these weeds retained their pathogenicity to sugar beet. Katan (10) isolated the tomato wilt pathogen, *F. oxysporum* f. sp. *lycopersici*, from symptomless pigweed, mallow, large crabgrass, and *Oryzopsis miliaceae* and found that isolates retained their pathogenicity to tomato seedlings. Clark and Watson (2) provided evidence that four species of Convolvulaceae, which are common weeds in agricultural land in Louisiana, are also symptomless hosts of the sweet potato wilt pathogen, *F. oxysporum* f. sp. *batatas*. Hepperly et al (8) isolated three potential soybean pathogens from velvetleaf in Illinois, including *Diaporthe phaseolorum* var. *sojae*, *C. dematium* var. *truncata*, and *C. gleosporioides*. Other fungi isolated included a *Fusarium* sp. at a 23% occurrence. The isolates of *Diaporthe* and *C. dematium* from velvetleaf were highly virulent to susceptible soybean pods and seed. Cerkauskas et al (1) also found that two common weeds found in soybean fields of southern Brazil serve as symptomless carriers of *Phomopsis* spp. pathogenic on soybean.

Persistence of pathogens in soil depends largely on their ability to survive in the absence of a suitable host. Most formae speciales of *F. oxysporum* can remain dormant in the soil and host tissue as chlamydospores until stimulated to germinate (15). *Fusarium* spp. have also been shown to survive in the absence of susceptible host plants by invasion and colonization of other plants that show few, if any, symptoms of disease (10,12,15).

Blight of soybeans caused by *F. oxysporum* has been increasing in the Delmarva region, which includes the Delaware, Maryland, and Virginia peninsula (4,11). We evaluated dicotyledonous weeds commonly found in soybean fields in Delaware as potential reservoirs of *F. oxysporum* pathogenic on soybeans. Preliminary results were reported previously (7).

MATERIALS AND METHODS

Field studies. Twenty Delaware soybean fields, 10 in Kent County and 10 in Sussex County, were included in the study and were representative of Delaware soybean culture including conventional and no-tillage practices. Three of the fields in Kent County and four in Sussex County were planted with no-tillage. Sampling began in June and continued monthly through September 1981, coinciding closely with planting through harvest of the soybean crop. At each location, 10 weed plants were collected on each sampling date. Of these 10, five were from within the field and five from the borders. The 10 plants were representative of the population of weeds for that particular location at that date. After collection, plants were sealed in polyethylene bags and transported to the laboratory in a cooler. Samples were refrigerated and prepared the next day as follows: Roots and basal stem tissue were rinsed in running tap water to dislodge soil. Four cross sections from each plant were aseptically excised and surface-disinfested for 45 sec in 70% (v/v) ethanol and 1 min in 10% Clorox (5.25% NaOCl, w/v), followed by a rinse for 1 min in sterile distilled water. Sections 0.2 cm or smaller were exposed for only 30 sec to each disinfestant. Sections were placed in 100-mm-diameter petri plates containing commercial acidified potato-dextrose agar (APDA). Plates were incubated in the dark for 7–10 days at 28 C, then macrocultural and microscopic characteristics were used to identify *F. oxysporum* recovered from plant tissue. When identification was difficult, cultures were exposed to natural light to induce greater sporulation. A random selection of tentatively identified *Fusarium* isolates was sent to the Fusarium Research Center, Pennsylvania State University, to confirm identifications.

Pathogenicity studies. *F. oxysporum* isolates were transferred to 100-mm-diameter petri plates with commercial potato-dextrose agar (PDA), three plates per isolate, then incubated 14 days. Disinfested 100-mm-diameter plastic pots were filled with an autoclaved greenhouse potting mix (2:3:4 vermiculite: peat moss:perlite, v/v/v). Certified seed of the soybean cultivar Essex, which is

Present address of first author: USDA-APHIS, PPQ, 522 N. Central Ave., Phoenix, AZ 85004.

Portion of an M.S. thesis submitted by the first author to the Office of Graduate Studies, University of Delaware, Newark 19711.

Published with the approval of the Director of the Delaware Agricultural Experiment Station as Miscellaneous Paper 1038. Contribution 163 of the Department of Plant Science, University of Delaware, Newark 19711.

Accepted for publication 13 February 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

susceptible to Fusarium blight, was seeded at the rate of 10 seeds per pot, 2–2.5-cm-deep. The pots were arranged in a randomized complete block design with three replicates and isolates as treatments. Plants were inoculated at the V-1 stage (3) using a soil-drench method (6,11). Wounding was facilitated by thinning the plants from 10 to five per pot and slicing through the soil with a sterile knife blade 1 cm from the plant stems. The inoculation procedure was as follows: Conidia and mycelial fragments from *Fusarium* cultures (three petri dishes per isolate) were removed with a rubber policeman into 200 ml of sterile distilled water, mixed in a blender for 30 sec at high speed, and diluted to 300 ml with sterile distilled water. One hundred milliliters of the fungal suspension (1.7×10^6 propagules per millilter) was transferred to each pot. Mycelial fragments as large as macroconidia were counted as propagules. Controls were root-wounded as before and treated with 100 ml of sterile distilled water. Plants were watered daily until the experiment was terminated 3 wk later.

Further pathogenicity tests were performed using a random sample of *F. oxysporum* isolates obtained from weeds collected after 1 August. Twelve isolates with proven pathogenicity from the first test were also included for a total of 29 isolates. Experimental design and inoculation procedures were identical to the first test. The experiment was terminated 4 wk after inoculation.

Plant roots were evaluated using a disease index from 1 to 5 based on symptoms of *Fusarium* blight as follows: 1 = healthy root, no evidence of infection; 2 = slight root damage and reduction, feeder roots darkened and new white lateral roots starting to emerge; 3 = moderate root damage and reduction; 4 = extensive cortical or vascular tissue destroyed; 5 = severe infection and/or wilt.

Roots were washed under running tap water to remove adhering soil before rating. The procedures used in the root evaluation were adapted primarily from Leath (11).

Weed inoculations. Five weed species—velvetleaf, ivyleaf morning glory, jimsonweed, lambsquarter, and pigweed—were planted in pots as described previously. Pots were heavily seeded and thinned to five plants per pot 10 days later. When the plants were 13 cm tall, weeds were inoculated with a pathogenic isolate of *F. oxysporum* obtained from soybean (4), using a soil-drench method (6,11). Inoculum was prepared as described previously and a concentration of 1.9×10^6 propagules per milliliter (viability level of 63%) was applied at the rate of 50 ml per pot. Plants were watered as needed until the experiment was terminated 5 wk later. Isolations from roots and stems of inoculated weeds were attempted after disinfestation with APDA. The experiment was repeated with the same design and methods, except the inoculum of *F. oxysporum* was incorporated into the soil mix immediately before planting the weed seeds.

The experimental design was a randomized complete block with three replicates per treatment. Data from all greenhouse tests were analyzed for differences in pathogenicity among *Fusarium* isolates.

RESULTS

Field studies. There were 21 weed species tested, including 15 annuals, 2 biennials, and 4 perennials (Table 1). Isolations were made from 800 samples, 400 each from both Kent and Sussex counties. Table 1 summarizes the *Fusarium* spp. isolated from weeds in conventional tillage and no-tillage fields throughout the growing season. The recovery rate for all *Fusarium* spp. from all root and stem isolations was 18.6%, of which 74.5% were identified as *F. oxysporum*. Of the 21 weed species assayed, 16 were shown to harbor *F. oxysporum* with frequencies ranging from 25 to 100% of all the *Fusarium* spp. isolated (Table 1). Frequency of isolations was much higher from roots than from stems for various species of *Fusarium*, mainly *F. oxysporum*. Other *Fusarium* spp. isolated at a very low frequency included *F. solani, F. graminearum, F. acuminatum, F. moniliforme, F. tricinctum, F. semitectum, F. equiseti,* and *F. avenaceum.*

A comparison of no-tillage versus conventional tillage with regard to isolation of *F. oxysporum* from selected weeds is shown in Table 2. No direct effect of tillage was noted, although the frequency of recovery of *F. oxysporum* was highest for tall morning glory in no-tillage fields (31.2%). It was followed closely by lambsquarter in conventional tillage (29.4%).

There were no patterns of species dominance noted from locations of soybean fields or for time of the season that *Fusarium* was isolated. Symptoms of Fusarium blight were not observed on any of the weeds.

Pathogenicity studies. Results of pathogenicity studies on Essex soybean with *F. oxysporum* isolates from weeds are summarized in Table 3. All isolates except isolate 4 from tall morning glory (rating of 1.5) caused significant disease on soybean, with isolate 1 from horsenettle the most pathogenic (rating of 3.7). There were small differences in pathogenicity among the remaining

Table 1. *Fusarium* isolated from dicotyledonous weeds in conventional and no-tillage soybean fields from June through September 1981[a]

Weed	*Fusarium* species		
	Conventional tillage	No-tillage	*F. oxysporum*[b]
Mustard (*Brassica* sp.)	2 (0)[c]	1 (0)	0.0
Bull thistle (*Cirsium vulgare* (Savi) Tenore)	2 (0)	0 (0)	0.0
Cocklebur (*Xanthium chinense* Mill.)	7 (1)	15 (1)	50.0
Dogbane (*Apocynum cannabinum* L.)	25 (2)	14 (0)	50.0
Giant ragweed (*Ambrosia trifida* L.)	1 (0)	24 (5)	40.0
Horsenettle (*Solanum carolinense* L.)	30 (6)	3 (0)	83.3
Ivyleaf morning glory (*Ipomoea hederacea* (L.) Jacq.)	119 (28)	70 (14)	78.6
Jimsonweed (*Datura stramonium* L.)	1 (1)	0 (0)	100.0
Lambsquarter (*Chenopodium album* L.)	51 (17)	15 (1)	88.9
Marestail (*Erigeron canadensis* L.)	20 (5)	21 (2)	57.1
Milkweed (*Asclepias syriaca* L.)	12 (2)	2 (1)	33.3
Pigweed (*Amaranthus retroflexus* L.)	28 (8)	18 (2)	80.0
Pokeweed (*Phytolacca americana* L.)	1 (1)	0 (0)	0.0
Prickly lettuce (*Lactuca serriola* L.)	0 (0)	3 (0)	0.0
Purslane (*Portulaca oleracea* L.)	1 (0)	4 (1)	0.0
Ragweed (*Ambrosia artemisiifolia* L.)	81 (12)	44 (5)	62.5
Smartweed (*Polygonum pensylvanicum* L.)	7 (4)	9 (2)	83.3
Tall morning glory (*Ipomoea purpurea* (L.) Roth)	56 (9)	16 (5)	92.8
Velvetleaf (*Abutilon theophrasti* Medic.)	67 (6)	20 (2)	87.5
Violet (*Viola* sp.)	3 (2)	1 (1)	100.0
Wild carrot (*Daucus carota* L.)	4 (4)	1 (0)	25.0

[a] Total of 20 soybean fields in Kent and Sussex counties in Delaware.
[b] The *F. oxysporum* value is a percentage of the total *Fusarium* spp. isolated for each weed.
[c] The first number indicates the number of samples from which isolations were made and the number in parentheses indicates the number from which *Fusarium* spp. was isolated.

Table 2. Comparison of conventional and no-tillage soybean fields for isolation of *Fusarium oxysporum* from selected weeds[a]

Weed	Conventional tillage	No-tillage
Ivyleaf morning glory	119 (21.8)[b]	70 (10.1)
Lambsquarter	51 (29.4)	15 (6.7)
Marestail	20 (20.0)	21 (0.0)
Ragweed	81 (9.9)	44 (4.5)
Tall morning glory	56 (14.3)	16 (31.2)
Velvetleaf	67 (7.5)	20 (10.0)

[a] Total of 20 soybean fields in Kent and Sussex counties, DE.
[b] The first number indicates the number of samples from which isolations were made and the number in parentheses indicates the percentage from which *F. oxysporum* was isolated.

Table 3. Mean disease ratings for Essex soybeans inoculated with isolates of *Fusarium oxysporum* obtained from different weeds

Isolate source	Isolate no.	Mean disease rating[x,y]
Horsenettle	1	3.7 ab[z]
Ragweed	1	3.5 ab
Ivyleaf morning glory	1	3.5 ab
Viola sp.	1	3.5 ab
Horsenettle	2	3.3 ab
Velvetleaf	1	3.3 ab
Tall morning glory	1	3.1 ab
Velvetleaf	2	3.1 ab
Lambsquarter	1	3.0 ab
Ivyleaf morning glory	2	3.0 ab
Lambsquarter	2	3.0 ab
Tall morning glory	2	2.9 ab
Ragweed	2	2.9 ab
Tall morning glory	3	2.6 abc
Ragweed	3	2.5 bc
Pigweed	1	2.4 bcd
Tall morning glory	4	1.5 cde
Control	...	1.0 e

[x] Mean disease rating for 15 soybeans, three replicates of five plants per pot.
[y] Disease scale of 1 = no evidence of infection to 5 = severe infection and/or wilt.
[z] Means followed by the same letter are not significantly different according to Duncan's new multiple range test at $P = 0.05$.

isolates, with a range from 2.4 to 3.5. Also, there was no apparent relationship between source of isolates and pathogenicity. Isolate 1 from ragweed gave one of the highest disease ratings, whereas isolate 3 from the same weed gave one of the lowest.

Pathogenicity of the isolates included in the first test increased upon second passage through Essex soybean. Attempts to reisolate *F. oxysporum* from inoculated soybeans using APDA were successful for all isolates except those giving the lowest disease ratings (tall morning glory 4, pigweed 1, and ragweed 3).

Weed inoculations. The percent recovery of *F. oxysporum* from inoculated weeds was low (0–8%) for those inoculated via the soil-drench method and 0–24% where inoculum was incorporated into the soil before planting, even though a high concentration of inoculum was used (1.9×10^6 propagules per milliliter). The highest rate of recovery in both tests was for ivyleaf morning glory and velvetleaf, with isolation from stem and root tissue, respectively. No symptoms were observed on any of the weeds inoculated in this experiment.

DISCUSSION

F. oxysporum was recovered from 16 weed species commonly found in Delaware soybean fields. Although *Fusarium* spp. are ubiquitous fungi (15), it has been shown that formae speciales of *F. oxysporum* are able to build up and persist on nonhost plants (10,15). We found that *F. oxysporum* can colonize roots of common weed species in soybean fields and retain their pathogenicity to the economic host. Further, it was found that pathogenicity of some isolates increased when inoculated into soybean a second time.

It was expected that *F. oxysporum* would be isolated with greater frequency from weeds in no-tillage versus conventional tillage fields, because *F. oxysporum* has been shown to build up on wheat and barley crop stubble in no-tillage fields and can be recovered from roots of these plants inoculated in the greenhouse (R. B. Carroll, *unpublished*). The expectation that this would influence the infection of weeds, especially in no-tillage fields with higher weed populations, was not substantiated except for tall morning glory.

The lack of any symptoms on weeds assayed from the field and when inoculated in the greenhouse with *F. oxysporum* is in agreement with findings of others (2,10,12) that these weeds may act as "symptomless" alternate hosts for the pathogen. It is also possible that weeds are symptomless because only less-aggressive isolates (with respect to soybeans) infect weed plants. The poor recovery of *F. oxysporum* from weeds inoculated in the greenhouse may have been caused by several factors that resulted in poor colonization of root tissue. Environmental conditions in the greenhouse are vastly different from the field and the autoclaved greenhouse potting mixture was quite different from the soil (loamy sand) in the field, or perhaps a wounding procedure should have been employed.

The importance of weeds as reservoirs of potential fungal pathogens was recognized by Garrett (5) when he stated, "Weeds exert their most directly harmful effects, perhaps, in the case of soil-borne diseases, because the roots of weeds infected by a particular parasite act as direct inoculum for the roots of any following crop susceptible to infection by that parasite." This could certainly be true for the weeds found to harbor *F. oxysporum* in this study.

One cannot conclude from the available data that weed control would prevent or reduce soilborne diseases, especially Fusarium blight on soybean, but it would seem in agreement with McGlohon's statement (13) that "the solution to many of our serious disease problems is often found in the area of simple management, yet we sometimes overlook this area while searching for more dynamic cures."

ACKNOWLEDGMENTS

We wish to thank Paul Nelson and Nancy Fisher, Pennsylvania State University Fusarium Research Center, for identification of representative *Fusarium* isolates. We also thank David Regehr for help in weed identification and for supplying weed seed for inoculation tests.

LITERATURE CITED

1. Cerkauskas, R. F., Dhingra, O. D., Sinclair, J. B., and Asmus, G. 1983. *Amaranthus spinosus*, *Leonotis nepetaefolia*, and *Leonurus sibiricus*: New hosts of *Phomopsis* spp. in Brazil. Plant Dis. 67:821-824.
2. Clark, C. A., and Watson, B. 1983. Susceptibility of weed species of Convolvulaceae to root-infecting pathogens of sweet potato. Plant Dis. 67:907-909.
3. Fehr, W. R., and Caviness, C. E. 1977. Stages of soybean development. Iowa State Univ. Coop. Ext. Serv. Spec. Rep. 80. 11 pp.
4. Ferrant, N. P., and Carroll, R. B. 1981. Fusarium wilt of soybean in Delaware. Plant Dis. 65:596-599.
5. Garrett, S. D. 1960. Inoculum potential. Pages 23-56 in: Plant Pathology. An Advanced Treatise. Vol. 3. J. G. Horsfall and A. E. Dimond, eds. Academic Press, New York. 675 pp.
6. Hart, L. P., and Endo, R. M. 1981. The effect of length of exposure to inoculum, plant age, root development, and root wounding on Fusarium yellows of celery. Phytopathology 71:77-79.
7. Helbig, J. B., and Carroll, R. B. 1982. Weeds as a source of *Fusarium oxysporum* pathogenic on soybean. (Abstr.) Phytopathology 72:707.
8. Hepperly, R. P., Kirkpatrick, B. L., and Sinclair, J. B. 1980. *Abutilon theophrasti*: Wild host for three fungal parasites of soybean. Phytopathology 70:307-310.
9. Karunakaran, P., Chandrasekharan Nair, M., and Gokulapalan, C. 1980. Survival of the clove pathogen *Colletotrichum gloeosporioides* on the weed Clerodendron in India. Plant Dis. 64:415-416.
10. Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. Phytopathology 61:1213-1217.
11. Leath, S., and Carroll, R. B. 1982. Screening for resistance to *Fusarium oxysporum* in soybean. Plant Dis. 66:1140-1143.
12. McDonald, J. D., and Leach, J. D. 1976. Evidence of an extended host range of *Fusarium oxysporum* f. sp. *betae*. Phytopathology 66:822-826.
13. McGlohon, N. E. 1982. Management practices that are controlling peach diseases. Plant Dis. 66:7.
14. McKeen, C. D., and Thorpe, H. J. 1973. Pathogenic species of *Verticillium* in horticultural crops and weeds in southwestern Ontario. Can. J. Plant Sci. 53:615-622.
15. Nelson, P. E. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. Pages 51-80 in: Fungal Wilt Diseases of Plants. M. E. Mace, A. A. Bell, and C. H. Beckman, eds. Academic Press, New York. 640 pp.
16. Wooliams, G. E. 1966. Host range and symptomology of *Verticillium dahliae* in economic, weed and native plants in interior British Columbia. Can. J. Plant Sci. 46:661-669.