Incidence and Distribution of Charcoal Rot of Sunflower Caused by *Macrophomina phaseolina* in Spain

R. M. JIMÉNEZ-DÍAZ, Professor, and M. A. BLANCO-LÓPEZ, Adjunct Professor, Departamento de Patología Vegetal, Escuela Técnica Superior de Ingenieros Agronomos, Universidad de Córdoba, Córdoba, Spain; and W. E. SACKSTON, Professor, Department of Plant Science, Macdonald Campus of McGill University, Ste. Anne de Bellevue, Quebec H9X 1C0, Canada

ABSTRACT

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Charcoal rot of sunflower caused by *Macrophomina phaseolina* occurred throughout the main areas of sunflower cultivation of Spain in 1976, 1977, and 1978. Disease incidence varied widely among regions and years of sampling; it was most prevalent where low rainfall and high temperature coincided with flowering time. Highest incidence was observed in 1976; the disease was present in 76 of 163 fields and 25% or more of the plants were affected in 25 fields. Symptoms were most conspicuous from late flowering to early ripening. They included a dark brown to black discoloration at the stem base, small and distorted heads with a zone of aborted flowers, and premature ripening.

Sunflower (*Helianthus annuus* L.) is the most important oilseed crop in Spain, with an area of about 600,000 ha (5). In preliminary observations, the crop was found to be affected by several diseases (20) but information on their relative importance was lacking. Consequently, we carried out investigations on the etiology, prevalence, and incidence of diseases affecting sunflower throughout the country. We report results concerning charcoal rot (CR) caused by *Macrophomina phaseolina* (Tassi) Goid. Information on other diseases has been published elsewhere (8,12,13).

CR of sunflower was first reported in the USSR in the 1930s (6,9) and was recorded in Texas in 1949 (24), but it was not widely recognized until 1957, after the importance of attacks in Uruguay was shown (17). Later, it was reported in Yugoslavia (1), and now it is considered one of the most important diseases affecting the crop throughout the world (18,19), reducing seed yields by 20–36% (1,2,23).

The disease is associated with drought and high temperatures. It is most conspicuous near the end of the host cycle; affected plants ripen prematurely, heads are small and distorted, and there is a gray to silvery discoloration at the stem base (2,9).

MATERIALS AND METHODS

Systematic disease surveys were carried out in the main areas of sunflower cultivation in Spain (Fig. 1) in 1976, 1977, and 1978. The methodology described by Sackston (18) was used. In 1976, surveys were made from 15 May to 20 June and from 15 to 30 July in Andalucia and during June, July, and August in other regions. In 1977, they were made from 1 to 15 July in Andalucia and from 10 to 30 August elsewhere; in 1978, they were made in June and July in Andalucia and in June and August elsewhere.

More than 600 fields were surveyed during the 3 yr. The numbers for the respective years were 301 in 1976 (10,100 ha, about 2% of the national sunflower area), 130 in 1977 (4,200 ha, 0.8% of the sunflower area), and 180 in 1978 (8,540 ha, 1.5% of the sunflower area).

Temperature and rainfall data were obtained from the Ministry of Agriculture, Madrid (4). The average values of the mean maximum temperatures in June and July and the annual precipitation each year were calculated for all provinces sampled in each region.

Isolations were made from roots and lower stems of affected plants. The sampled tissues were washed in running tap water, cut into small pieces, surface-disinfested in 0.5% sodium hypochlorite for 1–3 min, and plated onto potato-dextrose agar (PDA). Cultures were incubated at 23 or 30 C in the dark for 1–2 wk.

Pathogenicity of nine isolates of *M. phaseolina* was tested on 2-wk-old seedlings of sunflower hybrid SH-25 in a growth chamber adjusted to 24–30 C and with a 14-hr photoperiod of fluorescent light at 10,000 lux. Seedlings were grown in 15-cm-diameter plastic pots (three per pot) containing a mixture of perlite and

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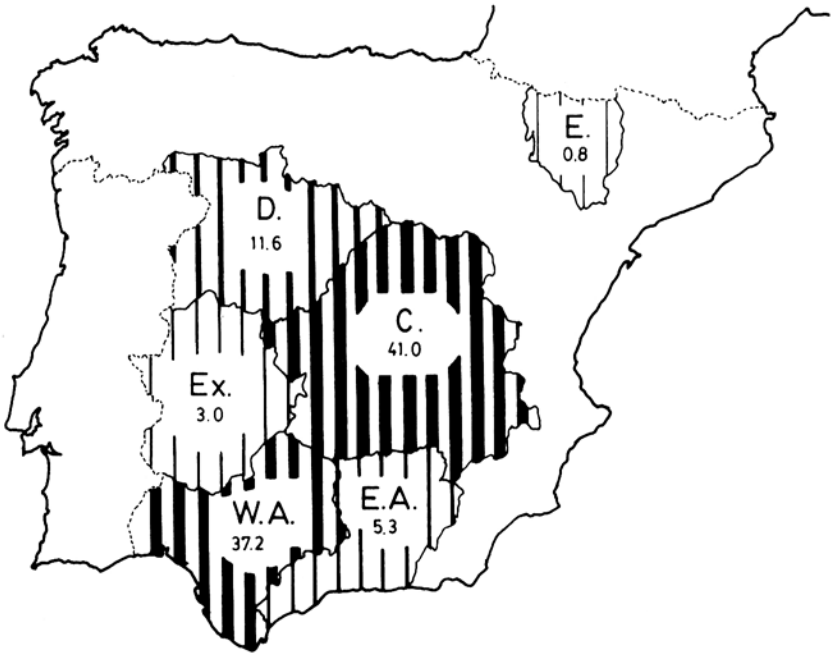

Fig. 1. Main areas of sunflower cultivation in Spain: (C.) Centro, (D.) Duero, (E.) Ebro, (E.A.) Eastern Andalucia, (Ex.) Extremadura, and (W.A.) Western Andalucia. Figures below letters are percentages of national sunflower acreage grown in given regions, averaged over the 1976–1978 period.

vermiculite (1:1, v/v) and watered with full-strength Hoagland and Arnon nutrient solution (10). Inoculum was increased from 4-day-old cultures grown on PDA at 30 C. Four 5-mm agar disks with mycelia and sclerotia were placed in 250-ml flasks containing 50 ml cornmeal sand medium (1,000 g cornmeal, 1,000 g washed white sand, and 1,500 ml water) (16) that were maintained at 30 C for 4 days. The flasks were shaken vigorously every day to facilitate homogeneous colonization of the substratum. Inoculation was done by the unwounded stem base inoculation (USBI) method (6); 2 g of inoculum was placed around the exposed base of each stem and covered with the perlite-vermiculite mixture. Control seedlings were treated similarly with sterile medium. Four pots were used for each isolate. Seedlings were observed daily for symptom development. The severity of disease reaction was assessed on a 0–3 scale (0 = no symptoms and 3 = dead plant) 4 and 12 days after inoculation. Data were subjected to analysis of variance and Duncan's multiple range test (22).

RESULTS

Symptomatology. The most conspicuous symptoms of the disease were observed in crops at growth stages 4.4–5.1, from anthesis to seed development (21). Affected plants often ripened prematurely, with thin stems and small recurved heads with a central zone of aborted flowers (Fig. 2A,B). Stems of less severely affected plants showed dark brown to black discoloration from the base to a height of 30–40 cm (Fig. 2C). Numerous sclerotia of the pathogen occurred under the epidermis as well as in the cortex and pith of the discolored zone. Roots were dark brown externally; inner tissues appeared grayish because of the large numbers of sclerotia embedded in them.

Mature plants were most severely affected. The stems had a silvery gray discoloration extending up from the base, and in many cases, the epidermis was split (Fig. 2D). The roots were black and mostly decomposed.

Distribution and incidence. CR of sunflower was not found in any of 260 fields surveyed before July in any of the 3 yr; therefore, data on disease occurrence (Table 1) are for the 353 fields sampled in July or later in the 3 yr.

The disease occurred in all the regions surveyed, with incidence varying from year to year (Table 1). In 1976, incidence of disease in fields sampled from July to September ranged from a trace to more than 50%, and in 1977 and 1978, from a trace to 25%. Distribution of CR also varied with the years. The disease was most prevalent in Andalucia in 1976, in Extremadura in 1977, and in Centro in 1978 (Table 1).

Weather records. Average mean

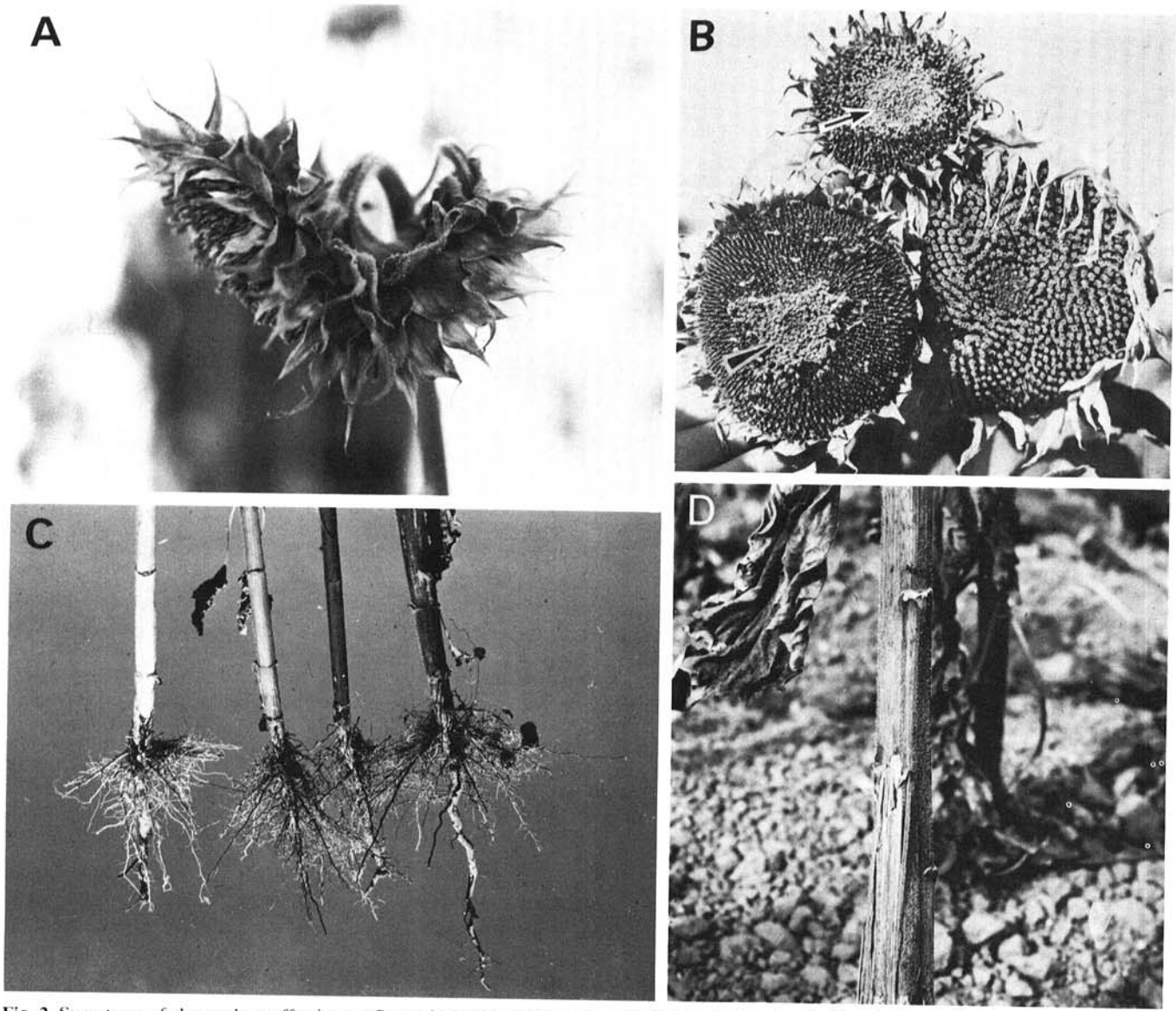

Fig. 2. Symptoms of charcoal rot affecting sunflower in Spain: **(A)** Small recurved head. **(B)** Heads of affected plants with central zone of aborted flowers (arrows). Head of a healthy plant on right. **(C)** Dark brown to black discoloration of roots and lower stems of affected plants. Healthy plant on left. **(D)** Split epidermis of stem of severely affected plant.

Table 1. Weather data[a] and distribution and incidence of charcoal rot (CR) on sunflower in Spain

Year	Region	Mean maximum temperature (C) in June–July	Annual precipitation (mm)	Numbers of fields					
				Sampled	CR present	Disease incidence[b] Trace to 10%	11–25%	25–50%	>50%
1976	Western Andalucia	32.1	425	52	44	17	11	9	7
	Eastern Andalucia	29.8	382	9	6	3	2	1	0
	Centro	29.8	396	65	9	5	3	1	0
	Duero	27.0	352	14	1	0	0	1	0
	Ebro	26.0	471	7	4	0	1	1	2
	Extremadura	32.3	396	16	12	6	3	0	3
		Mean 29.5	404	Total 163	76	31	20	13	12
1977	Western Andalucia	28.7	688	37	4	3	0	1	0
	Eastern Andalucia	28.0	506	23	4	4	0	0	0
	Centro	25.0	522	37	1	1	0	0	0
	Duero	21.5	517	16	0	0	0	0	0
	Ebro	27.0	496	3	1	1	0	0	0
	Extremadura	29.0	596	6	4	2	1	1	0
		Mean 26.3	554	Total 122	14	11	1	2	0
1978	Western Andalucia	32.0	500	23	2	1	1	0	0
	Eastern Andalucia	27.5	411	8	0	0	0	0	0
	Centro	27.8	402	23	10	6	3	1	0
	Duero	23.1	498	14	4	4	0	0	0
	Ebro	...	...	...	...	...	...	...	...
	Extremadura	...	...	...	...	...	...	...	...
		Mean 27.6	453	Total 68	16	11	4	1	0

[a] Average values for all provinces sampled in given regions (4).
[b] Based on counts of plants with CR symptoms in each field using a standardized sampling method (18).

maximum temperatures in June and July and annual precipitation in the regions surveyed from 1976 through 1978 are presented in Table 1. Temperature and rainfall varied widely among regions and from year to year.

Pathogenicity of *M. phaseolina* isolates. Isolations from lower stems and roots of affected plants consistently yielded cultures of *Sclerotium bataticola* Taub., the sclerotial state of *M. phaseolina*. We did not observe the pycnidial state of the fungus either in affected sunflower tissues or in pure cultures obtained from them.

Our isolates grew better at 30 C than at lower temperatures. Colonies developed profuse aerial grayish mycelia; sclerotia formed within 72 hr. The diameters of main hyphae were 5–11 μm, and of secondary hyphae, 2.3–6.5 μm. Black, more or less spherical sclerotia ranging from 28 to 162 μm in diameter developed abundantly in cultures from single hyphae or at the intersection of several hyphae. Occasionally, hyphal cells near a sclerotium were barrel-shaped.

All nine isolates tested were pathogenic to sunflower. The first symptoms developed within 48 hr of inoculation; they were dark brown necrotic lesions from 1 cm or less to several centimeters long at the stem base of seedlings and in some cases, induced damping-off. Inoculated seedlings also showed localized water-soaked leaf spots that became necrotic and coalesced to affect entire leaves. Necrosis of stem and leaves resulted in seedling death, as reported by others (6,16).

Table 2. Virulence to sunflower of isolates of *Macrophomina phaseolina* from sunflower growing in various locations in Spain[x]

Isolate	Location and region of sample	Disease reaction at days after inoculation[y] 4	12
1	Saelices (Centro)	3.0 a[z]	3.0
2	Cordoba (Western Andalucia)	2.3 ab	2.7 a
3	Iznalloz (Eastern Andalucia)	2.5 ab	2.6 a
4	Lora (Western Andalucia)	2.4 ab	2.4 a
5	Carmona (Western Andalcuia)	2.0 b	2.4 a
6	Arcos (Western Andalucia)	0.6 c	1.5 b
7	Cuenca (Centro)	0.3 c	1.3 b
8	Cordoba (Western Andalucia)	0.8 c	1.2 b
9	Ecija (Western Andalucia)	0.6 c	1.2 b

[x] Two-week-old seedlings were inoculated by the unwounded stem base inoculation method (6).
[y] Based on a 0–3 scale (0 = no symptoms, 3 = dead plant). Each figure is mean of 12 values.
[z] Values followed by the same letter within columns are not significantly different ($P = 0.05$) according to Duncan's multiple range test (22).

DISCUSSION

In Spain, *M. phaseolina* was first found infecting soybean (14; E. Mateo-Sagasta, *unpublished*) and later was reported on sunflower (18,20). Charcoal rot is now widely distributed and may affect high percentages of plants in all of the sunflower-growing areas of Spain (Table 1), as in other countries (18).

Severity of disease reactions in seedlings inoculated by the USBI method with isolates of *M. phaseolina* from sunflower in various locations in Spain varied widely (Table 2), as reported for isolates from other crops (7,11). The relative virulence of isolates was not associated with their geographical origin.

It must be stressed that much of the sunflower area of Spain has a typical Mediterranean climate. Most of the rain falls between October and April, with very little if any from May to September. Sunflowers sown from February to April normally benefit from spring rains but are usually dependent on moisture stored in the soil during the critical periods of flowering and filling of seed.

Incidence of CR of sunflower varied widely from year to year (Table 1). Much of the variation can be attributed to the influence of environment on the disease (2,3,9,20, R. M. Jiménez-Díaz et al, *unpublished*). It was more prevalent and severe in years with low rainfall and high temperatures in June and July (Table 1), when sunflowers are in bloom in most regions of Spain. Some variation may also be attributed to number and stage of

development of fields surveyed in the various districts. Symptoms of the disease are apparent only after flowering, which occurred at different times because of variation in cropping practices within as well as among various regions.

The symptoms we observed in CR-affected sunflowers were similar to those reported by other authors (2,9); however, the silvery gray discoloration of stems described as a typical symptom of the disease (2) was observed by us only in already mature plants and was preceded by a dark brown discoloration at the stem base of plants in the early phases of ripening.

Results of our disease surveys are only a general indication of the economic importance of CR on sunflower in Spain because we did not determine the reduction in seed yield experimentally. Nevertheless, the small, light seeds in small heads with a zone of aborted flowers (Fig. 2A,B) that developed in affected plants, together with the wide distribution of the pathogen and the high incidence of infection in hot dry years (Table 1), provide convincing evidence that this disease seriously limits seed yields of dry land sunflower in Spain.

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