Photoperiod Influence on the Susceptibility of Sunflower to Sclerotinia Stalk Rot

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ABSTRACT

Photoperiod affected reaction of the annual sunflower *Helianthus annuus* to *Sclerotinia sclerotiorum*, a soilborne pathogen that causes stalk and head rot. Cultivars grown in the growth chamber at approximately 1,900 lux and 22/20 C day/night temperatures under short days on an 8- and 14-hour photoperiod were susceptible. In contrast, cultivars grown under long days on an 18- and 24-hour photoperiod were tolerant. Annual sunflowers grown in the greenhouse were highly susceptible, in both the seedling and adult stages. The susceptibility of the annual sunflower was associated with the proneness of succulent hypocotyls to fungal infection in response to short photoperiods. Tolerance in *H. annuus* was associated with enhanced growth and lignification in response to long photoperiods. The perennial *H. tuberosus* and interspecific hybrids *H. tuberosus* × *H. annuus* and *H. tuberosus* × *H. annuus* × *H. strumosus* were resistant to stalk rot, irrespective of photoperiodic treatment.

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Additional key words: Whetzelinia sclerotiorum.

Putt (19) indicated that cultivars and lines of sunflower, *Helianthus annuus* L., differ in susceptibility to stalk and head rot caused by *Sclerotinia sclerotiorum* (Lib.) d By. *(Whetzelinia sclerotiorum* (Libert) Korf et Dumont). This disease continues to be highly destructive in the largest sunflower seed-producing countries, namely, the Soviet Union, Argentina, and Romania, as well as in the United States. Resistance to Sclerotinia stalk rot exists among the North American native perennial *Helianthus* spp. It has been used by Pustovoit (18) in the production of resistant interspecific artificial hybrids with the cultivated sunflower.

Robinson (20) and Geise (5) have studied the influence of latitude on the phenology of the annual cultivated sunflower, but little is known on how natural daylength affects the susceptibility of this plant to various pathogens. Preliminary experiments indicated that the reaction of sunflower to Sclerotinia stalk rot was influenced by photoperiod (15). The purpose of this study was to determine the effect of the photoperiod on the stalk rot phase of the disease.

MATERIALS AND METHODS.—*Fungal isolates.*—*Sclerotinia sclerotiorum* isolates used in preliminary experiments were S-G and S-EP obtained, respectively, from field-infected sunflowers collected at Gonvick, Minnesota, in 1966 and at El Paso, Texas, in 1971 (16). Both isolates have similar pathogenicity to sunflower. In vivo and in vitro, they produce black, 10- to 15-mm diameter subspherical sclerotia that develop freely on the mycelium. We used only the S-EP isolate in subsequent experiments.

Sunflower cultivars.—In the greenhouse, stalk rot was studied on the following annual and perennial cultivated sunflowers: *H. annuus* 'Krasnodarets', 'Manchurian', 'Peredovik', 'Mingren', 'Graystripe', 'Commander', 'Mennonite', 'Arrowhead', 'VNIINK 8931', 'Romania HS 52', 'CM 144', 'CM 162', 'S 37 388', and USDA-Texas inbred selections P 21, HA 61, HA 65, and HA 89. In the growth chamber, stalk rot was studied on open-pollinated annual Krasnodarets and Manchurian (early- and late-maturing, respectively); the Canadian inbred lines CM 144 and CM 162 (moderately early- and late-maturing, respectively); the perennial, less cultivated tuber-bearing Jerusalem artichoke or topinambur *H. tuberosus* L. 'Nakhodka' (P.I. 357300), 'Skorospelka' (P.I. 357301), 'Vadium' (P.I. 357302), and 'Volga 2' (P.I. 357303); and the interspecific experimental hybrids *H. tuberosus* × *H. annuus* (P.I. 274517) and *H. tuberosus* × *H. annuus* × *H. strumosus* L. (P.I. 274518). These perennial accessions were obtained from the Soviet Union by the U.S. Regional Plant Introduction Station at Ames, Iowa.

Experimental growing conditions.—Greenhouse tests were conducted at Beltsville, MD, during fall and winter, with daylight supplemented to about 1,500 lux by means of 200-w incandescent lamps. Day temperatures were kept at about 22 and night temperatures at about 20 C. Experiments in a controlled environment were conducted in Sherer-Gillett growth chambers with 8-, 14-, 18- or 24-hours per day of fluorescent-incandescent light of about 1,900 lux. A distance of 20 to 30 cm between the overhead light source and the plant tops was kept throughout the growing period (62 days after plant emergence). Temperatures were 22 ± 2 C during light and 20 ± 2 C during dark periods with relative humidity of 60 to 70%. The annual sunflowers were grown from surface-disinfested seed in clay loam soil in triplicate 15-cm diameter pots, with three plants per pot. The perennial sunflowers were grown from tubers in duplicated pots, with one tuber per pot. We watered the plants daily, without exceeding the soil's water-holding capacity.

Growth measurements and histochemical tests.—Dry weights calculated as percentages of fresh weights were determined on the stem, hypocotyl, and roots of the annual sunflower and on stems and tubers of the perennial sunflower. Measurements were determined on the visible-flower-primordium stage of the annuals, and at 62 days after emergence in the perennial cultivars. In the perennial cultivars a flower primordium did not

TABLE 1. Influence of photoperiod on the growth and development of the annual cultivated sunflower *Helianthus annuus*, the perennial *H. tuberosus*, and interspecific hybrids

	Visible bud (days)		Dry wt. / Fresh wt. × 100[a] (%) of plant parts daily exposed to photoperiods[b] of:					
			Stem		Hypocotyl		Root	
	8	24	8 hr	24 hr	8 hr	24 hr	8 hr	24 hr
H. annuus:								
Krasnodarets	43	46	5.8 A	6.3 A	7.3 A	12.5 C	8.9 AB	12.0 C
Manchurian	0	0	9.9 B	11.1 BC	13.9 C	14.3 C	9.4 AB	16.3 D
CM 144	45	46	5.5 A	12.2 C	10.4 B	12.2 C	7.4 A	10.5 BC
CM 162	0	0	4.8 A	5.5 A	7.3 A	12.5 C	8.1 A	11.8 C
H. tuberosus:								
Nakhodka	0	0	18.9 A	26.1 C	. . .	. . .	17.3 AB[c]	19.3 BC[c]
Vadium	0	0	24.2 BC	21.4 AB	. . .	. . .	16.5 A	18.2 ABC
Volga 2	0	0	19.6 A	24.8 BC	. . .	. . .	17.8 ABC	20.0 C
Skorospelka	0	0	22.2 ABC	19.8 A	. . .	. . .	19.5 BC	16.8 AB
Hybrids:								
H. tuberosus × *H. annuus*	0	0	18.1 B	12.2 A	. . .	. . .	29.1 B	20.2 A
H. tuberosus × *H. annuus* × *H. strumosus*	0	0	19.9 B	17.8 B	. . .	. . .	17.7 A	16.2 A

[a]Calculated as percentage of fresh weight of indicated plant part. Values are averages of two measurements per plant part sampled. Duncan's multiple range test: values followed by the same capital letter within each species or within the hybrid group are not significant, $P = 0.05$.

[b]Plants grown in growth chambers at 1,500 lux light intensity, 22 C day and 20 C night, and relative humidity which ranged 60-70%.

[c]Tuber.

TABLE 2. Influence of photoperiod on the susceptibility of four cultivars of the annual cultivated sunflower *Helianthus annuus* to stalk rot, artificially incited by *Sclerotinia sclerotiorum* and expressed by a calculated disease severity index (DSI)

Photoperiod (hours)	Reaction of indicated cultivars expressed by DSI values[a]			
	Krasnodarets	Manchurian	CM 144	CM 162
8	4.8	4.5	4.1	5.0
14	4.1	2.6	2.0	4.3
18	2.7	2.0	1.5	2.5
24	0.9	0.8	0.4	1.0

[a]Averages of nine plants per cultivar. DSI = (number of infected plants) × (severity class) ÷ (number of inoculated plants). Severity classes: 0 = no disease symptoms; 5 = plants killed.

develop in 62 days after emergence. Histochemical diagnostic staining tests of freshly cut transections of noninoculated hypocotyls and stems were made with 0.5% phloroglucinol in 95% ethanol followed by 20% HCl and with 0.2% syringaldazine in ethanol followed by 0.5% H_2O_2 by the method of Harkin and Obst (7) with slight modification. Also, the transections were examined under ultraviolet light and tested for resistance to disintegration in concentrated H_2SO_4.

Inoculation methods.—Seedlings and adult plants of the annual sunflower in the visible-flower-primordium stage, at about 43 days of age, grown in the greenhouse or the growth chamber, were inoculated with *S. sclerotiorum* on the lower hypocotyl, without wounding. I attached an agar plug cut from a 2-week-old PDA culture of the fungus and then covered the inoculated part with soil. Plants of the perennial sunflower and the interspecific hybrids were inoculated, with and qithout wounding, on the lower or upper stem, 1, 2, or 3 weeks after plant emergence. The inoculating agar plug was covered with cloth and aluminum foil. A disease severity index (DSI) to express relative disease reaction was calculated as described in Table 2.

RESULTS.—*Growth and disease reaction in the greenhouse.*—Annual sunflower plants grown in the greenhouse for 52 days ranged from 90 to 100 cm tall and had hypocotyls 5-8 cm long and 12-15 mm in diameter at the visible-bud stage, 43-52 days after plant emergence. Growth of perennial sunflowers was comparable to that of annual cultivars, except that the former failed to bloom within 62 days.

All the annual sunflowers inoculated in the greenhouse

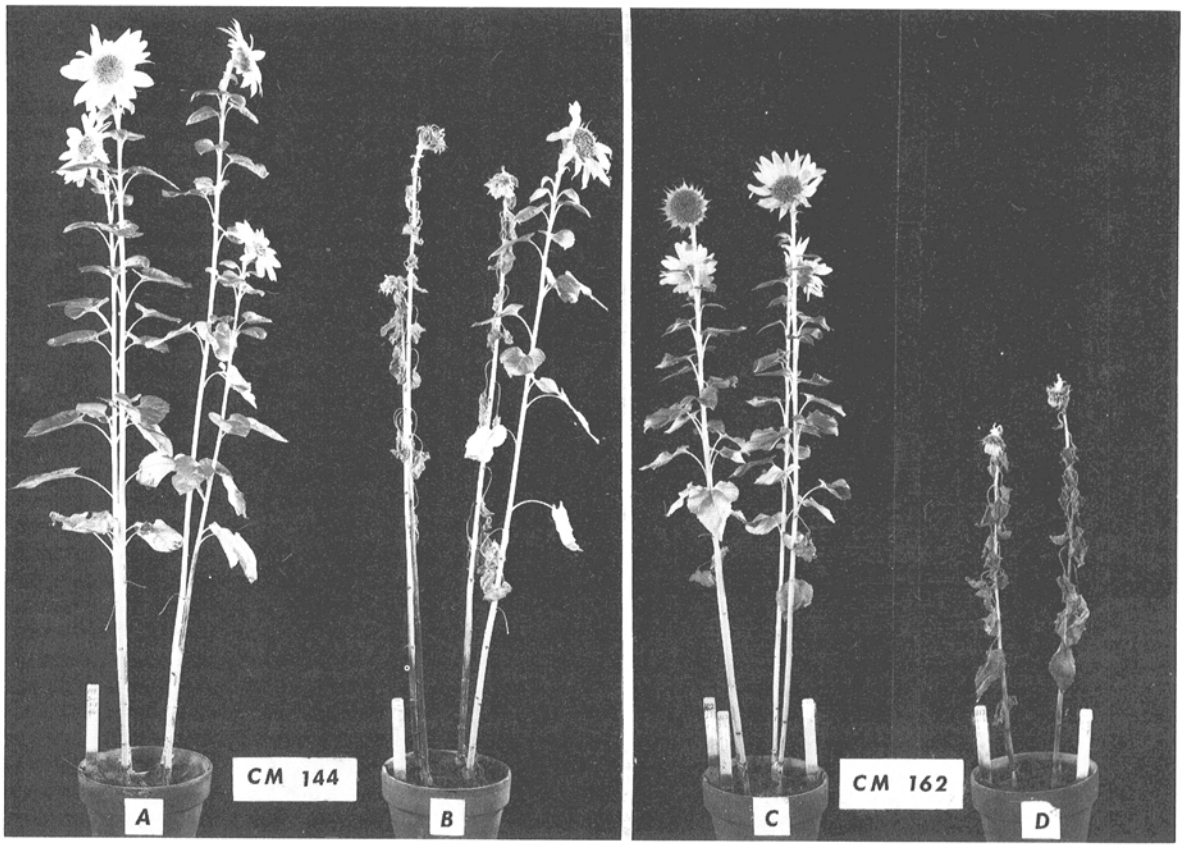

Fig. 1-(A to D). Sclerotinia stalk rot reactions of the annual cultivated sunflower *Helianthus annuus* CM 144 grown on a 24-hour **A)** and a 14-hour **B)** photoperiod and of CM 162 grown on a 24-hour **C)** and an 8-hour **D)** photoperiod at about 1,900 lux and 23 C. Plants were inoculated on the hypocotyl without wounding at the onset of the flower primordium. Note dark basal stem region in B and D due to the disease.

were highly susceptible to infection with Sclerotinia stalk rot, both in the seedling and in the adult stage. Most plants collapsed within 7-12 days after inoculation. The symptoms first appeared as a brown discoloration of the inoculated area and spread upward, causing stem rot and plant collapse. Disease severity among annual cultivars differed only until the flower bud appeared; thereafter, most inoculated plants developed rot. Sclerotia developed occasionally on rotting tissue, but were not seen below ground level. In contrast, the perennial *H. tuberosus* and hybrids failed to become infected, despite two successive reinoculations. Localized necrosis, however, was induced on these sunflowers by epidermal wound-inoculation of young shoots.

Growth and disease reaction in the growth chamber.—The percentage of dry matter harvested from plants grown in the growth chamber on an 8- and a 14-hour photoperiod was consistently lower for the annuals Manchurian, Krasnodarets, CM 144, and CM 162 after 62 days of treatment than on an 18- or a 24-hour photoperiod at nearly the same light intensity, ambient temperature, and watering regime (Table 1). Hypocotyls of plants grown on short days were succulent and easily injured, whereas those from plants grown on long days were woody and hard. Manchurian and CM 162 failed to bloom on either short or long days, Krasnodarets reached the visible-primordium stage in 43 and CM 144 in 45 days on an 8-hour photoperiod and both in 46 days on a 24-hour photoperiod. The percentage of dry matter accumulated in the stem and tuber tended to be higher for each of the perennial cultivars grown on long days than for those grown on short days, although none bloomed within the experimental period.

The influence of four photoperiodic regimes on the reaction of the annual sunflowers Krasnodarets, Manchurian, CM 144, and CM 162 to stalk rot incited by *S. sclerotiorum*, and expressed by a calculated DSI (Table 2) was striking. All four cultivars were susceptible when grown under the 8-hour photoperiod and tolerant under either the 18- or 24-hour photoperiods. Under the 14-hour photoperiod, the early-maturing Krasnodarets and CM 162 were susceptible, whereas the late-maturing Manchurian and CM 144 were tolerant. The stalk rot reaction of CM 144 and CM 162 is depicted in Fig. 1.

Stem inoculation without wounding and reinoculation of the perennial sunflowers and of the interspecific hybrids grown on short and long days failed to incite stalk rot. Thus, their resistance appeared to be independent of photoperiodic control. Wound inoculation of young shoots of the perennial species induced a severe necrotic reaction, but this reaction was confined to the internodal area of the stem, and the wound healed rapidly.

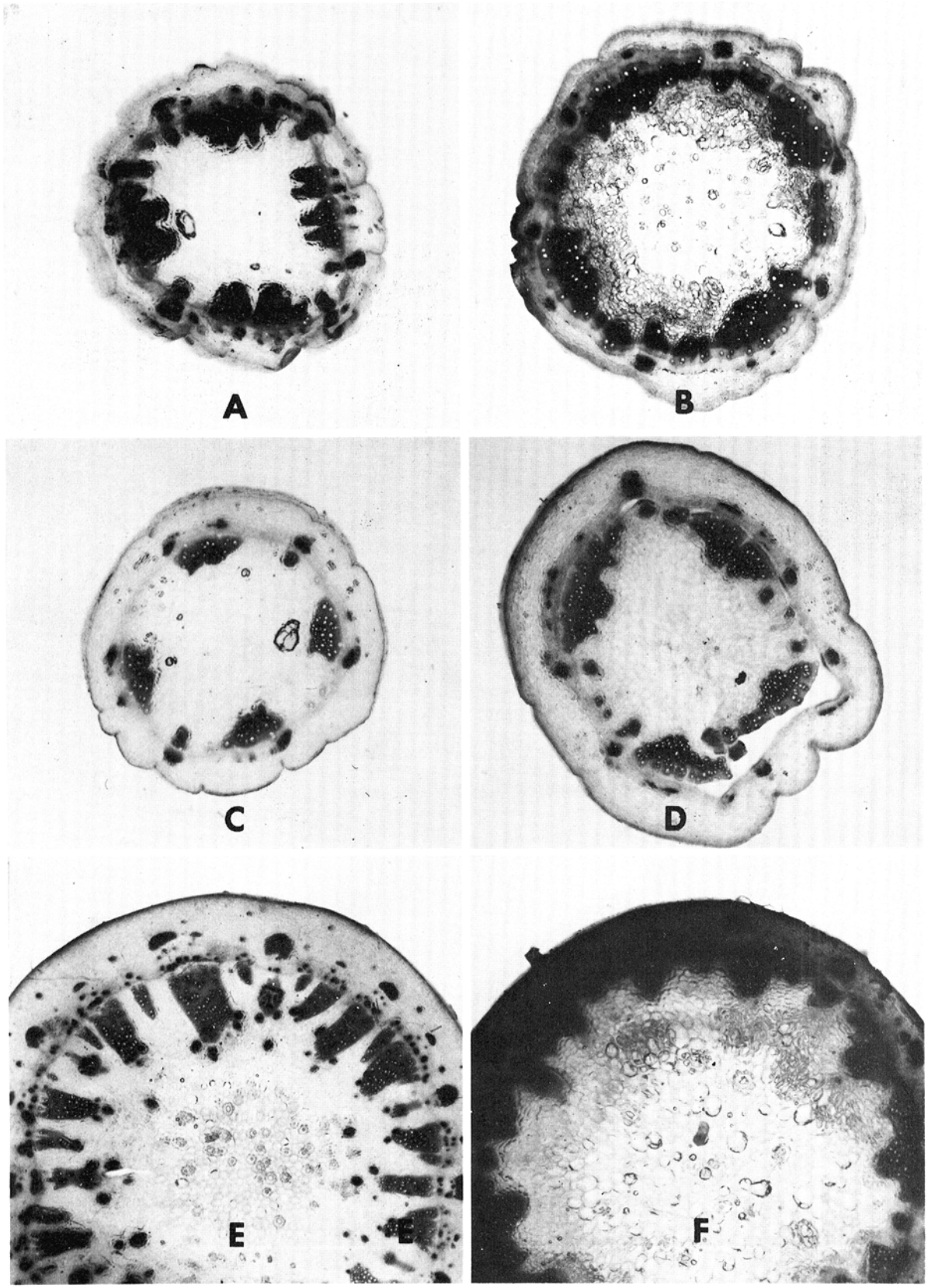
A
B
C
D
E
F

Fig. 2-(A to F). Phloroglucinol-HCl stain reaction of lignified tissues of hypocotyl transections of noninoculated annual sunflower *Helianthus annuus* 'Krasnodarets' (A, B) and 'Peredovik' (C, D), grown on an 8-hour **A)** and **C)** and on a 24-hour **B)** and **D)** photoperiod at the onset of the flower-primordium stage (× 3.5). The lower figures are cross-sections (× 5.0) of the basal stalk of noninoculated, nonblooming perennial artichoke *Helianthus tuberosus* 'Vadium' grown on an 8-hour **E)**, and a 24-hour **F)** photoperiod.

Histochemical staining.—Noninoculated hypocotyl transections of Manchurian, CM 144, and CM 162 plants grown on 24-hour days and treated with phloroglucinol-HCl produced a strong dark-purple reaction. This reaction indicated an advanced stage of cell lignification of the cortical fiber, the phloem, and particularly, the xylem. Similarly, hypocotyls of sunflower grown on the 8-hour photoperiod produced a reaction that was less extensive and not as strong in color, indicating a less advanced stage of lignification (Fig. 2). Positive reaction to syringaldazine-H_2O_2, as indicated by a medium reddish-purple, coincided with the phloroglucinol-reacting areas. Thereby, the reaction confirmed the advanced lignification of the sclerenchymatous cortical tissue, the phloem, and particularly, the xylem. This photoperiodically enhanced lignification appeared to account for the toughening of the hypocotyl of the annual sunflower. Also, the lignification was indicated by the hypocotyl's absorbance of ultraviolet light and resistance to disintegration in concentrated H_2SO_4. Hypocotyls grown on an 8-hour photoperiod were poorly lignified and disintegrated almost completely in acid.

DISCUSSION.—Photoperiod has a profound influence on the reaction of the annual cultivated sunflower, *H. annuus*, to stalk rot caused by *S. sclerotiorum*. Photoperiod, however, hardly influenced the resistance of the perennial sunflower (Jerusalem artichoke or topinambur) *H. tuberosus*.

The susceptibility of the annual cultivated sunflower appeared to be associated with its growth response to 8- and 14-hour days. Plants grown on these short days had succulent hypocotyls that were highly prone to fungal attack. Susceptibility of tomatoes to Fusarium wilt was increased by short days (4). Susceptibility of *Phaseolus* spp. to tobacco mosaic virus (8), and of wheat to both *Gaeumannomyces graminis* (23) and *Septoria tritici* (1) was increased by short days also. The reaction of the photosynthetic capacity in peppers to infection by *Xanthomonas phaseoli* (21) and the light dependence of resistance to *X. cyamopsidis* in guar (*Cyamopsis tetragonoloba*) (17) have also been demonstrated.

My results show that the tolerance of the annual cultivated sunflower to *S. sclerotiorum* was associated with enhanced growth and intense lignification of the host tissue in response to long-day treatment. The possibility that the reaction of sunflowers to stalk rot might be related to light as it affected the activity of antifungal host metabolites was indicated by chromatographic analysis of hypocotyls of plants grown on an 8- or 24-hour photoperiod (Orellana, *unpublished*). This study showed that methanol extracts of healthy and diseased tissues from plants grown on 24-hour days contained predominantly two compounds having R_f values the same as those of chlorogenic and isochlorogenic acids. However, these compounds were only in traces in the hypocotyl of plants grown on short days. The relative antifungal activity of eluates of these compounds was indicated by inhibition of *S. sclerotiorum* and *Macrophomina phaseolina* in vitro. It is significant that Koeppe et al. (9) showed a close relation between phenolic compounds and lignin synthesis in the wild *H. annuus*. This relation suggests a similar relation in the annual cultivated sunflower. Photocontrol of phenylalanine deaminase has been reported by Nitsch and Nitsch (14) in tubers of *H. tuberosus*, and that of flavonoid biosynthesis has been reported by Smith (22) in plants. Hemicellulose enzymes secreted by *S. sclerotiorum* were present in sunflower (*H. annuus*) hypocotyls (6). The fungistatic properties of chlorogenic acid and caffeic acid in potatoes were reported by Kuć et al. (11). The activities of polyacetylenes, which Bu'Lock (2) found abundant in the Compositae and especially in *H. tuberosus*, need to be studied, particularly for disease reaction in the cultivated sunflower. Krizek and Milthorpe (10) have studied the effect of photoperiodic induction in *Xanthium*, which is, like *Helianthus*, a Compositae. However, we lack information on the effect of photoperiodic induction on disease susceptibility in the sunflower.

A necrotic reaction can be induced by wound inoculation of young shoots of the perennial *H. tuberosus* with *S. sclerotiorum*. Thus, the host tissue may have elicited a postinfectional antifungal response on or around the necrotized area. This may have prevented (3, 13) the further spread of the fungus. The response of *H. tuberosus*, of other perennial species of *Helianthus*, and of several interspecific hybrids to artificial inoculation by *S. sclerotiorum* has been reported by Pustovoit (18). Therefore, my results and those of Leclercq (12) indicate that this response is an example of a high degree of resistance, rather than immunity.

Until the stalk rot phase of this disease is studied more, and until the resistance of the perennial species is incorporated in the annual cultivated sunflower in the production of commercial interspecific hybrids, alternate methods of disease control should be sought. Robinson (20) suggested that the vegetative growth period of the annual cultivated sunflower might be a guide to predict dates of future growth stages, pesticide applications, and cultural practices.

LITERATURE CITED

1. BENEDICT, W. C. 1971. Differential effect of light intensity on the infection of wheat by Septoria tritici Desm. under controlled environmental conditions. Physiol. Plant Pathol. 1:55-56.
2. BU'LOCK, J. D. 1964. Polyacetylenes and related compounds in nature Pages 86-134 *in* James Cook and W. Corruthers, eds. Progress in organic chemistry, Vol. 6. Butterworths and Co., London. 256 p.
3. CRAFT, C. C., and W. V. AUDIA. 1962. Phenolic substances associated with wound-barrier formation in

vegetables. Bot. Gaz. 123:211-219.
4. FOSTER, R. E., and J. C. WALKER. 1947. Predisposition of tomato to Fusarium wilt. J. Agric. Res. 74:165-185.
5. GEISE, H. A. 1967. Sunflower development at latitudes ranging from 31 to 49 degrees. Crop Sci. 7:134-136.
6. HANCOCK, J. G. 1967. Hemicellulose degradation in sunflower hypocotyls infected with Sclerotinia sclerotiorum. Phytopathology 57:203-206.
7. HARKIN, J. M., and J. R. OBST. 1973. Lignification in trees: indication of exclusive peroxidase participation. Science 180:296-298.
8. HELMS, K., and G. A. MC INTYRE. 1967. Light-induced susceptibility of Phaseolus L. to tobacco mosaic virus infection. I. Effect of light intensity, temperature, and the length of the preinoculation dark period. Virology 31:191-196.
9. KOEPPE, D. E., L. M. ROHRBAUGH, E. I. RICE, and S. H. WENDER. 1970. Tissue age and coffeoylquinic acid concentration in sunflower. Phytochemistry 9:297-301.
10. KRIZEK, D. T., and F. L. MILTHORPE. 1973. Effect of photoperiodic induction on the transpiration rate and stomatal behaviour of debudded Xanthium plants. J. Exp. Bot. 24:76-86.
11. KUĆ, J., R. E. MENZE, A. J. ULLSTRUP, and F. W. QUACKENBUSH. 1956. Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with Helminthosporium carbonum. J. Am. Chem. Soc. 78:3123-3125.
12. LECLERCQ, P. 1968. Cytoplasmic male sterility in sunflower. Pages 40-44 *in* Proc. Third Intern. Sunflower Conf., Crookston, Minn. 139 p. (1968).
13. MC CLURE, T. T. 1960. Chlorogenic acid accumulation and wound healing in sweet potato roots. Am. J. Bot. 47:277-280.
14. NITSCH, C., and J. P. NITSCH. 1966. Effet de la lumière sur l'induction de la phenylalanine-deaminase dans les tissus de tubercule d' Helianthus tuberosus L. C. R. Acad. Sci. Paris. 262:1102-1105.
15. ORELLANA, R. G. 1974. Photoperiod-induced tolerance of sunflowers to Sclerotinia sclerotiorum. Page 121 *in* Abstracts of papers. Sixth Int. Sunflower Conf., Bucharest, Romania. 144 p.
16. ORELLANA, R. G., C. D. FOY, and A. L. FLEMING. 1975. Effect of soluble aluminum on growth and pathogenicity of Verticillium albo-atrum and Whetzelinia sclerotiorum from sunflowers. Phytopathology 65:202-205.
17. ORELLANA, R. G., and C. A. THOMAS. 1968. Light and nitrogen affect reaction to guar to bacterial blight caused by Xanthomonas cyamopsidis. Phytopathology 58:250-251.
18. PUSTOVOIT, G. 1967. Selection of sunflowers for resistance to Sclerotinia by interspecific hybridization. Bull. Nauch. Tek. Inform. Masl, Kult. Vypusk 1:5-10.
19. PUTT, E. D. 1958. Note on differences in susceptibility to Sclerotinia wilt in sunflower. Can. J. Plant Sci. 38:380-381.
20. ROBINSON, R. G. 1971. Sunflower phenology—year, variety, and date of planting effects on day and growing degree—day summations. Crop Sci. 11:635-638.
21. SASSER, M., A. K. ANDREWS, and Z. U. DOGANAY. 1974. Inhibition of photosynthesis diminishes antibacterial action of pepper plants. Phytopathology 64:770-772.
22. SMITH, H. 1972. The photocontrol of flavonoid biosynthesis. Pages 433-481 *in* K. Mitrakos and W. Shropsire, eds. Phytochroms. Proc. Symp., Eretria, Greece, 648 p. (1971).
23. WILKINSON, V. 1970. Light and infection of wheat by Ophiobolus graminis. Trans. Br. Mycol. Soc. 54:331-332.