Effect of Irrigation on Susceptibility of Sunflower to *Macrophomina phaseoli*

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ABSTRACT

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Inoculations with *Macrophomina phaseoli* were done in 1977 and 1978 in flood-irrigated field plots. Inoculating with infested toothpicks was more effective than infesting soil at planting. Highest incidence and severity occurred in unirrigated plots. Irrigating at flowering or at flowering and ripening stages decreased disease incidence. In both years, inoculated plants matured earlier than uninoculated controls, but infection by *M. phaseoli* hastened plant senescence to a lesser extent than drought.

Sackston (10) first reported charcoal rot (CHR), induced by *Macrophomina phaseoli* (Maubl.) Ashby, as a severe disease of sunflowers (*Helianthus annuus* L.). CHR causes severe losses of seed yield in some countries (1,2,16). It is one of the most important sunflower diseases throughout the world (11,12).

In Spain, CHR of sunflowers occurs widely and is particularly severe in hot, dry years (R. M. Jiménez-Díaz, M. A. Blanco-López, and W. E. Sackston, *unpublished*;13). Under such conditions, affected plants developed small heads and ripened prematurely. From similar field observations, others (2,3,7) have suggested the disease is influenced by dry soil and high air temperatures. Water stress predisposes grain sorghum (5) and cotton (6) to CHR experimentally, but the effect of these factors on CHR of sunflower has not been tested.

This report presents results of experiments done in irrigated field plots with plants artificially inoculated with *M. phaseoli*. The objectives were to determine whether soil moisture influences susceptibility of sunflower to *M. phaseoli* and to study the role drought and infection by the pathogen might have in premature ripening of affected plants. A summary of part of this work has been published (8).

MATERIALS AND METHODS

Experimental design. Two field experiments were conducted at the INIA Research Station near Córdoba, southern Spain, in 1977 and 1978 on a sandy loam soil, pH 8.5, that contained about 1% organic matter. Fertilizer (780 kg/ha of 8-15-15, NPK) was broadcast before sowing. Calcium-ammonium-nitrosulfate (275 kg/ha, 26% N) was applied after stand establishment. Rootworms and weeds were controlled by preplant application of heptachlor and trifluralin at 40 kg/ha and 1.5 L/ha, respectively. Long-cycle hybrid SH-25 (Semillas Pacifico, El Arahal, Sevilla, Spain) was used in both years. Plots were drill-sown in rows 75 cm apart and thinned after emergence to about 65,000 plants per hectare. Irrigation was by flooding. Weather during the experiments was monitored with a hygrothermograph and a pluviometer. Mean temperatures in June, July, and August and annual precipitation were 23.1 C and 675 mm in 1977 and 24 C and 591 mm in 1978, respectively.

The 1977 experiment was a split-plot 3×2^2 factorial randomized complete-block design with two replicates. Six-row plots 8 m long sown on 22 April were used for the irrigation treatments. Plots were irrigated at either 800 or 400 m^3/ha at the flowering and/or ripening stages. Greatest and least irrigation depths corresponded to 80 and 40% of the water evapotranspired in a Thornthwaite lysimeter at the plots. Three irrigations spanning 7–10 days were applied during the flowering stage, starting 62 days after emergence (AE), and two were applied during the ripening stage, starting 91 days AE. An unirrigated plot was included as a control. Each irrigation plot was divided in half (subplots) and each half was randomly assigned to one of the two inoculation treatments. Half of each subplot served as an uninoculated control.

The 1978 experiment was a split-plot $7 \times 2 \times 3$ factorial randomized complete-block design with four replicates. Plots consisted of paired rows 8 m long sown on 28 April. Plots were irrigated with either 700 or 350 m^3/ha at the budding, flowering, or ripening stages or at any two or all three stages. Greatest and least irrigation depths corresponded to 90 and 45% of the water evapotranspired. Plots were irrigated at weekly intervals, three times for every stage, starting 34 days AE for budding, 56 days AE for flowering, and 74 days AE for ripening. An unirrigated plot was included as a control. Each irrigation plot was divided into three subplots 2.2 m long, with 11 plants each, and randomly assigned to the three inoculation treatments. One of the paired rows served as an uninoculated control.

Inoculum and inoculations. A virulent isolate of *M. phaseoli* obtained from affected sunflowers in southern Spain was used. The fungus grew at 30 C in soybean seed-extract broth (4) for 5 days or on sterile toothpicks (17) for 6 days. Mycelium and microsclerotia from the broth culture were comminuted in a Waring Blendor at high speed for 1 min. The suspension was diluted twofold with sterile distilled water and used for soil infestation.

In the 1977 experiment, plants were inoculated with infested toothpicks placed in a hole made with a disinfested, pointed stick at the stem bases before inoculation. Toothpicks cut level with the stem surface were covered with sterile Vaseline. Plants were inoculated at growth stages described by Siddiqui et al (14), 3.4–4.1 (flowering, 60 days AE) or 5.1 (ripening, 75 days AE). Uninfested toothpicks were used in control plants. In 1978, inoculation was by placing 40 ml of inoculum suspension on the soil at each sowing site or by the toothpick method at stages 3.1 (budding, 43 days AE) or 4.2 (flowering, 62 days AE).

Plants were rated for incidence of disease and severity (spread of necrotic lesion around the inoculation point) 90 days AE. This was 29 days after flowering inoculation and 14 days after ripening inoculation in 1977 and 43 and 24 days after budding and flowering inoculation, respectively, in 1978. Plants were considered ripe when about one-fourth of the surface on the back of the head turned from pale yellow to brown. Ripening was monitored at 3-day intervals starting 80 days AE.

Data were analyzed for covariance to account for heterogeneity among plants. Statistical analyses were performed with program BMDP2V, University of California, Los Angeles, in a CDC-172 computer. Percentage data were transformed to arc sine before analysis. Mean comparisons were made by Duncan's multiple range test (15).

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RESULTS

Symptomatology. Dark brown lesions developed around stems inoculated with infested toothpicks (Fig. 1A). Necrosis was the same in 1977 and 1978. It was not influenced by the irrigation regime or the plant stage at inoculation. No symptoms developed around the uninfested toothpicks in control plants, and discoloration was not found in the stem bases of inoculated plants in 1977 at 90 days AE. In 1978, however, a dark brown to black discoloration developed upward for various lengths at the stem bases of uninoculated plants. This discoloration was observed by the time the final data were recorded, about 90 days AE, although it was not apparent in plants examined 1 wk earlier. Plants inoculated by infesting soil at planting in 1978 showed discoloration in the stem bases similar to that found in uninoculated plants, but it was found 1 wk earlier than in the controls.

Lower leaves of inoculated and uninoculated plants affected by stem necrosis became yellow and dried but remained firmly attached to the stem (Fig. 1B). Plants with severe stem necrosis developed small heads with a central zone of aborted flowers and ripened prematurely. Numerous microsclerotia of the pathogen formed under the epidermis as well as in inner stem tissue. Affected stems were brittle.

Incidence and severity of infections. In 1977, disease incidence and severity in irrigated plots were lowest for plants irrigated at ripening and/or flowering stages (Table 1). No significant effect was found for irrigation depth. In irrigated plots, disease incidence was higher for plants inoculated at flowering than for those inoculated at ripening (Table 2). No difference occurred in disease severity. Plant stage at inoculation did not influence disease incidence and severity in unirrigated plots. Nevertheless, values in unirrigated plots were higher than those in irrigated plots (Table 2).

In 1978, disease incidence in plants

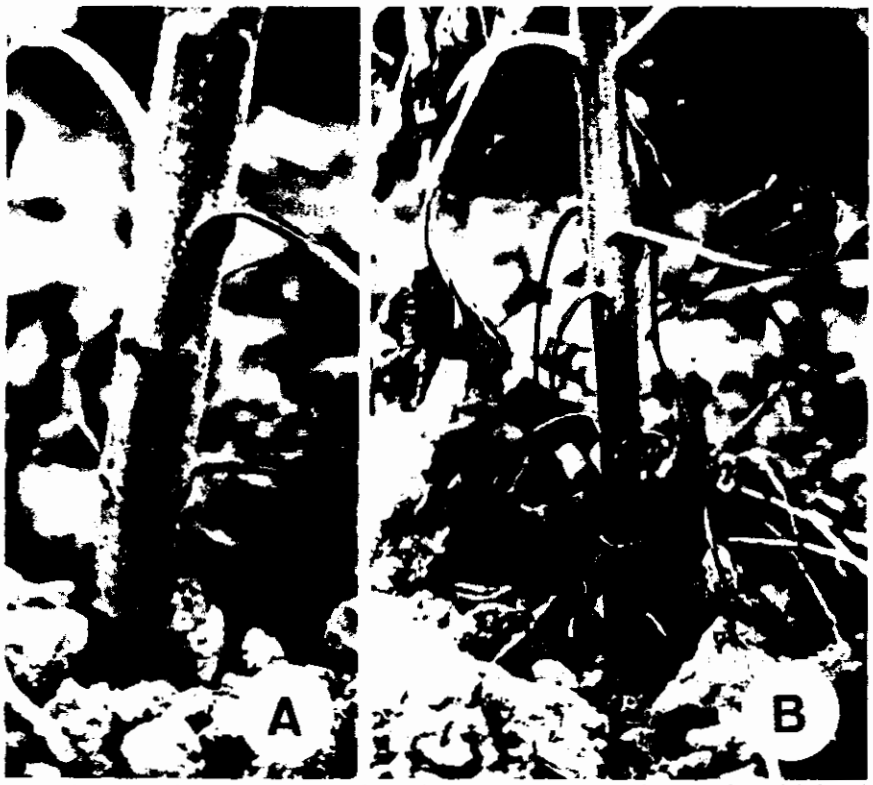

Fig. 1. Symptoms that developed in sunflower inoculated with *Macrophomina phaseoli*-infested toothpicks: **(A)** Stem necrosis around inoculation point. **(B)** Drying of leaves near necrotic area in inoculated stem.

Table 1. Effect of plant stage at irrigation on susceptibility of sunflower to *Macrophomina phaseoli*[y]

Plant stage at irrigation	1977 Experiment[z]		1978 Experiment			
	Incidence (%)	Mean length of necrotic lesion in stem (cm)	Incidence (%)		Mean length of necrotic lesion in stem (cm)	
			Inoculated	Uninoculated	Inoculated	Uninoculated
Budding	···	···	35.0 a	9.5 d	12.8 a	4.7 b
Flowering	40.4 a	4.6 a	28.5 ab	4.6 d	8.4 a	3.3 b
Ripening	79.4 b	7.7 b	39.1 a	8.7 d	13.2 a	6.2 b
Budding + flowering	···	···	29.7 ab	6.1 d	13.1 a	4.8 b
Budding + ripening	···	···	18.2 bc	7.8 d	10.1 a	4.0 b
Flowering + ripening	37.0 a	4.7 a	14.6 c	3.0 d	6.6 a	1.7 b
Budding + flowering + ripening	···	···	12.1 c	2.3 d	8.4 a	1.6 b

[y] Numbers within columns or rows for a given year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Necrosis developed at the base of the stem of uninoculated plants in 1978 but not in 1977.

Table 2. Effect of plant stage at inoculation on susceptibility of sunflower to *Macrophomina phaseoli*[y]

Year of experiment	Plant stage at inoculation	Irrigated plots				Unirrigated plots			
		Incidence (%)		Mean length of necrotic lesion in stem (cm)		Incidence (%)		Mean length of necrotic lesion in stem (cm)	
		Inoculated	Uninoculated[z]	Inoculated	Uninoculated[z]	Inoculated	Uninoculated[z]	Inoculated	Uninoculated[z]
1977	Flowering	69.5 a	···	5.6	···	80.0	···	7.5	···
	Ripening	35.2 b	···	5.7	···	80.3	···	9.6	···
1978	Sowing	7.4 a	6.6 a	4.7 a	3.3 a	27.3 a	44.0 a	10.1 a	9.1 a
	Budding	31.4 b	4.4 a	11.0 b	2.9 a	81.8 b	31.8 a	21.9 b	8.2 a
	Flowering	37.0 b	6.9 a	15.4 c	5.1 a	97.7 b	38.6 a	23.8 b	8.4 a

[y] Numbers within columns or rows for a given year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Necrosis developed at the base of the stem of uninoculated plants in 1978 but not in 1977.

Table 3. Effect of plant stage at inoculation on ripening of sunflowers[y,z]

Year of experiment	Plant stage at inoculation	Irrigated plots		Unirrigated plots	
		Inoculated	Uninoculated	Inoculated	Uninoculated
1977	Flowering	103.6 a	105.7 b	96.5	98.5
	Ripening	101.9 a	105.1 b	94.5	97.0
1978	Sowing	107.7 a	100.9 a	93.7 a	93.2 a
	Budding	98.4 b	101.9 a	88.2 b	96.2 a
	Flowering	98.3 b	101.1 a	87.7 b	93.2 a

[y] Plants were considered ripe when about one-fourth of the surface on the back of the head turned from pale yellow to brown.
[z] Numbers within columns or rows for a given year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 4. Effect of plant stage at irrigation on ripening of sunflowers[y,z]

Plant stage at irrigation	1977 Experiment		1978 Experiment	
	Inoculated	Uninoculated	Inoculated	Uninoculated
Budding	···	···	96.4 a	98.7 c
Flowering	104.1 b	106.6 c	96.3 a	98.1 c
Ripening	97.8 a	102.3 c	100.8 b	105.1 d
Budding + flowering	···	···	95.7 a	98.3 c
Budding + ripening	···	···	102.7 b	103.3 bd
Flowering + ripening	106.4 b	107.3 bc	101.2 b	103.0 d
Budding + flowering + ripening	···	···	100.5 b	102.8 d

[y] Plants were considered ripe when about one-fourth of the surface on the back of the head turned from pale yellow to brown.
[z] Numbers within columns or rows for a given year followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

irrigated and inoculated was highest in plots irrigated at budding or ripening stages and lowest in plots irrigated at flowering and ripening or at all three plant stages (Table 1). No difference occurred in disease severity. Disease incidence was lower in plots irrigated with 700 m^3/ha than in plots irrigated with 350 m^3/ha, but no difference occurred in disease severity. Disease incidence and severity were highest in plants inoculated at budding or flowering stages for either irrigated or unirrigated plots, but values in unirrigated plots were higher than those in irrigated ones (Table 2). Soil infestation at planting did not increase incidence and severity of symptoms significantly for either irrigated or unirrigated plots (Table 2).

Irrigation and infection effects on plant ripening. Unirrigated plants matured earlier than irrigated plants in 1977 and 1978 (Table 3). In 1977, inoculated plants matured earlier in plots irrigated at ripening than in plots irrigated at ripening and/or flowering stages (Table 4). Infection by *M. phaseoli* shortened the time to maturation in plants irrigated at the flowering or ripening stages but not in plants unirrigated or irrigated at both stages (Tables 3 and 4). No significant effect was found for either irrigation depth or plant stage when inoculated.

In 1978, inoculated or uninoculated plants irrigated at budding and/or flowering stages matured earlier than those irrigated at ripening or at ripening and any other plant stage (Table 4). Also, in irrigated plots, inoculated plants matured earlier than uninoculated plants (Table 4). Inoculated or uninoculated plants irrigated with 350 m^3/ha matured earlier than those irrigated with 700 m^3/ha. Plants toothpick-inoculated at budding or flowering stages ripened earlier than those not inoculated or those inoculated by infesting soil at planting for either irrigated or unirrigated plots (Table 3). Mean time to maturation was significantly correlated with disease incidence in 1977 ($r = -0.62$, no symptoms in uninoculated plants) and 1978 ($r = -0.73$ and $r = -0.57$ for inoculated and uninoculated plants, respectively).

DISCUSSION

Our results showed that irrigating sunflowers at flowering or at flowering and ripening stages decreases the incidence of infection by *M. phaseoli* under natural conditions (Table 1). Results with toothpick inoculation also indicate that higher incidence and severity in unirrigated plots are caused by increased plant susceptibility. Thus, drought predisposes sunflower, as well as other crops (5,6), to *M. phaseoli*. Increased susceptibility in sunflower occurs naturally when using saline water for irrigation as well (9).

Soil infestation at planting resulted in dark brown to black discoloration at the stem base that later evolved to silvery gray as described by Alabouvette and Bremeersch (2). The same symptoms developed in uninfested control plots. In both cases, incidence and severity of those symptoms were higher in unirrigated compared with irrigated plots (Table 2). Predisposition of plants by water stress or increase of inoculum potential by drought conditions could cause this. Interestingly, symptoms developed in uninoculated plants in 1978 but not in 1977. This could be caused by the effect of environmental factors on the disease because annual precipitation was higher in 1977 than in 1978 and mean temperatures in July and August were 2 and 3.4 C lower, respectively, in 1977.

Although number of days to maturation was negatively correlated with disease incidence, plant maturation is enhanced by drought more than by infection by the pathogen. Consequently, the premature ripening of CHR-affected sunflowers found during disease surveys (R. M. Jiménez-Díaz, M. A. Blanco-López and W. E. Sackston, *unpublished*; 7,11) may reflect conditions of severe drought.

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