Alternaria helianthi on Sunflower in Ohio

L. J. HERR, Professor, and P. E. LIPPS, Assistant Professor, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster 44691, and Ohio State University, Columbus 43210

ABSTRACT

Herr, L. J., and Lipps, P. E. 1982. *Alternaria helianthi* on sunflower in Ohio. Plant Disease 66: 509-512.

A leaf and stem spot disease of oilseed sunflower (*Helianthus annuus*) caused by *Alternaria helianthi* was found in Ohio for the first time in 1980. In severely affected fields, many sunflower plants were defoliated and lodged. In greenhouse tests, inoculation with suspensions of 15,000 conidia per milliliter resulted in death of most plants. *A. helianthi* was seedborne and overwintered in diseased sunflower residues.

Oilseed sunflower (*Helianthus annuus* L.), a relatively new crop for Ohio, was severely affected with leaf and stem spots during the 1980 season, resulting in extensive defoliation and lodging of plants. Whereas bacteria of the *Pseudomonas syringae* group and *Septoria helianthi* Ell. & Kell. were among the pathogens found associated with leaf spotting (7), most of the foliar and stem lesions were attributable to *Alternaria helianthi* (Hansf.) Tubaki and Nishihara (13). Although a brief description of the symptoms has been published (7), this is the first detailed report on the occurrence of *A. helianthi* in Ohio. This pathogen has been reported from Mississippi (12), Minnesota (12), North Dakota (12), Wisconsin (11), and Florida (11).

The pathogen was first described as *Helminthosporium helianthi* Hansf. in 1943 by Hansford (5) as the cause of blackish brown, zonate spots on leaves of sunflower in Uganda. In 1950, Wallace and Wallace (14) reported a severe outbreak of *H. helianthi* on sunflower in Tanganyika; stem-breaking of sunflower due to *H. helianthi* was reported from northern Rhodesia in 1962 (2). In 1964, Pavgi and Upadhyay (10), unaware of Hansford's report, described *H. helianthi* Pavgi as a new species causing a leaf spot on sunflower in northern India. Tubaki and Nishihara (13) discovered the pathogen in Japan and in 1969 renamed it *Alternaria helianthi* because of the occurrence of longitudinal septa in the conidia and the porogenous conidial development. This name is currently accepted.

According to Zimmer and Hoes (15), *A. helianthi* has been reported on sunflower from various African countries, Argentina, India, Japan, Yugoslavia, and Brazil. Additions to this list include Rumania (6) and Bulgaria (3). A serious head blight occurs in Yugoslavia (13), as well as leaf and stem spotting. Shane et al (12) recently reported that *A. helianthi* causes a severe seedling blight of sunflower in Minnesota. This seedling-blight phase had not been reported previously. The pathogen is seedborne (11,15) and is capable of initiating disease over a wide range of temperatures (1). It thus constitutes a potential threat to sunflower producing regions worldwide (11,15).

Our objectives were to describe the disease symptoms on sunflower and the characteristics of the pathogen and to investigate the origin of the disease outbreak in Ohio.

MATERIALS AND METHODS

Isolation. A disease survey of Ohio sunflower fields was made during late July, August, and early September 1980. Specimens of sunflower plants exhibiting leaf and stem spotting were examined microscopically when collected and after incubation in moist chambers for 24–48 hr at 25 C. The most successful isolations of *A. helianthi* were made directly from conidia developing on plant lesions. A finely drawn glass needle was used to transfer the large, characteristic conidia to 2% water agar (WA) plates. Cultures were maintained on potato-dextrose agar (PDA) test tube slants.

Growth in culture. Three culture media —Difco PDA (DPDA), PDA freshly prepared from potatoes (FPDA), and V-8 juice agar (9)—were used to study the growth and sporulation of *A. helianthi*. Agar inoculum disks (4 mm diameter) were transferred from 1-wk-old DPDA cultures to six plates of each medium. Half the plates were incubated in darkness and half under continuous fluorescent light at 25 C for 1 wk. Colony diameters were measured (two measurements at right angles), and the amount of sporulation was estimated by microscopic examination.

Pathogenicity. To enhance production of conidia by *A. helianthi* for pathogenicity tests, 1- to 2-wk-old FPDA culture plates were flooded with 1–2 ml of sterile water, and the fungal colonies were rubbed with a wire loop to suspend the conidia and disperse them over the agar surface. This procedure resulted in uniform growth and production of numerous conidia after 1 wk of incubation at 25 C. Sunflower plants (cv. RBA 300G) were inoculated by spraying water suspensions of conidia on plant foliage with an atomizer or, in some cases, brushing comminuted mycelium or conidial suspensions on leaves with a camel's hair brush. Inoculated plants were immediately placed in a dew chamber at 27 C for 24 or 48 hr. Four replicate, 10-cm-diameter pots, with two plants per pot, were used per treatment. Generally, 3- to 4-wk-old plants, grown in the greenhouse at about 26 C and 8,500 lux supplemental lighting for 12 hr/day, were used in the tests. After inoculation and incubation in the dew chamber, plants were returned to the greenhouse for disease development.

Seed assays. Seeds of 11 sunflower cultivars from 10 commercial seed producers were assayed for the presence of *A. helianthi* by plating 1,000 seeds of each cultivar on one or more agar media. These media were acidified PDA (APDA; acidified to pH 4.5 with 85% lactic acid), APDA plus 1.0 g of oxgall per liter, APDA plus 1.0 g of oxgall and 1.0 g of sodium propionate per liter, and WA. Generally, 15 seeds (not surface sterilized) were plated per petri plate. Seeds of one sunflower cultivar (cv. A) were surface-sterilized in 1:3 (v:v) solution of 5.25% sodium hypochlorite and distilled water for 1 min and then plated on APDA. Seeds plated on media containing APDA were examined after 2 days of incubation at 25 C for *Rhizopus*

Approved for publication as Journal Article 84-81 of the Ohio Agricultural Research and Development Center, Wooster.

Accepted for publication 12 January 1982.

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.

0191-2917/82/06050904/$03.00/0

sp. and after 3 days for *Alternaria alternata* (Fr.) Keissler and *A. helianthi*. Examination of seeds plated on WA was generally delayed for an additional day.

Overwintering. Discolored sunflower stems, standing or lying on the surface of the soil, were collected on 24–25 March 1981 from two fields severely affected with *A. helianthi* leaf and stem spot in 1980. Stem segments were plated without any further treatment on WA plus streptomycin sulfate and chloramphenicol, each at 50 μg/ml. Usually, four to five pieces (~0.5 × 2.5–3.0 cm) of each stem were plated per petri dish. After incubation for 1–2 days at 25 C, the stem pieces were examined under a stereomicroscope for conidia of *A. helianthi*. Conidia were removed with a needle from the sporulation of the pathogen on overwintered stem pieces. The conidia that were picked up were suspended in water and used to inoculate sunflower plants in the greenhouse by use of a camel's hair brush.

Fig. 1. Diseased plants within a sunflower field severely affected by leaf and stem spotting caused by *Alternaria helianthi*. Note extensive defoliation, stem blackening, and stem breakage.

RESULTS AND DISCUSSION

Symptoms. Diseased plants within a sunflower field severely affected by *A. helianthi* are depicted in Figure 1. Note the extensive damage to the foliage. Coalescence and enlargement of leaf spots resulted in withering, drying, and loss of leaves. Severely affected plants were completely defoliated (Fig. 1). Many stems bore blackened lesions, and some stalk breakage was also apparent. Stem lesions started as black flecks or streaks (Fig. 2) that enlarged into elliptic spots that coalesced to form large, blackened areas involving much of the stem. Leaf spots were at first small, but some later enlarged up to 1.5 cm in diameter. The spots had dark brown borders and brown to grey centers, sometimes zonate, and were frequently surrounded by chlorotic halos (Fig. 3).

Zimmer and Hoes (15) noted that the symptoms caused by *A. helianthi* are quite similar to those caused by *A. zinniae* Pape on sunflower. This similarity of symptoms may have obscured recognition of *A. helianthi* on sunflower in North America for some time.

Pathogen characteristics. Several conidia and conidiophores of *A. helianthi* in a leaf spot on the upper surface of a leaf of an inoculated plant are shown in Figure 4. The conidia were solitary, nonbeaked, and borne on simple or (rarely) branched conidiophores. Under higher magnification, conidia (as illustrated in Fig. 5) were cylindric to elongated-elliptic, straight or slightly curved, yellowish brown in color, septate, with four to 11 transverse or (occasionally) longitudinal septa, constricted at septa, and rounded at both ends. Measurements of conidia from leaf spots on inoculated sunflower plants (from greenhouse pathogenicity tests of *A. helianthi* isolates from sunflower seeds) and measurements of conidia reported in the literature are given in Table 1. The range in size of conidia isolated from the seeds of the three different cultivars was quite similar but appeared to be slightly larger than the size ranges reported by others (5,10, 12,13). Differences in conidial size may be the result of measurements taken from conidia produced on different substrates (ie, on diseased plants or on artificial media). More information on the sources of conidia (plant lesions or culture media) would be required to establish whether true differences exist.

Fig. 2. Portion of stem from field-infected sunflower, showing black flecks and streaking associated with early symptoms of stem disease caused by *Alternaria helianthi*.

Growth in culture. Best growth of *A. helianthi* on culture media was on V-8 juice agar with average colony diameters of 18.7 mm in darkness and 30.8 mm under continuous fluorescent light after 1 wk of incubation at 25 C. FPDA was superior to DPDA in both darkness and light. Colony diameters in darkness were: FPDA = 10.0 mm, DPDA = 8.7 mm; under fluorescent light, FPDA = 22.0 mm, DPDA = 12.0 mm. Estimated sporulation densities (observed under a stereomicroscope) were judged to be equal and very dense on all media in both darkness and light. The observed stimulation of growth under continuous fluorescent light is similar to the report of Shane et al (12) that growth of *A. helianthi* was enhanced under 12 hr of fluorescent light per day, as compared with growth in total darkness.

Pathogenicity. The virulence of a field isolate of *A. helianthi* was tested by inoculating 3-wk-old sunflower plants by spraying them with either 1,500 or 15,000 conidia per milliliter, after which the plants were kept in a dew chamber for 48 hr. Leaf and stem spots were evident on plants sprayed with the higher spore concentration when they were removed from the dew chamber. Most of these plants died after several days of incubation in the greenhouse. Plants inoculated with the lower conidial concentration were heavily spotted on stems and leaves but were not killed. The field isolate was extremely virulent on sunflower cultivar RBA 300G tested under these conditions.

The three isolates of *A. helianthi* obtained from seed lots of three commercial sunflower cultivars (A, B, and C) were pathogenic on 4-wk-old sunflower plants. Reduction of the

incubation time from 48 to 24 hr in the dew chamber slowed symptom expression as compared with the earlier pathogenicity test, although the plants were heavily spotted (Fig. 3). Foliar symptoms varied considerably, apparently depending upon the age of leaves at the time of inoculation, as seen in the density of spots per leaf. Prominent chlorotic halos were evident on the youngest leaf (Fig. 3, left). Field leaf-spot symptoms also exhibited some variability, making it advisable to determine the pathogen involved by means other than symptomatology.

Seed assay. Of 11 sunflower cultivars assayed, *A. helianthi* was isolated from seeds of three commercial cultivars (Table 2). *A. helianthi* was detected in seed lots of two cultivars, designated A and B, on all four media, but only once from seeds of cultivar C on APDA plus oxgall. Percentages of seeds with *A. helianthi* were low (0.2–0.6%), but for cultivars A and B the percentage was fairly consistent regardless of the medium used in the assay. *Rhizopus* sp. and *A. alternata* colonies also were recorded. They were universally present in seeds of all 11 cultivars and interferred with the assay of *A. helianthi*. Oxgall and sodium propionate were added to APDA in an attempt to inhibit the *Rhizopus* sp. (4,8). Both substances restricted growth of the *Rhizopus* sp. but did not reduce the frequency of occurrence. Surface sterilization of seed did not eliminate *Rhizopus* sp. or *A. alternata*, although some reduction was noted. Surface sterilization might be more deleterious to *A. helianthi* than to the interfering, associated fungi (cv. A seed not sterilized, 0.5% vs 0% for cv. A seed surface sterilized) (Table 2).

A. helianthi was confined to the seed (fruit) coat or to plant fragments present in seed lots and did not grow out onto the agar surface to any extent during the assay period. Plates were examined macroscopically and with a stereomicroscope. *A. helianthi* colonies had a reddish brown color and were less aerial (fuzzy) than *A. alternata*. WA provided conditions suitable for detection of *A. helianthi* on seed, while reducing growth of *Rhizopus* sp. and *A. alternata*. Some additional contamination with a *Bacillus* sp. and other bacteria was noted, however. All media used were suitable for detection of *A. helianthi* if examined within 3–4 days after plating (Table 2), but detection was facilitated when growth of *Rhizopus* sp. and *A. alternata* was retarded. Because occurrence of *A. helianthi* was infrequent and only 1,000 seeds per cultivar per medium were examined for conidia, we could not be certain that seeds of the other eight cultivars were free of this pathogen.

Overwintering. *A. helianthi* overwintered in diseased sunflower stems under Ohio conditions. In two fields, 25 of 25 and 21 of 21 stems assayed yielded *A. helianthi*, respectively. Conidia obtained from overwintered sunflower stem pieces were highly virulent on the sunflower cultivar (RBA 300G) used in greenhouse tests.

We have demonstrated that *A.*

Table 1. Dimensions and septations of conidia of three isolates of *Alternaria helianthi* originally obtained from seeds of three sunflower cultivars compared with dimensions and septations reported in the literature

Source of isolate	Septa (no.)	Length × width[a] (μm)
Seeds from Ohio		
cv. A	4–11	54–126 × 18–31[a]
cv. B	5–10	84–129 × 21–36[a]
cv. C	5–11	69–132 × 16–30[a]
Average	8	100.4 × 25.1
Literature		
Hansford (5)	2–8	30–90 × 11–16
Pavgi and Upadhyay (10)	8–11	69–116 × 13–20
Tubaki and Nishihara (13)	1–11	40–110 × 13–28
Shane et al (12)	0–11	20–97 × 5–23

[a] Based on measurements of 20 conidia per isolate obtained from lesions produced on leaves of sunflower plants in greenhouse pathogenicity tests of isolates from Ohio.

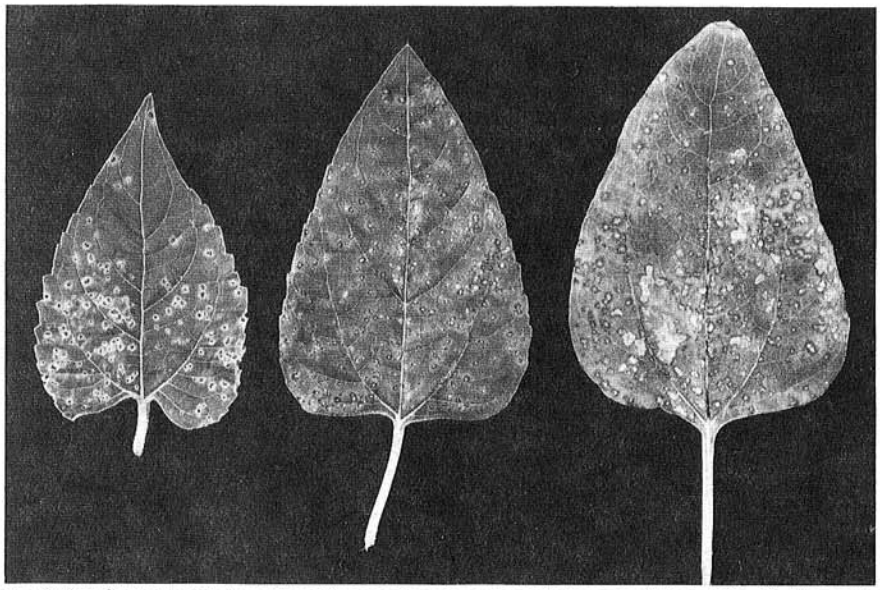

Fig. 3. Sunflower leaves inoculated in greenhouse pathogenicity tests with conidia of *Alternaria helianthi*, showing a range of leaf spot symptoms. **(Left)** Chlorotic halos surrounding lesions. **(Right)** Large necrotic areas resulting from coalescence of lesions. These lesions have dark brown borders and light grey centers.

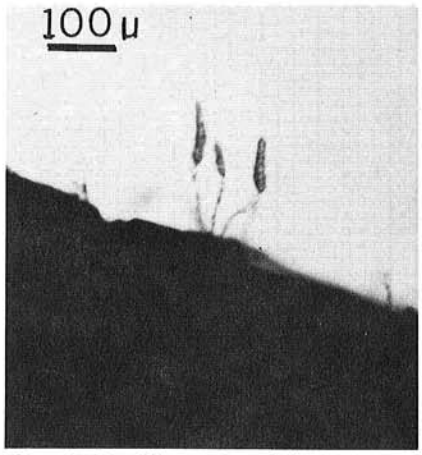

Fig. 4. Conidia and conidiophores of *Alternaria helianthi* in a lesion on upper surface of a leaf. Conidia are solitary and nonbeaked. Conidiophores illustrated are unbranched.

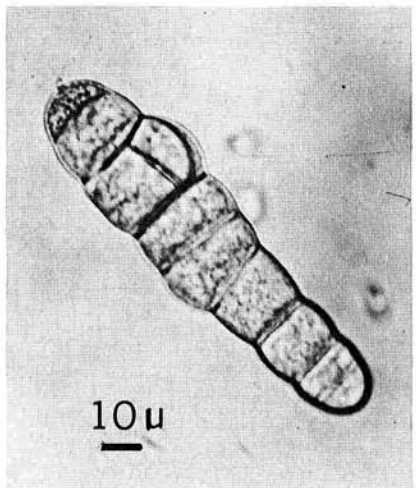

Fig. 5. Conidium of *Alternaria helianthi* with seven transverse and one longitudinal septa and with constrictions at transverse septa.

Table 2. *Alternaria helianthi, A. alternata,* and *Rhizopus* sp. obtained from seeds of three sunflower cultivars not surface sterilized and (in one case) following surface sterilization

Medium	Cultivar	Seeds bearing fungi (%)[a]		
		A. helianthi	*A. alternata*	*Rhizopus* sp.
APDA[b]	A	0.5	90	2
	B	0.6	80	34
APDA + oxgall	A	0.2	95	3
	B	0.5	93	33
	C	0.2	99	23
APDA + oxgall + sodium propionate	A	0.4	96	4
	B	0.3	92	42
	C	0.0	96	41
Water agar	A	0.5	99	5
	B	0.4	70	16
	C	0.0	76	23
APDA (seeds surface sterilized)	A	0.0	71	1

[a] Based on 1,000 seeds per cultivar.
[b] Acidified potato-dextrose agar (pH 4.5).

helianthi was present on seed, and infected seed was probably responsible for the introduction of *A. helianthi* into Ohio. This is likely because many of the affected sunflower fields were in their first year of production. Zimmer and Hoes (15) and Sackston (11) indicated that the seedborne means of dispersal constitutes a serious threat to sunflower production in North America. Furthermore, overwintering of *A. helianthi* on sunflower residue suggests that this inoculum source may be of great significance in maintaining the disease once the pathogen has been introduced into an area.

LITERATURE CITED

1. Acimovic, M. 1974. [Effects of some ecological factors on sporulation of *Alternaria helianthi* (Hansf.) Tub. and Nish. and on infection of sunflower.] Zast. Bilja 30:59-63. Taken from: Rev. Plant Pathol. 59:401 (abstr.).
2. Anonymous. 1962. Annual report of the Ministry of African Agriculture, Northern Rhodesia. Taken from: Rev. Appl. Mycol. 43:531 (abstr.).
3. B'chvarova, R. 1977. [Alternaria blight of sunflower, a new disease in Bulgaria.] Rastit. Zasn. 25:23-24. Taken from: Rev. Plant Pathol. 57:5077 (abstr.).
4. Crook, P., Carpenter, C. C., and Kleins, P. F. 1950. The use of sodium propionate in isolating actinomycetes from soils. Science 112:656.
5. Hansford, C. G. 1943. Contributions towards the fungus flora of Uganda. V. Fungi imperfecti. Proc. Linn. Soc. London 1:34-67. Taken from: Rev. Appl. Mycol. 23:409 (abstr.).
6. Hulea, A., Illiescu, H., and Bunescu, S. 1973. [Detection of an unusual fungal complex pathogenic on sunflower in Rumania.] Probl. Prot. Plant. 1:73-85. Taken from: Rev. Plant Pathol. 54:5035 (abstr.).
7. Lipps, P. E., and Herr, L. J. 1981. Major diseases may affect Ohio sunflower production. Ohio Rep. 66:19-22.
8. Littman, M. L. 1947. A culture medium for the primary isolation of fungi. Science 106:109-111.
9. Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
10. Pavgi, M. S., and Upadhyay, H. P. 1964. Parasitic fungi from north India. II. Mycopathol. Mycol. Appl. 24:347-354.
11. Sackston, W. E. 1981. The sunflower crop and disease: Progress, problems, and prospects. Plant Dis. 65:643-648.
12. Shane, W. W., Baumer, J. S., and Sederstrom, S. G. 1981. *Alternaria helianthi*: A pathogen of sunflower new to Minnesota. Plant Dis. 65:269-271.
13. Tubaki, K., and Nishihara, N. 1969. *Alternaria helianthi* (Hansf.) comb. nov. Trans. Br. Mycol. Soc. 53:147-149.
14. Wallace, G. B., and Wallace, M. M. 1950. Tanganyika fungus list: Recent records. XIV. Mycol. Circ. Dep. Agric. Tanganyika 29. 6 pp. Taken from: Rev. Appl. Mycol. 30:490 (abstr.).
15. Zimmer, D. E., and Hoes, J. A. 1978. Diseases. Pages 225-262 in: Sunflower Science and Technology. J. F. Carter, ed. Agronomy 19, Am. Soc. Agron., Madison, WI. 505 pp.