Effects of Isolate Virulence, Plant Age, and Crop Residues on Seedling Blight of Sunflower Caused by *Alternaria helianthi*

K. K. Jeffrey, P. E. Lipps, and L. J. Herr

Graduate research associate, assistant professor, and professor, respectively, Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691. Present address of first author: Buckman Laboratories, Inc., 1265 N. McLean Boulevard, Memphis, TN 38108.

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ABSTRACT

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Isolates of *Alternaria helianthi* obtained from infested sunflower seed, wild *Helianthus annuus*, and a commercial sunflower hybrid varied in virulence as determined by the number of lesions produced on leaves. Differences in the number of lesions per leaf caused by the various isolates could only be detected at the highest conidial concentration used (1,500/ml). Young sunflower plants, ranging in age from 1 to 21 days, were equally susceptible to stem infection when conidia (20,000/ml) were deposited on the stem base. Conidia of *A. helianthi* were produced on sunflower stem residue that overwintered in the field. A greater proportion of stem residue (92%) yielded conidia when collected from the soil surface on 20 May compared to residues that were buried (13%) or those that were collected on 27 July from the soil surface (7%) or buried (0%). In greenhouse mist-chamber tests, infested residue placed on the soil surface resulted in a high amount of seedling disease, whereas residues buried above or below the seed resulted in very little and no disease, respectively. In a field trial, extensive seedling death occurred when early planted (14 June) seedlings emerged in plots covered with infested sunflower residues as compared to the level of disease that occurred in plots without residues or in plots planted later in the season (12 July). Results indicate that crop residues are a significant source of inoculum for Alternaria blight and that reduced inoculum levels could be achieved by clean plowing, 1-yr crop rotation, and/or delayed planting.

Alternaria helianthi (Hansf.) Tubaki and Nishihara is an important pathogen of sunflower (*Helianthus annuus* L.) throughout the world (1–5, 7–11). In general, wet weather, high humidity, and warm temperatures during the growing season favor sporulation of the pathogen (1,3) and disease development (3,4,8,10,11). *A. helianthi* has been detected on seed (5,7,8,10,11) and on overwintered crop residues (2,8–11). In regions where sunflower has only recently been grown (ie, Ohio), infested seed was apparently the means of introduction of the pathogen (8,10,11). Due to the very low level (<0.5%) of contaminated seed detected in seed lots (8), favorable weather conditions would possibly be needed for severe disease to occur the first year of planting sunflower. However, infested crop debris appears to be the major source of inoculum for epidemics (2,8–11).

Sunflowers are most susceptible to infection by *A. helianthi* during anthesis and the seed filling stage of growth (4,9). However, *A. helianthi* can cause seedling blight, which reduces stand, and can infect both leaves and stems of 10- to 32-day-old plants (11). These reports indicate that sunflowers are susceptible to infection at any stage of growth. Although pathogenicity tests of several different isolates have been performed (8), no information is available regarding differences in virulence among isolates of *A. helianthi*.

Objectives of this study were to determine the virulence of different isolates of *A. helianthi* on sunflower, to determine differences in susceptibility among young sunflower plants of different ages, and to demonstrate the importance of infested crop debris as a source of inoculum for seedling blight.

MATERIALS AND METHODS

Isolates. Ten isolates of *A. helianthi* were obtained from different sources including infested seed (8) (isolate L26, L27, L28, L29, L30 and L31; isolated in 1981), a commercially grown sunflower hybrid cultivar RBA 300G, Ashtabula County, OH (H101; isolated in 1980), and three wild plants of *Helianthus annuus* in Darke County, OH (H102, H103, H104; isolated in 1982). Isolate H101 has been deposited in the American Type Culture Collection as ATCC 52588. These isolates, obtained over a 3-yr period, were maintained on fresh potato-dextrose agar (FPDA) (6).

Isolate virulence. Cultures were grown at room temperature (24 ± 2 C) under continuous fluorescent light (1,000 lux) on FPDA in 9-cm-diameter petri dishes. Conidial suspensions were prepared from 4-wk-old cultures by adding 10 ml of distilled water to each plate and rubbing the agar surface with a rubber policeman. Conidia were counted with a hemacytometer and suspensions were adjusted to 1,500, 150, and 15 conidia per milliliter by dilution. Cultivar RBA 3101 sunflower plants (5 wk old, 10-leaf stage), grown in the greenhouse under supplemental fluorescent lighting (8,000 lux) for 12 hr per day at 23 ± 5 C, were used in the test. Plants were inoculated by atomizing (703 gm/cm^2 pressure) conidial suspensions for 5 sec on the third through sixth leaf from the bottom of each plant. Four replicate plants, one plant per pot, were inoculated with each concentration of each isolate. The plants were immediately placed in a mist chamber for 48 hr at about 24 C, and then returned to the greenhouse. The number of lesions per leaf on leaves three through six were counted 3–4 days later.

Effect of plant age. Seeds of sunflower (cultivar Sun Hi S304) were planted on different days to obtain plants 1, 2, 3, 4, 5, 6, 7, 10, 14, and 21 days old. Plant ages were expressed as the number of days following emergence. Eight seeds were planted per 15-cm-diameter pot containing a mixture of sand and Marengo silty clay soil (1:1, v/v). Emerging seedlings were thinned to five plants per pot. Plants were grown on a greenhouse bench at 23 ± 5 C using supplemental fluorescent lighting (9,300 lux) for 12 hr per day. Eight pots were used per age group: four were inoculated with *A. helianthi* and four were uninoculated controls.

A conidial suspension (~20,000 conidia per milliliter) of isolate H103 was prepared as described previously. Five plants per pot were inoculated by depositing 2 ml of the conidial suspension on the stem surface just above the soil surface with a Cornwall syringe.

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The plants were immediately placed in a mist chamber at 24 C for 48 hr then returned to the greenhouse. The plants with stem lesions were counted 1 wk later.

Winter survival on infested residue. Naturally infected sunflower stems with blackened lesions, randomly collected from a field after harvest (10 November 1981), were used to monitor survival of *A. helianthi*. Portions of each stem (~0.5 × 2.5 cm) were placed in moist chambers to confirm the presence of *A. helianthi*. Petri dish moist chambers contained two moistened 9-cm-diameter filter-paper disks and a glass microscope slide upon which the stem pieces were placed to avoid excessive absorption of moisture. These chambers were placed in plastic bags and maintained for 2 days at 24 C. The stem pieces were then examined under a stereo microscope (×20) for the presence of conidia of *A. helianthi*.

Winter survival of *A. helianthi* on stems was studied by placing two sets of 20 infested stalks (25–30 cm long) flat on the soil surface and burying two sets of 20 stalks at a depth of about 15 cm. Each set of stem residues was secured between two sheets of 12.7 mm mesh hardware cloth screen (100 × 75 cm) to facilitate recovery of the residue. Residues were placed in a harvested sunflower field near Wooster, OH, on 3 December 1981, and retrieved at dates corresponding to the approximate planting times of single- and double-crop sunflower. Two sets of residue, one buried and one left on the surface, were collected 20 May 1982 (single crop) and on 27 July 1982 (double crop). Five 1 × 4-cm pieces of the stem rind were cut from each stalk piece, washed with tap water to remove adhering soil, and then incubated for 48 hr in a petri dish moist chamber as previously described. After 2 days, 100 pieces of stem residue from each set of 20 stalks were microscopically examined for sporulation.

Infested residues as a source of inoculum. *Greenhouse studies.* Stem residues with blackened lesions, typical of those caused by *A. helianthi*, were collected in a field after harvest for use in a series of greenhouse tests. The residues were stored in plastic bags in a cold room at 5 C until used. Before and after each test, 25 and 50 stem pieces, respectively, were assayed for the presence of *A. helianthi* in petri dish moist chambers and examined microscopically for sporulation.

Four separate tests were conducted to determine the effect of burying residues on the incidence of disease. In the first two tests, 10 seeds (cultivar Sun Hi S304) were planted 1 cm deep in each of 40 flats (16 × 12 × 6 cm) in an autoclaved (121 C for 6 hr) greenhouse soil mixture consisting of Wooster silt loam and peat (5:1, v/v). Stem residue was broken in small pieces (~1 × 2 cm) and spread over the soil surface. In the third test, after the seeds were planted 1 cm deep in 40 flats, the residue was placed over the soil layer, and then covered with an additional 1 cm of soil. In the fourth test, the residue was spread over an ~3-cm-deep layer of soil in 40 flats, covered with 1 cm of soil, upon which the seeds were placed and covered with an additional 1-cm layer of soil.

Flats were placed in a greenhouse bench-top mist chamber (215 × 92 × 92 cm) made of a wooden frame and enclosed with plastic sheeting. A centrifugal humidifier (Herrmidifier, model 500; Herrmidifier, Co., Inc., Lancaster, PA) provided moisture after every 2 min for 20-sec periods 15–16 hr daily. The chamber front wall was tightly closed during the time the humidifier was running to ensure maximum wetness. The front plastic wall was opened to allow drying during the 8–9 hr daily that the humidifier was turned off. During the drying period, an oscillating three-speed fan (Vortalex, 32-cm diameter, General Electric Co.) was used to direct airflow over the flats within the mist chamber. The fan was operated at low speed and placed on an adjacent greenhouse bench about 2 m from the mist chamber.

Each test was maintained for 2 wk at 23 ± 5 C. The total number of plants emerging, the number with stem lesions, with leaf lesions, and with both stem and leaf lesions were recorded.

Field studies. The role of sunflower plant residue as a source of inoculum was tested in the field by using infested stalks from an inoculated field plot. Following fall harvest, sunflower stalks were removed from the field and distributed onto individual adjacent plots in a split-plot design, where residue plus nitrogen were whole plots and planting dates were split-plots, with four replications (blocks). Two plots per block received residue (residue plots) and two plots per block did not (no-residue plots). One of each residue and no-residue plot per block was then treated with urea (112 kg N/ha) plus nitrapyrin (0.55 kg/ha) to determine if nitrogen application would reduce the level of *A. helianthi* surviving the winter on residue. The following spring ammonium nitrate (112 kg N/ha) was applied to those plots that were not treated with nitrogen in the fall. Sunflower (cultivar Stauffer 3101) was planted at a rate of 59,300 seeds per hectare with a no-till planter where plots consisted of 12 rows, 12.5 m long with 76 cm between rows. The first planting date was 14 June 1983 and the number of surviving plants per row from the center two rows in each plot was determined 3 wk later. Plots were then treated with glyphosate (1.7 kg/ha) to kill remaining sunflowers and then replanted into the same plots on 12 July. The number of surviving plants per row and the percentage of diseased plants with at least one lesion per plant per row were recorded 3 wk after replanting. Twenty-five stem pieces were collected from each residue plot on 11 May and 4 August and assayed in moist chambers for presence of *A. helianthi* as previously described.

RESULTS

Isolate virulence. Considerable variability was detected in the virulence of the 10 isolates of *A. helianthi* (Table 1). There were significant differences among the isolates, concentrations, and also their interaction was significant ($P = 0.05$). Isolates L30 and H103 were highly virulent, whereas isolate L27 was the least virulent. Greater differences among isolates in the mean number of lesions per leaf were obtained by using the highest concentration (1,500 conidia per milliliter) of inoculum. Lower (150 and 15 conidia per milliliter) conidial concentrations did not reveal a large significant difference in virulence among isolates.

Effect of plant age. Inoculation of stems with conidia just above the soil surface resulted in the development of stem lesions on 75–85% of the plants in all 10 age groups of sunflower seedlings. No statistical differences were detected in the number of plants having stem lesions among the age groups. Although most plants had stem lesions, few plants were killed. Death usually occurred when the stem was completely girdled by a lesion.

TABLE 1. Virulence differences among isolates of *Alternaria helianthi* on sunflower

Isolate	Mean no. of lesions per leaf at inoculum concentration (conidia/ml)		
	1,500	150	15
L30	41.2[z]	4.1	1.1
H103	38.6	4.1	1.3
L29	23.2	3.5	0.6
H104	22.9	2.4	0.5
L28	21.0	2.0	0.4
L31	20.7	2.3	0.4
H102	19.7	3.2	0.9
L26	18.2	1.4	0.3
H101	13.8	1.4	0.4
L27	6.2	0.8	0.2
LSD ($P = 0.05$) = 1.4			

[z] Means based on the number of lesions developing on the third through sixth leaf of each of four plants inoculated per inoculum concentration.

TABLE 2. Winter survival of *Alternaria helianthi* on infested sunflower stalk residues from 3 December 1982 to dates corresponding to planting times of single- and double-crop sunflower

Recovery date	Percentage of stem pieces with sporulation[z]	
	Soil surface	Buried
20 May 1982	92	13
27 July 1982	7	0

[z] Percentages determined by assaying 100 pieces of stem residue in petri-dish moist chambers.

Winter survival on infested residue. Conidia of *A. helianthi* developed on all of the stems collected from a diseased sunflower field and subsequently used to test winter survival of the pathogen. Ninety-two of 100 stem pieces from the 20 stalks placed on the soil surface from 3 December 1981 to 20 May 1982 yielded conidia of *A. helianthi* (Table 2). Sporulation was observed on only 13 of 100 pieces from the buried stem residue. Residue placed in the field from 3 December 1981 to 27 July 1982 had substantially fewer stem pieces yielding *A. helianthi* than those collected on the previous date. In the case of stalks placed on the soil surface, sporulation occurred on only seven of the 100 stem pieces. No sporulation of *A. helianthi* developed on the 100 stem pieces from the buried residue.

Infested residues as a source of inoculum. *Greenhouse studies.* The infested residues were examined for development of conidia of *A. helianthi* before and after each test. Before each of the four tests, over 92% of 25 stem pieces examined had visible sporulation. After use in the different tests, detectable differences in the number of stem pieces with sporulation were associated with specific treatments of the residue. *A. helianthi* sporulated on 80 and 88% of the 50 stem pieces placed on the soil surface after tests 1 and 2, respectively, and only 6% of the stem pieces buried above the seed (test 3) had conidia present. No sporulation was observed on any of the stem pieces buried below the seed (test 4).

The level of disease incited by *A. helianthi* was significantly ($P = 0.05$) greater when the stalk residue was placed on the soil surface than when it was buried. Significantly fewer plants became diseased when the residue was buried above the seed and no disease occurred when the residue was buried below the seed (Table 3).

Field studies. Application of nitrogen plus the denitrification inhibitor nitrapyrin had no effect on the level of *A. helianthi* overwintering on infested residues or on the incidence of Alternaria blight in the field plot; therefore, only the planting date and residue treatment data are presented in Table 4.

Analysis of variance indicated a significant ($P = 0.05$) interaction between planting date and the presence or absence of residues in plots on the number of plants surviving seedling blight (Table 4). In plots planted 14 June, seedling blight was severe in residue plots as compared to the plots without residues. There were no differences in the number of surviving plants among the residue and no-residue plots planted on 12 July. There was a significant difference in the percentage of diseased plants in plots planted 12 July between the residue plots and the no-residue plots; however, the level of disease detected in both plots was high (90 and 78%, respectively). Data on the percentage of diseased plants in plots planted on 14 June were not recorded due to the low number of surviving plants in the residue plots.

The percentage of residues yielding sporulation of *A. helianthi* was significantly greater (94%) from stalk sections collected 11 May than those collected 4 August after the later planting date (8%).

DISCUSSION

In this study, isolates of *A. helianthi* obtained from seedlots, a commercial sunflower hybrid, and wild plants of *Helianthus annuus* were all pathogenic to sunflower. Isolates obtained from the same source, and isolated the same time, varied in virulence (Table 1), indicating differences in virulence existed among these isolates of *A. helianthi*. For example, isolates H102, H103, and H104, though obtained from the same source at the same time (1982), differed markedly in virulence. Herr and Lipps (4) tested isolate H101 in 1981 and reported that this isolate was highly virulent on sunflower (cultivar RBA 300G). In the present study, however, isolate H101 ranked ninth for virulence among 10 isolates (Table 1), indicating that isolate H101 may have lost virulence after 2 yr in culture. Development of culture storage methods that minimize isolate transfer should help retain virulence characteristics.

Detection of differences in virulence among isolates depended on conidial concentration. At the lower concentrations (15 and 150 conidia per milliliter), only a few leaf lesions developed and separation of lesion number means among the isolates was limited. With the highest concentration (1,500 conidia per milliliter), greater separation of means occurred. A problem with using higher conidial concentrations was the difficulty in counting individual leaf lesions due to coalescence and development of large necrotic areas.

Results of greenhouse tests indicated that from 0 to 14 days after emergence, plants were equally susceptible to infection. Stem lesions were common on most plants, but most lesions were small. Few plants were killed in contrast to the report by Shane et al (11). The reason for differences in results may be due to differences in inoculum levels among tests.

Results from the greenhouse residue-placement studies and the early-planted field study demonstrated that inoculum from infested stem residue could initiate disease. These studies indicated that stem residue placed on the soil surface resulted in severe disease on emerging sunflower plants. Greenhouse residue studies also demonstrated that survival of *A. helianthi*, as determined by sporulation, was greatly reduced on buried stem residue. Some disease symptoms were observed when the stem residue was buried above the seed, but this may have occurred because as the seedlings emerged, some pieces of stem residue were pushed to the soil surface allowing conidia to develop.

Once introduced into an area, survival of *A. helianthi* from season to season probably occurs mainly on infested sunflower plant residues. Herr and Lipps (8) attributed the introduction of the pathogen into Ohio to contaminated seed and indicated that the inoculum from the overwintering of *A. helianthi* on sunflower plant residue may be of great significance in maintaining the pathogen in an area. Other researchers (2,5,9,11) have also indicated the importance of these inoculum sources. Although residue may be the most important source of primary inoculum, there also may be other sources. Three of the isolates studied were obtained from wild *Helianthus annuus* in southwestern Ohio. These wild plants were abundant in that area and all were heavily

TABLE 3. Effect of surface and buried sunflower plant residues infested with *Alternaria helianthi* on the incidence of leaf and stem spot on sunflower in greenhouse tests

	Mean number of plants with lesions[x]		
Residue position	Stem	Leaf	Both
Soil surface, test 1	6.4 a[y]	7.8 a	6.1 a
Soil surface, test 2	5.8 a	6.7 b	5.6 a
Buried 1 cm above seed, test 3	0.3 b	0.6 c	0.1 b
Buried 1 cm below seed, test 4[z]	0	0	0

[x] All values are the mean number of diseased plants/flat (10 plants per flat, 40 flats per treatment).
[y] Column means followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.
[z] Data were not included in the analysis because no disease occurred.

TABLE 4. Effect of infested residues and planting date on the incidence of Alternaria blight of sunflower in the field in 1982

Planting date	Residue treatment[v]	Surviving plants (no.)[w]	Diseased plants[x] (%)	Residue with sporulation[y] (%)
14 June	Residue	5 a[z]		94 a
	No residue	31 b		
12 July	Residue	29 b	90 a	8 b
	No residue	34 b	78 b	

[v] Residue = infested residues placed in plot area the preceding fall; no residue = residues removed from plot area the preceding fall.
[w] Total number of nonkilled plants per 12.5 m row.
[x] Mean percentage of plants with one or more lesions on leaves or stems 3 wk after planting.
[y] Mean percentage of stalks (25 per plot) collected on 11 May (first planting date) and 4 August (second planting date) with sporulation of *Alternaria helianthi*.
[z] Column means followed by the same letter are not statistically different ($P = 0.05$) according to Duncan's new multiple range test.

infected with *A. helianthi*. Other weed hosts, including closely related Compositae, need to be investigated as possible sources of inoculum.

In Ohio, sunflowers may be planted as a full-season crop following fall or spring plowing or some form of reduced tillage in late May or early June, or may be double-cropped after wheat in July using reduced or no-till residue management systems. Because of this split planting season and the different tillage practices used, it was necessary to determine the survival of *A. helianthi* on infested, overwintered residues at different times during the growing season and on residues either buried or placed on the soil surface. More stem pieces yielded conidia of *A. helianthi* early in the season (20 May 1982 and 11 May 1983) than later (27 July 1982 and 4 August 1983) (Tables 2 and 4). Moreover, on both recovery dates, residues left on the soil surface had a substantially greater percentage of stem residue with sporulation of *A. helianthi* than did the buried stem residue (Table 2). These results indicate that the fungus survived poorly on buried residues and that delayed planting may allow sufficient time to reduce levels of *A. helianthi* surviving in residues. Clean plowing after sunflower or a 1-yr rotation away from sunflowers should reduce the level of inoculum for both single- and double-crop sunflower.

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