Fungal Wilt and Root Rot Diseases of Chickpea in Southern Spain

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ABSTRACT

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Disease surveys in 1979–1981 indicated that chickpeas in southern Spain were severely affected by a wilt and root rot complex. Symptoms included vascular wilt or yellowing, nonvascular yellowing, collar and root rots or cortical collar and root necrosis, and yellow stunt. Plants in all but one of 108 fields inspected had at least one of the symptoms. Vascular wilt and yellowing were the most prevalent. *Fusarium oxysporum*, *F. solani*, *F. eumartii*, and *Macrophomina phaseolina* were associated with the wilt and root rot complex. Salmon-pigmented isolates of *F. oxysporum* induced vascular wilt or yellowing, and reddish-pigmented isolates induced nonvascular yellowing and cortical collar and root necrosis. *F. solani* and *F. eumartii* induced nonvascular yellowing and black collar and root rot. *M. phaseolina* induced dry collar and root rot. Isolates of *F. oxysporum* that induced vascular wilt or yellowing were not pathogenic to alfalfa, bean, broadbean, lentil, lupine, pea, and soybean, but they could be reisolated from all inoculated legumes except soybean and yellow lupine. Isolates of *F. solani* and *F. eumartii* caused symptoms on all of the legumes except alfalfa and soybean. Those most severely affected were chickpea, broadbean, and pea. Isolates of *F. oxysporum* that induced vascular yellowing in a local chickpea cultivar were not pathogenic to cultivar JG-62, which was highly susceptible to the vascular wilt isolates.

Additional key words: *Cicer arietinum*, *Fusarium oxysporum* f. sp. *ciceri*.

Approximately 55,000 ha are sown annually to chickpea (*Cicer arietinum* L.) in Andalucía, southern Spain. This area amounts to about 65% of the total national acreage of chickpea and more than half of it is concentrated in the Córdoba and Sevilla provinces of Andalucía (2). For a long time "Rabia" or Ascochyta blight has been considered the most important disease of chickpea in Spain (3,30). However, recent observations (8) indicated that this disease occurred rarely whereas there was an increased incidence of the "Seca" or "Fusariosis" disease, which is reported to be associated with *Fusarium* species (3). Little is known about the etiology and relative importance of the "Seca" disease in Spanish chickpea crops. Preliminary observations suggested that it might include a disease complex similar to the wilt and root rot (WRR) complex that severely reduces seed yields in most chickpea-growing countries (17).

Several fungi and viruses have been reported to be agents of the WRR complex of chickpea (1,7,26). Wilt, including flaccidity, yellowing, and vascular discoloration, induced by *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans. f. sp. *ciceri* (Padwick) Snyd. & Hans., is regarded as the most important disease in the complex (26). This disease was first found in India and it has since been reported in Burma, California, Ethiopia, Malawi, Mexico, Pakistan, Peru, Tunisia, Turkey, and the USSR (1,26,33). Wilt of chickpea also may be induced by *Verticillium albo-atrum* (11) and *Acrophialophora fusispora* (31). Black root and collar rot induced by *F. solani* (Mart.) Appl. & Wr. f. sp. *pisi* (Jones) Snyd. & Hans. was first reported from Washington in 1969 (22) and later found in California (33) and India (13). Infected plants show yellowing without vascular discoloration, growth reduction, and eventually death (13,22,33). *F. solani* f. sp. *pisi* is considered one of the most important pathogens associated with the WRR of chickpea in California (33) and in some states of India (18). A dry root rot induced by *Macrophomina phaseolina* (Tassi) Goid. is also a component of the WRR complex (26). It has been reported from Australia, California, Ethiopia, India, Iran, Lebanon, Mexico, Pakistan, Syria, and Turkey (1,26,33). Affected plants develop dark brown to black roots and dry up without showing any flaccidity (26,28). Six plant viruses have been associated with chickpea affected by stunting, chlorosis, and yellowing: alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), cucumber mosaic virus (CMV), lettuce necrotic yellows virus (LNYV), pea enation mosaic virus (PEMV), and pea leaf roll virus (PLRV) (1,20,21,26).

This paper presents results of research to determine the etiology, incidence, and prevalence of the "Seca" disease of chickpea in Western Andalucía. A preliminary report of some of this work has been made (19).

MATERIALS AND METHODS

Disease surveys. Systematic surveys were carried out in the main chickpea areas of Córdoba and Sevilla provinces from May through June from 1979 to 1981. A total of 108 fields were inspected: 40 in 1979, 30 in 1980, and 38 in 1981. Some fields were visited repeatedly each year to observe progressive symptom development. Disease incidence was assessed by counting the number of plants showing symptoms in a minimum of three representative 10-m lengths of row (60–100 plants) which were randomly chosen in each field. Disease severity was assessed for each plant on a 0–4 scale according to the percentage of foliage with yellowing or necrosis in an acropetal progression (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, and 4 = dead plant).

Isolations from affected plants. From each field, at least 12 plants representative of each symptom type and two symptomless plants were used for isolation of potential fungal pathogens. Initially isolations were made from all organs of an affected plant,

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but later only collar tissues were used because more consistent results were obtained. The tissues were washed in running tap water, cut into 5- to 10-mm pieces, surface disinfested in 1% sodium hypochlorite for 30 sec to 2 min, blotted dry on sterile filter paper, and plated on water agar (WA), potato-dextrose agar (PDA), acid-potato-dextrose agar (APDA) (PDA acidified to pH 4.5–5.0 with lactic acid), and Nash and Snyder PCNB medium (24). Cultures grown for isolation, identification, and inoculum production were incubated at 23–27 C and received a 12-hr photoperiod of fluorescent and near-UV light at 2,000 lux. Isolates of *Fusarium* spp. were identified according to Booth (4) by using five monoconidial cultures of each isolate. Identifications of isolates of *Fusarium* were verified by C. Booth, Commonwealth Mycological Institute, Kew, Surrey, England.

Pathogenicity tests. A local chickpea cultivar of the "kabuli" type, widely grown in the area surveyed and named PV-24 hereafter, was used in all pathogenicity tests. Its seeds are large (average 0.56 g), pale cream and rugose. Seeds were surface-disinfested, air-dried, germinated and then planted (three per pot) in 15-cm-diameter clay pots in autoclaved (twice at 121 C for 1.5 hr) potting mixture (clay loam, sand, and peat; 1:1:1, v/v). Plants were grown in a growth chamber adjusted to a 14-hr photoperiod of fluorescent light at 12,000–15,000 lux. Temperature and relative humidity, respectively, were 22–28 C and 50–90% during the light period, and 18–24 C and 60–100% during the dark period. Plants were watered daily and fertilized with 100 ml of nutrient solution (16) at weekly intervals.

Pathogenicity of isolates of *Fusarium* was tested in several experiments by using the water- and pot-culture inoculation methods developed by Nene and Haware (27) to screen chickpea for resistance to wilt. Inocula for water cultures were increased in potato-dextrose broth (PDB) (27). Liquid cultures containing mostly conidia were diluted to 2.5% with sterile distilled water. Conidium concentrations (estimated with a hemacytometer) varied from 0.8 to 5.0×10^6 conidia per milliliter depending upon isolates. Inoculum consisted mostly of microconidia for isolates of *F. oxysporum* and both macroconidia and microconidia for isolates of *F. solani*. For inoculation, plants grown for 7–10 days in sterile sand were removed, washed free of sand, and transferred (without intentional wounding) to 6-cm-diameter cylindrical glass bottles (four per bottle) each containing 200 ml of inoculum or sterile PDB for controls. The plants were held in position by strips of adhesive paper and the bottles were placed on a rotary shaker which was run at 110–120 rpm. Sterile distilled water was added every 3 days to replace the water loss.

Inocula for pot cultures were increased in a cornmeal-sand (CMS) mixture (27). Infested CMS was thoroughly mixed with an autoclaved soil mixture (1:12, w/w) and the seeds were sown in pots filled with that mixture. Control plants were grown in a comparable mixture of uninfested CMS and autoclaved soil.

Pathogenicity of isolates of *M. phaseolina* was tested according to the method of Chang (9) with inoculum increased in a CMS mixture. Eight-day-old plants were inoculated by placing 4 g of infested CMS around the exposed base of each stem and covered with the sterile soil mixture. Control plants were treated similarly with sterile medium.

Plants were observed daily for symptom development. Severity of disease reactions to isolates of *Fusarium* was assessed on the 0–4 scale (0 = no symptoms and 4 = dead plant) at 5, 10, and 15 days after inoculation by the water culture method, and at 20, 40, and 60 days after inoculation by the pot culture method. Reactions to isolates of *M. phaseolina* were assessed 50 and 60 days after inoculation. Isolations were attempted from all inoculated and control plants after the final disease evaluation. Experiments were designed as randomized complete blocks. For isolates of *Fusarium* there were four replicated bottles for water culture inoculations, and three replicated pots for pot culture inoculations; for *M. phaseolina* isolates there were five replicated pots. Analysis of variance and mean comparisons were performed on the data.

Virulence and host range of isolates of *Fusarium*. Differences in virulence of four isolates of *F. oxysporum* and two of *F. solani* to susceptible and resistant chickpea cultivars were tested by the pot culture inoculation method. One isolate each of *F. oxysporum* f. sp. *ciceri* and *F. solani* obtained from Y. L. Nene, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India, were included as a comparison. Cultivars P-678 and WR-315 are resistant, and cultivar JG-62 is susceptible, to race 1 of *F. oxysporum* f. sp. *ciceri* (15,27). Cultivars PV-24 and P-2245 were found least and most susceptible to the WRR complex, respectively, in a naturally infested plot near Córdoba. Seeds of these cultivars are small (average 0.2 g or less), pale cream, and scarcely rugose (P-2245), or dark brown and rugose (JG-62, P-678, and WR-315). All cultivars except PV-24 were obtained from Y. L. Nene. Plants were observed for symptom development periodically and severity of reactions was assessed on the 0–4 scale 20, 40, and 60 days after inoculation.

Information on host range was obtained by inoculating (pot culture method) the following legumes with two isolates of *F. oxysporum* and three of *F. solani*, and with the isolates of each species obtained from Y. L. Nene: alfalfa (*Medicago sativa* L. 'Aragon'), bean (*Phaseolus vulgaris* L. 'Contender'), chickpea P-2245, broadbean (*Vicia faba* L. 'Alameda'), lentil (*Lens esculenta* Moench, cultivar unknown), white lupine (*Lupinus albus* L. 'Multulupa'), blue lupine (*L. angustifolius* L. 'Unicrop'), yellow lupine (*L. luteus* L. 'Tremosilla'), andine lupine (*L. mutabilis* Sweet. 'Potosi'), pea (*Pisum sativum* L. 'Lancet'), and soybean (*Glycine max* (L.) Merr. 'Amsoy'). Plants were observed for symptom development periodically for 2 mo. Then they were carefully removed from the pots, washed free of soil and observed for symptoms on below-ground tissues. Symptom severity was assessed on a 0–4 scale according to the percentage of necrotic tissue present in the below-ground epicotyl and hypocotyl tissues (0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100%). Isolations were attempted from all inoculated and control plants when experiments were completed. For the host range experiments, pathogenicity of cultures obtained by reisolation was tested on the PV-24 cultivar by the water culture method. All experiments had three replicates in a randomized complete block design. Analysis of variance and mean comparisons were performed.

RESULTS

Disease symptoms in the field. Several symptom complexes were found affecting chickpeas during disease surveys, which we have designated as wilt, foliar yellowing, dry collar and root rot, yellow stunt, and iron chlorosis. Those different complexes were discernible in the early stages of development but later they became difficult to distinguish. In the wilt complex, individual leaves showed flaccidity followed by a dull-green discoloration and dessication. Shortly after, symptoms developed on all the foliage and the plants died; necrotic leaflets remained attached to the petioles. Vascular and pith tissues were colored dark-brown. Wilt symptoms were occasionally observed on 20-day-old plants, but they were most conspicuous at the onset of flowering 50 days after planting.

Foliar yellowing was most conspicuous from onset of flowering. Yellowing gradually progressed upwards on the plant and necrotic leaflets fell off. Plants with foliar yellowing had either dark-brown vascular and pith tissues, a cortical necrosis of collar and root, or a severe black collar and root rot. Some plants affected by foliar yellowing also showed wilt symptoms.

Dry collar and root rot occurred most frequently at the seed development stage. Stems and leaves dried up and became straw colored, and roots and collars became dry and brittle. Sclerotia of *M. phaseolina* occurred under the epidermis as well as in inner tissues of the lower stem, which appeared grayish. Affected plants produced pods that were either empty or contained small, poor-quality seed.

Plants affected by yellow stunt had shortened internodes and a bunchy appearance due to excessive branching. A golden yellow discoloration of leaflets developed basipetally on an affected plant, followed by necrosis of the stem apex. In some instances, stems grew in a zig-zag pattern and had longitudinal grooves. Lower

stems were purplish-white with brown phloem tissue. Such a discoloration was diagnostic and could be easily observed on transverse sections or by stripping the epidermis from stems. Most affected plants died before producing any seed. Frequently, plants affected by yellow stunt also showed symptoms of vascular wilt or foliar yellowing.

Plants with iron chlorosis were characterized by bright yellow upper leaves which maintained a dark green color of the leaflet midveins. This symptom was most conspicuous at early stages of plant growth and in moist, foggy weather. Some long-cycle cultivars and breeding lines of "desi" type became severely affected in a breeding nursery but recovered following foliar sprays with an iron-chelate.

Prevalence, incidence, and severity of disease symptoms. Except for iron chlorosis, all symptom complexes associated with WRR of chickpea occurred in the 3 yr of the surveys. No correlations were observed between incidence and cultivar, soil type, or management practices.

Foliar yellowing occurred in 86 of the 108 fields sampled. Of the affected fields, over 87% had a disease incidence higher than 50%. In most cases, severity of symptoms was low, with average disease scores of 1–2 within a range of 0–4.

In 54 of the fields sampled, most affected plants were either dying or dead. The incidence of dead or dying plants was lower than 5% in 56% of the affected fields and ranged from 5 to 20% in 26% of them. Most fields with an incidence of dead or dying plants higher than 20% had been sown to chickpea within the previous 2–4 yr. Dry collar and root rot was most prevalent in 1981, when severe drought and high temperatures prevailed. That year, 26 of the 38 sampled fields were affected, and 69% of them had an incidence higher than 50%. Disease incidence was lower than 5% in 6 of 70 fields sampled in 1979 and 1980. Yellow stunt occurred in 26 of the 108 fields sampled. Incidence of stunted plants was lower than 5% in 81% of affected fields. Iron chlorosis was found in 9 of 70 fields sampled in 1979 and 1980 with an incidence lower than 5% in 89% of them. All but one of the 108 fields sampled were affected by at least one symptom complex; in 55 and 21 fields, respectively, two or three symptom complexes were present in the same field.

Isolation, identification, and pathogenicity of isolates. *F. oxysporum*, *F. solani*, and *M. phaseolina* were most consistently isolated from plants in chickpea fields affected by WRR in 1979–1981 (Table 1). Frequently, more than one of these fungi were isolated from an affected plant, with the association *F. oxysporum* and *F. solani* being the most common. *F. oxysporum* was mainly isolated from dead or dying plants, and it was the only fungus isolated from plants with early symptoms of wilt. Dying plants affected with black collar and root rot also yielded *F. solani* in 47% of the cases. *F. oxysporum* also was isolated commonly from plants affected by foliar yellowing or by yellow stunt. Plants with foliar yellowing and vascular discoloration yielded mostly *F. oxysporum*, and those with foliar yellowing, black collar, and root rot yielded mostly *F. solani*. Both species were isolated from the same plant in 18% of the cases. Yellowing plants sampled from fields with high plant density or with poor, dry, shallow soils usually did not yield any fungus except that *F. oxysporum* was isolated in few instances. No fungi were isolated from plants with symptoms characteristic of yellow stunt. However, either *F. oxysporum*, *F. solani*, or most frequently both, were isolated from yellow-stunted plants which also had black collar and root rot or yellowing symptoms (Table 1). *M. phaseolina* predominated in plants affected with dry collar and root rot, although both *F. oxysporum* and *F. solani* were also isolated from them.

Isolates of *F. oxysporum* formed either salmon-pigmented or reddish-pigmented colonies on PDA when incubated under near-UV light. Salmon isolates were obtained mostly from dead or dying plants and from plants affected by foliar yellowing with vascular discoloration, and to a lesser extent from those showing dry collar and root rot. Those isolates consistently grew from all plant organs in contrast to the reddish isolates that originated only from root and collar tissues. Only reddish isolates were obtained from yellowing plants in poor, dry, and shallow soils or in densely planted crops, and from plants affected by yellow stunt or iron chlorosis. They also were obtained from dead or dying plants, and from plants affected with foliar yellowing or dry collar and root rot, but in these instances they were usually associated with salmon isolates or with *F. solani*.

Isolates of *F. solani* were uniform in morphology except for one identified by C. Booth as *F. eumartii* Carpenter (12). No efforts were made subsequently to distinguish between the two species, and all of our isolates are referred to as *F. solani* unless otherwise stated. They all were obtained from necrotic roots or collars and in some instances from the base of a stem.

Representative isolates of *F. oxysporum* (IMI-249635, 263812, and 263813 for the salmon type; IMI-249636 for reddish type), *F. solani* (IMI-249637, 263814), and *F. eumartii* (IMI-263815) have been deposited in the CMI culture collection.

Sclerotium bataticola Taub., the sclerotial state of *M. phaseolina*, was identified among cultures. Pycnidia of the fungus formed in tissue that was plated but not when cultures were grown on agar media. Sclerotia in pure cultures were black, more or less spherical, and from 36 to 180 μm in diameter. Sclerotia formed under the epidermis and in the cortex of the root and collar were more regular in shape and ranged from 36 to 110 μm in diameter.

All isolates of *Fusarium* were pathogenic to chickpea (Table 2). Symptoms for a given isolate were similar whether inoculation was by the water- or pot-culture method, but a severe reaction occurred sooner with the former. No symptoms developed in controls. Isolates of *F. oxysporum* varied in the nature and severity of symptoms that they induced. Of 30 isolates tested, two induced wilt with vascular discoloration (vascular wilt), 15 induced foliar yellowing with vascular discoloration (vascular yellowing), and 13 induced foliar yellowing without vascular discoloration (nonvascular yellowing) along with cortical collar and root necrosis. Symptoms developed by 15 days after inoculation by the pot-culture method. With vascular wilt isolates, flaccidity of leaves occurred first and was followed by a dull green discoloration and dessication of leaves and stem. Roots showed no external symptoms. All plants were necrotic by 1 mo after inoculation. First symptoms developed from water-culture inoculations within 10 days and all plants were dead within 15 days.

Yellowing of lower leaves was the first symptom in plants inoculated with vascular yellowing isolates. This yellowing progressed upward and was followed by leaflet necrosis and drop, but no plant died by 60 days after inoculation by the pot-culture method, or by 15 days after inoculation by the water-culture method. No external symptoms were observed in roots.

Although the salmon isolates of *F. oxysporum* induced vascular wilt or yellowing (Table 2), the reddish isolates induced only a shallow necrosis in roots and collars and mild foliar yellowing. No vascular discoloration occurred. Overall, wilting isolates were the most virulent, and nonvascular yellowing isolates the least virulent, to cultivar PV-24. No statistically significant differences in virulence were found among isolates inducing vascular wilt or yellowing, but some differences occurred among isolates that

TABLE 1. Frequency of fungi isolated from chickpea affected by symptom complexes associated with the "Seca" disease in southern Spain in 1979–1981[1]

Symptom complex	Percentage of plants from which indicated fungi were isolated			
	Fusarium oxysporum	*F. solani*	*Macrophomina phaseolina*	*Fusarium* spp.[2]
Foliar yellowing	52	21	4	3
Dead or dying plants	93	56	13	–[3]
Dry collar and root rot	19	7	80	27
Yellow stunt	59	46	4	–
Iron chlorosis	26	3	6	–

[1] Plants were sampled from 108 fields during disease surveys in 1979–1981 in Córdoba and Sevilla provinces.
[2] Species not identified because cultures were overgrown by *M. phaseolina* or by contaminants.
[3] *Fusarium* spp. not isolated.

induced nonvascular yellowing. Salmon isolates were reisolated from roots and stems of all plants, while reddish isolates were recovered routinely from roots and collars but only occasionally from the lowermost stems.

Isolates of *F. solani* and *F. eumartii* induced foliar yellowing that progressed upward in the plant, and extensive black rotting of roots, collars, and lowermost stems, but no vascular discoloration. Inoculation by the pot-culture method resulted in death of most plants by 20 days after inoculation, or in progressive yellowing without plant death by 60 days after inoculation. Plant death occurred for most isolates by 15 days after inoculation by the water-culture method. Small but significant differences in virulence to cultivar PV-24 were found among isolates of *F. solani*. The isolate of *F. eumartii* was as virulent as any isolate of *F. solani*. *F. solani* and *F. eumartii* were reisolated only from roots and collars of inoculated plants.

All 15 isolates of *M. phaseolina* tested were pathogenic to chickpea. Severity of disease reaction was mild for most isolates; a low proportion of plants showed yellowing or necrosis of lower leaves by 50 days after inoculation. Severe leaf yellowing and small to large necrotic lesions in roots and collars developed when plants were grown at higher temperature and irrigated every other day which caused periodic wilting. *M. phaseolina* was consistently reisolated from collar tissue of inoculated plants.

Virulence and host range of *Fusarium* isolates. The results of three experiments with five isolates of *F. oxysporum* and two experiments with three isolates of *F. solani* were similar. Severity of infections varied with isolates of *F. oxysporum* and with chickpea cultivars. Cultivars WR-315 and P-678 were highly resistant to all isolates and did not show symptoms by 60 days after inoculation, but a differential interaction occurred between other cultivars and isolates. Cultivars JG-62, P-2245, and PV-24 were highly susceptible to vascular wilt isolates; all plants died within 20–30 days after inoculation. Both vascular and nonvascular yellowing isolates were not pathogenic to cultivar JG-62. Cultivar PV-24 was significantly less susceptible than cultivar P-2245 to vascular yellowing isolates. Most P-2245 plants died by 40 days after inoculation; 20 days later, PV-24 exhibited a severe disease reaction. Plants of cultivars WR-315, P-678, and JG-62 inoculated with nonpathogenic vascular isolates had a vascular discoloration in the roots and lower stems. *F. oxysporum* was reisolated from the roots and collars of 58–78% of resistant plants depending upon the cultivar-isolate combination. Reisolations were positive from all susceptible plants inoculated.

Isolates of *F. solani* were not pathogenic to cultivars WR-315 and JG-62, and they did not differ in virulence to cultivars P-2245 or PV-24. Cultivar PV-24 was significantly more susceptible than cultivar P-2245. About 50% of PV-24 plants died by 20 days after inoculation, but only progressive leaf yellowing and small to large necrotic lesions on collars and roots developed on plants of cultivar P-2245. Some superficial and small necrotic lesions were observed on collars and roots of resistant WR-315 and JG-62 cultivars. *F. solani* was reisolated from collars and roots of all susceptible and resistant plants.

TABLE 2. Pathogenicity to "kabuli" chickpea cultivar PV-24 of isolates of *Fusarium* spp. associated with wilt and root rot of chickpea in southern Spain

Fusarium spp.	Colony type	Symptom	Number of isolates tested	Severity of disease reaction[w]			
				Water culture[x]		Pot culture[y]	
				Range	Mean	Range	Mean
F. oxysporum f. sp. *ciceri*[z]	Salmon	Wilt	1	3.0–4.0	3.5	···	4.0
F. oxysporum	Salmon	Wilt	2	3.0–4.0	3.5	···	4.0
F. oxysporum	Salmon	Foliar yellowing and vascular discoloration	15	1.2–2.9	1.7	1.4–3.1	2.2
F. oxysporum	Reddish	Foliar yellowing, cortical collar and root necrosis	13	0.5–3.5	1.7	0.8–1.7	1.0
F. solani[z]	–	Foliar yellowing, black collar and root rot	1	···	4.0	2.7–4.0	3.4
F. solani	–	Foliar yellowing, black collar and root rot	9	2.1–4.0	3.7	2.0–3.4	3.0
F. eumartii	–	Foliar yellowing, black collar and root rot	1	3.9–4.0	4.0	3.5–4.0	3.7

[w] Pooled results of several experiments, based on a 0–4 scale according to percentage of foliage with yellowing or necrosis in acropetal progression (0 = 0%, 1 = 1–33%, 2 = 34–66%, 3 = 67–100%, 4 = dead plant).
[x] Seven- to 10-day-old plants were placed in cylindrical glass bottles containing 200 ml of a diluted suspension of liquid cultures in potato-dextrose broth. Disease reaction was assessed 15 days after inoculation.
[y] Disinfested seeds were sown in autoclaved soil mixture infested with isolates grown in cornmeal-sand (1:12, w/w). Disease reaction was assessed 40 days after sowing.
[z] Isolates provided by Y. L. Nene, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India.

TABLE 3. Severity of necrotic lesions in the belowground epicotyls and hypocotyls of legumes inoculated with isolates of *Fusarium eumartii* and *F. solani*[v]

Fusarium spp.	Origin	Legume										
		Alfalfa	Bean	Broadbean	Chickpea	Pea	Lentil	Andine lupine	Blue lupine	Yellow lupine	White lupine	Soybean
F. solani	India	0.0 a[w]	1.5 a	3.3 a	4.0 a	4.0 a	2.8 a	1.5 a	2.2 a	2.0 a	1.7 a	0.0 a
F. solani	Sevilla	0.0 a	1.1 a	1.4 b	3.5 a	1.7 b	1.1 b	0.9 b	1.3 b	0.8 b	1.2 b	0.0 a
F. solani	Sevilla	0.0 a	0.3 b	1.4 b	3.8 a	1.1 c	0.3 c	0.6 b	0.6 c	2.3 a	1.2 b	0.0 a
F. eumartii	Córdoba	0.0 a	2.2 c	3.6 a	4.0 a	4.0 a	3.4 a	2.0 a	2.2 a	2.2 a	2.0 a	0.0 a

[v] Disinfested seeds were sown in an autoclaved soil mixture infested with isolates grown in cornmeal-sand (1:12, w/w). Disease reaction was assessed 60 days after sowing according to percentage of necrotic tissue present in the belowground epicotyl and hypocotyl tissues: 0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100%. Each figure is mean of nine plant values. All legumes except broadbean, chickpea, and pea were free of foliar symptoms.
[w] Values in a column followed by the same letter are not significantly different according to Student's *t*-test, $P = 0.05$.

Isolates of *F. oxysporum* were pathogenic to chickpea but not to any of the other 10 legume species, which had developed no symptoms by 60 days after inoculation. *F. oxysporum* was reisolated from roots or collars of 100, 92, and 80% of inoculated beans, peas, and white lupines, respectively, and from 20% or less of other legumes except soybeans and yellow lupines from which all isolations were negative. Isolates recovered from nonsuscepts were pathogenic to chickpea and reproduced symptoms like those induced by the original isolates.

Isolates of *F. solani* and *F. eumartii* were pathogenic to all the legume species except alfalfa and soybean. The isolate of *F. eumartii* and that of *F. solani* provided by Y. L. Nene were significantly more virulent than two other isolates of *F. solani*. Severity of reaction varied among plant species (Table 3). Chickpea, broadbean, and pea, in that order, were most susceptible and differed in degrees of foliar symptoms and root and collar necrosis. Beans, lentils, and all lupines were free of foliar symptoms but had cortical necrotic lesions on roots and collars (Table 3). *F. solani* and *F. eumartii* were reisolated from all inoculated plants except alfalfa and lentils, for which the percentage of reisolation varied among isolates. Recovered isolates were pathogenic to chickpea.

Based on the morphology and results from pathogenicity and host-range tests, the fungus inducing vascular wilt or yellowing was identified as *F. oxysporum* f. sp. *ciceri*.

DISCUSSION

The "Seca" disease severely affected chickpeas in southern Spain where it is widespread. It is the most important disease of chickpeas in that area. Ascochyta blight, rust, or broomrape were observed occasionally and with low incidence during our disease surveys. Although measurements of seed yield losses were not made, an annual loss of 12–15% was estimated based upon disease incidence and severity in surveyed fields.

The "Seca" of chickpea is a disease of complex etiology. It has as a main component a wilt and root rot similar to that reported from other countries (17,28,33). Symptoms observed in plants affected by yellow stunt appear to correspond closely to those of chickpea stunt in India (29), but other plant viruses have been found associated with similar symptoms (21). Also, the apical necrosis, longitudinal grooves, and zig-zag pattern that we observed in stems of affected plants have not been described as characteristic of chickpea stunt. No efforts were made to further characterize the nature of yellow stunt.

F. oxysporum, *F. solani*, and *M. phaseolina* were consistently isolated from diseased plants and proven to be pathogenic to chickpea under specific controlled conditions (Table 2). Isolates of *F. oxysporum* differed in colony morphology and in virulence to, and symptoms induced in, chickpea cultivars. Isolates that formed salmon-pigmented colonies induced wilt or foliar yellowing with vascular discoloration and showed pathogenic specialization to chickpea and differential pathogenicity to chickpea cultivars. Isolates that formed reddish colonies were weakly pathogenic to chickpea and induced foliar yellowing and cortical collar and root necrosis. No efforts were made to determine pathogenic specialization for reddish isolates. Infection of vascular tissues by *F. oxysporum* in other suscepts may also result in wilt or foliar yellowing (5,6,25). For chickpea, Prasad and Padwick (29) first distinguished a rapid flaccidity and dessication from progressive yellowing in infections by *F. oxysporum* f. sp. *ciceri*. However, in the literature only the first symptom type has been generally recognized (1,10,26,28), except for the foliar yellowing which progressed upwards as described by Westerlund et al (33). Our results are the first to show that wilt or foliar yellowing with vascular discoloration of chickpea are induced by different biotypes of the pathogen. Vascular wilt isolates occurred at a lower frequency than vascular yellowing isolates, and except for the vascular nature of the infections, no close correlation occurred between symptoms found in surveyed fields and those induced by artificial inoculations. Several factors may account for this, including methods used in pathogenicity tests, multiple infections by *F. solani* and reddish isolates of *F. oxysporum* as shown for interactions of these fungi in other plants (6,23), and environmental factors (25).

Isolates identified as *F. solani* induced foliar yellowing and black collar and root rot. One of them, later identified as *F. eumartii*, was among the most virulent to chickpea. Our isolates were also pathogenic to broadbean and to a lesser extent to pea, and they induced restricted root necrosis in other legumes. Kraft (23) and Westerlund et al (33) found that chickpea isolates of *F. solani* were pathogenic to pea; they did not include broadbean in pathogenicity tests. On the other hand, Yu and Fang (34) showed that broadbean isolates of *F. solani* were pathogenic to broadbean, but not to pea. We therefore believe that our isolates should not be identified as *F. solani* f. sp. *pisi*.

M. phaseolina was isolated with highest frequency from plants affected by dry root rot in a year of severe drought and high temperatures, and symptoms were induced in a growth chamber only when plants were subjected to stresses of low soil water and high temperature. This is similar to results of Westerlund et al (33) in California, which suggests that incidence and severity of infections of chickpea by *M. phaseolina* could be reduced by maintaining adequate soil moisture.

The "Seca" disease of chickpea in southern Spain illustrates complexities associated with root diseases in field situations. In many instances, more than one pathogenic fungus was isolated from affected plants, which suggests that interactions may occur and may influence disease incidence and severity (14,32). Also, isolations from plants showing foliar yellowing without vascular discoloration, sampled in fields with poor, shallow soils or high plant density, were frequently negative or yielded weakly pathogenic reddish isolates of *F. oxysporum*. This suggests that edaphic factors may play a role in the etiology of the "Seca" disease, as was indicated in California (33), and thus contribute to its complexity (14,32).

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Ecology and Epidemiology

Epidemiology and Yield Losses Associated with Alternaria Blight of Sunflower

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ABSTRACT

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Epidemics of Alternaria blight, caused by *Alternaria helianthi*, were initiated at different plant growth stages on two sunflower genotypes (cms HA89 and hybrid 894) during the 1981 and 1982 growing seasons at Brookings, SD. Yield losses as great as 51 and 60% were observed on hybrid 894 and cms HA89, respectively, when seed yields were compared to those of check plots sprayed with fungicide. The 100-seed weights and seed oil contents were also significantly reduced in some inoculated treatments. Yield losses, losses in 100-seed weight, and oil content were consistently greater on the inbred line cms HA89 than hybrid 894, indicating that Alternaria could be a greater problem in seed production fields than in commercial hybrid fields. The logistic growth model consistently fit Alternaria blight progress curves better than the Gompertz model in 1981, but in 1982 no consistent difference between the models was detected. Selected critical-point, and multiple-point regression yield loss models gave coefficients of determination of 0.65 and 0.70, respectively, when disease severity was used to predict percent yield reduction due to *A. helianthi*.

Additional key words: *Helianthus annuus*, yield loss assessment.

Alternaria blight of sunflower, caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara has been recognized as a potentially destructive disease in India, Yugoslavia, Australia, Tanganyika, Uganda, and South Africa (5-7,9,16,17,22,23). In the United States, Alternaria blight has been particularly destructive on sunflower in Ohio, Florida, and Mississippi, and has been reported from Minnesota, North and South Dakota, and Wisconsin (12,15,18,20,21). *A. helianthi* can cause severe leaf and stem spots resulting in premature defoliation and stem breakage. In addition, a seedling blight due to *A. helianthi* has also been reported (20). Estimates of yield losses in sunflower due to Alternaria blight in India range upward to 80% (2,6,7,10,14,17). The disease significantly reduces head diameters, numbers of seeds produced per head, 1,000-seed weight, and percent oil content of seed (6,7,10,17). Disease severity is negatively correlated with yields ($r = -0.76$ and -0.62 for two cultivars, respectively) (6). Estimates of yield losses due to Alternaria blight of sunflower at two locations in Australia in the 1977-1978 growing season were 26 and 17%, respectively (5). Thousand-seed weights were significantly reduced by Alternaria blight at both sites, and percent oil content was significantly reduced at one site.

Although destructive levels of Alternaria blight have been reported in the warmer, more humid areas of the United States (12,18,21), no information is available on the potential

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