Fungal Root Rots and Wilt of Chickpea in California

F. V. Westerlund, Jr., R. N. Campbell, and K. A. Kimble

Research Assistant, Professor of Plant Pathology, Staff Research Associate IV, Department of Plant Pathology, University of California, Davis 95616.

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ABSTRACT

At least five fungi, *Fusarium oxysporum* f. sp. *ciceri*, *F. solani* f. sp. *pisi*, *Pythium ultimum*, *Rhizoctonia solani*, and *Macrophomina phaseoli* cause root rot and wilt of chickpeas in the central coastal area of California. Inoculation with the different fungi in greenhouse studies reproduced disease symptoms observed in the field. *Fusarium solani* f. sp. *pisi* and *F. oxysporum* f. sp. *ciceri* caused similar yellowing and wilting of the shoots, but the former caused distinctive black root lesions and the latter, vascular discoloration extending to the top of the shoot. *F. oxysporum* f. sp. *ciceri* was found in only 6% of the wilted plants whereas *F. solani* f. sp. *pisi* was found in 47%. This is the first report that *F. solani* f. sp. *pisi* is an important chickpea pathogen in California. *Fusarium oxysporum* f. sp. *ciceri* may require wounding for efficient infection, but *F. solani* f. sp. *pisi* does not. Also, *F. solani* f. sp. *pisi* was carried on a low percentage of seeds, but there was no evidence that *F. oxysporum* f. sp. *ciceri* was seedborne. No virus diseases were observed during 1971-1972.

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Chickpea (*Cicer arietinum* L.), known as Garbanzo bean or gram, is a high protein food crop in many areas of the world. Ninety-five percent of the chickpea production in the United States is grown on about 7,500 acres in the central coastal area of California, either as a dryland crop, or occasionally as an irrigated crop. Chickpeas were grown in the south coastal area of California, but production has shifted to the central coastal area such as the Santa Maria Valley, where they were introduced in 1935 when 454 kg of seeds were brought from Mexico.

Several diseases have been described on chickpeas in California. Snyder et al. (18) found that bean yellow mosaic virus caused a yellowing disease, characterized by phloem discoloration, leaf yellowing, and eventual plant death. Erwin (8, 9, 10) described Verticillium and Fusarium wilts of chickpeas caused by *Verticillium albo-atrum* Reinke & Berth. and *Fusarium lateritium* (Nees) emend Snyder and Hans. f. sp. *ciceri* (Padwick) Erwin. These pathogens caused wilting, yellowing, and vascular discoloration. Elsewhere, Kraft (16) reported chickpeas as a host for *Fusarium solani* (Mart.) Appel and Wr. f. sp. *pisi* (E. R. Jones) Synd. and Hans. in trial plantings in Washington. *Fusarium oxysporum* caused wilting, yellowing, vascular discoloration, and the eventual death of chickpeas in Peru (6). In Iran, Kaiser and Danesh (13, 14) identified four viruses causing losses in chickpeas: alfalfa mosaic virus (AMV), bean yellow mosaic virus (BYMV), cucumber mosaic virus (CMV), and pea leaf roll virus (PLRV). The present work was done to determine the important chickpea pathogens in the central coastal area of California so these pathogens could be used to screen introductions for resistance.

MATERIALS AND METHODS. – Fungal isolations from diseased plants were made by surface-sterilizing host stem or root tissue pieces in 0.525% NaOCl for 10 to 20 min. Stem tissue was then cut into 5- to 10-mm-long sections and placed on water agar (WA) (21), potato-dextrose agar (PDA), acid-potato-dextrose agar (APDA) (PDA acidified to pH 4.5-5.0 with 25% lactic acid), Nash and Snyder PCNB Medium (NSA) (21), and Difco corn meal agar (CMA). Fungi were also cultured from root lesions by removing surface-sterilized tissues from the margins of lesions and placing them on culture media. Tissues suspected of harboring water molds were surface-sterilized in NaOCl (0.525%) for 3 min, floated in water for 24 h, and examined for fungi. If fungi were detected, the tissue pieces were washed in running water for 24-48 h and placed on pimaricin-vancomycin medium (PVM) (20) or CMA. When fungi began to grow from host tissue, mass transfers of mycelium were placed in culture tubes. Single spores of fusaria were also isolated and transferred to agar from sporodochia or mycelium growing from the host tissue.

Pathogenicity tests and inoculum preparation. – Two methods were used to test pathogenicity of *Fusarium* spp. isolates. First, chickpea seeds were surface-sterilized with 0.525% NaOCl for 10 to 20 min and planted in 13-cm diam plastic pots filled with pasteurized U.C. mix, type C- 2 (1). One ml of a conidial suspension was pipetted onto each seed before covering it with pasteurized river sand. In a second method, chickpeas grown in vermiculite for 7 days were removed and the roots carefully washed free of vermiculite, trimmed, and dipped into conidial suspensions.

Each isolate of *Fusarium* used in experimental work originated from a single spore. Conidial inoculum was produced on PDA slants incubated 7 to 14 days under 400-w fluorescent tubes. Suspensions of conidia were prepared by washing cultures with sterile distilled water followed by filtration through

two layers of cheesecloth and standardized at 1 X 10^6 conidial/ml.

Inoculum of *Pythium* sp. was prepared from cultures grown in petri dishes on CMA for 14 days. Cultures were cut into blocks approximately 5 X 5X 7 mm with a scalpel and mixed into moist, pasteurized, Yolo fine sandy loam soil in metal flats (30 X 45 cm). Chickpea seeds were sown 2 wk later and covered with pasteurized river sand.

Inoculum of *Rhizoctonia solani* and *Macrophomina phaseoli* was prepared by growing the fungi on PDA plates for 14 days and incorporating sections of mycelial mats into the soil as described for *Pythium* sp. Inoculum was also prepared in 0.95-liter (one-quart) Mason jars containing rice hulls enriched with a solution of 1% dextrose, 1% calcium nitrate, and 0.25% monobasic potassium chloride. After sterilization, they were inoculated with several plugs of PDA containing rapidly growing mycelium. After 35 days at room temp, one-to-five rice hulls covered with sclerotia were placed next to roots of 7-day-old chickpeas.

Chickpea seedlings were harvested 7-30 days after inoculation depending on conditions of the experiment. Noninoculated plants were used as controls for all pathogenicity trials. Each treatment consisted of four or five replicate pots each with four plants. All experiments were repeated at least three times.

Germination trials were done by the Crop Improvement Seed Laboratory at the University of California, Davis. Epicotyl length was measured 7 and 10 days after the initiation of germination trials to estimate seedling vigor.

Four methods were used to detect the presence of fungi within or upon chickpea seeds. Several hundred chickpea seeds were washed in 200 to 300 ml of sterile distilled water. The wash water was then centrifuged at 8,000 rpm for 10 to 15 min, the supernatant decanted, and the resuspended sediment examined for presence of *Fusarium* conidia. Small amounts of this suspension were also pipetted onto the surface of agar media (APDA and NSA) and onto roots, hypocotyls, and epicotyls of chickpea seedlings. In the second method, chickpea seeds were surface-sterilized by gentle agitation in 0.525% NaOCl for 10 min with or without two periods of reduced pressure. The seeds were then rinsed in sterile distilled water and placed on malt-salt agar (MSA) (21) and APDA. In the third method, chickpea seeds without surface sterilization were placed on APDA and MSA. In the fourth method, chickpea seedlings were planted in sterile white quartz sand, vermiculite, or U. C. mix. One-half of the seed samples were surface-sterilized while the other half were not. These seeds were planted; after several days, those seedlings having root lesions were dissected and cultured for fungi. Seedlings with clean roots were transplanted to U. C. mix. Some plants that wilted and yellowed were removed, examined for vascular discoloration and root rot, and cultured for fungi.

RESULTS. – *Disease survey.* – Only one plant with virus-like symptoms was collected. It and 21 symptomless plants were assayed for viruses by sap transmission to a host range consisting of: *Datura stramonium* L.; *Nicotiana glutinosa* L.; *N. tabacum* L. 'Turkish' and 'Havana 425'; *Gomphrena globosa* L.; *Chenopodium amaranticolor* Coste and Reyn.; *Cucumis sativus* L. 'National Pickling'; *Cicer arietinum* L.; *Phaseolus vulgaris* L. Bountiful'; *Pisum sativum* L. 'Dwarf Telephone'; *Vicia faba* L.; and *Vigna sinensis* (Stickm) Savi ex Hassk. 'Blackeye.' All mechanical inoculations were done by rubbing Carborundum-dusted leaves with a finger dipped into homogenates prepared by grinding leaves in 0.05 M phosphate buffer at pH 7.2 or 7.6. In some instances, graft inoculations were done by cleft-grafting scions from field plants into 2-wk-old chickpeas. Grafted plants were then placed in mist chambers for 3 days. No virus was detected by electron microscopic examination of dip preparations or from symptom development in assay plants. All nongrafted controls remained healthy.

Chickpea plants showing wilt and foliar yellowing were collected from seven separate fields in the central coastal area of California from mid-June to July in 1971 and 1972. Five fields had been previously cropped to chickpeas for several yr, but two fields had no history of chickpea plantings. These plants had two types of obvious symptoms, vascular discoloration and a severe black root rot. Some plants showed both types of symptoms, while others had no apparent symptoms. The frequency of isolation of pathogens from these plants is in Table 1.

Fusarium oxysporum was the only fungus isolated from the stems of wilted chickpeas with vascular discoloration extending above the soil line. It was not isolated from plants that lacked vascular discoloration. *Fusarium solani* was isolated from plants with severe black root rot, and was the most common pathogen isolated from chickpeas grown in the five fields previously cropped to chickpeas (Fields C-G, Table 1). *Macrophomina phaseoli* was isolated from wilted plants lacking vascular discoloration. These plants were growing in an unusually dry field (Field E, Table 1). In addition, *F. oxysporum,* later shown to be nonpathogenic, was frequently isolated from roots along with the pathogenic *F. solani* and *M. phaseoli. Rhizoctonia solani* and *Pythium ultimum* were isolated most frequently from diseased plants in the fields not previously cropped to chickpeas (Fields A and B, Table 1).

Soil was collected from beneath and around the roots of chickpea in four areas that had a previous history of chickpea production. When this soil was planted with surface-disinfested chickpeas in the greenhouse, *F. solani* and *P. ultimum* were consistently isolated and shown to be pathogenic.

Pathogenicity trials and identification. – The 37 isolates of *Fusarium solani* and the five isolates of *F. oxysporum* (Table 1) were identified using the criteria of Toussoun and Nelson (19) and were shown to be pathogenic to chickpeas in greenhouse inoculation trials. Symptoms developed 10 to 14 days after inoculation with *F. solani* and 14 to 21 days after inoculation with *F. oxysporum*. In both cases,

TABLE 1. The frequency of isolation of pathogenic fungi from chickpeas in seven fields in northern Santa Barbara and southern San Luis Obispo counties in California in 1971-72.

	1971-72					
Field	No. of plants tested	*Fusarium solani* f. sp. *pisi*	*Fusarium oxysporum* f. sp. *ciceri*	*Pythium ultimum*	*Rhizoctonia solani*	*Macrophomina phaseoli*
A	18	2	-	7	5	-
B	9	2	-	3	5	-
C	10	9	-	2	-	-
D	11	10	1	-	-	-
E	17	5	3	2	-	5
F	6	6	-	-	-	-
G	8	3	1	-	-	-
Total	79	37	5	14	10	5

yellowing of lower leaves of young plants was the first symptom. This yellowing progressed uniformly upward. When older chickpeas were inoculated with *F. oxysporum*, the yellowing was apparent on one side of the plant; i.e., only some of the main branches showed symptoms. Although symptoms caused by *F. oxysporum* or *F. solani* were similar on the aerial parts of the plants, each caused distinct internal and below ground symptoms. Vascular discoloration and normal-appearing roots were associated with *F. oxysporum*, whereas *F. solani* caused extensive black root lesions without vascular discoloration.

Trials were done to compare the effects of inoculum levels and wounding on pathogenicity of *F. oxysporum* and *F. solani*. Application of 1×10^6 *F. oxysporum* conidia/ml onto seeds at sowing or onto young seedlings growing in U. C. mix caused disease in only three of 80 plants; these plants wilted and the fungus was recovered from their stems. With less than 1×10^6 conidia/ml there were no symptoms and the fungus was not recovered from stems. If seedling roots were trimmed and dipped into conidial suspensions containing from 1×10^4 to 1×10^6 conidia/ml, symptoms developed and the fungus was reisolated. When 1×10^4 to 1×10^6 *F. solani* conidia/ml were applied to intact seeds or nonwounded seedlings, as described above, typical black rot lesions developed. This suggests that *F. oxysporum* requires a wound for efficient infection whereas *F. solani* does not.

Blackeye cowpea, Bountiful bean, 'Little Marvel' pea, 'Kanrich' soybean [*Glycine max* (L.) Merr.] and chickpea were inoculated with five *Fusarium oxysporum* or ten *F. solani* isolates to determine their host ranges. Controls consisted of noninoculated plants. *F. oxysporum* isolates infected chickpeas, caused wilt, and were easily reisolated. Peas and cowpeas inoculated with *F. oxysporum* isolates were free of symptoms and were similar in vigor to the controls. All the isolates infected these hosts, however, and invaded the above ground stems. This was demonstrated by making thin-sections that were either stained in 0.5% cotton blue in lactophenol or cultured. Fungal mycelium was seen in the vascular elements of both plant species and the pathogens were reisolated from each species. After being recovered from peas and cowpeas, the isolates were retested on chickpeas and were pathogenic although they seemed to be less virulent to chickpeas than the original isolates.

The *F. solani* isolates were pathogenic to chickpeas and common peas, causing a root rot in both hosts. Thin-sections were made of infected tissues and isolations were made as described above for plants inoculated with *F. oxysporum*. Typical *F. solani* cultures were reisolated and chlamydospores and mycelium were seen inter- and intracellularly in the cortical tissue of these two plants species.

Based on the morphology and results from pathogenicity tests, the fungus which caused the vascular wilt was identified as *Fusarium oxysporum* Schl. f. sp. *ciceri* (Padw.) Matuo and Sato. and the root rot pathogen was identified as *F. solani* (Mart.) Sacc. f. sp. *pisi* (Jones) Snyder and Hansen.

Pythium ultimum and *Rhizoctonia solani* caused pre- and postemergence damping-off, and death of small roots. *Macrophomina phaseoli* caused discrete root lesions only when chickpeas were grown under a stressed water regime that caused periodic wilting. This is similar to the situation observed with cotton and sorghum inoculated with *M. phaseoli* (7, 12). Symptoms produced in the greenhouse by these three fungi were similar to those seen in the field and each fungus was consistently reisolated.

In 1972 in the Santa Inez Valley, there was a 70% loss of stand in two fields that had not previously been cropped to chickpeas. *Rhizoctonia solani* and *Pythium* spp. were isolated from surviving plants. The grower applied Arasan and Demosan as a seed treatment, replanted the field, but again losses of 50% were sustained. Low percentages of *R. solani* and *P. ultimum* were recovered from surviving seedlings, so seed germination rate and germination vigor were examined. The seed used in this field (designated as seedlot 4) had a germination rate of only 72% compared with 94, 92, and 80% for three check seedlots (designated as seedlots 1, 2, and 3, respectively). An estimate of vigor was made by measuring epicotyl length. In seedlot 4, 70% of the seedlings were less than 5 cm tall in 7 days, whereas,

in seedlot 1, 90% of the seedlings were more than 11 cm tall in 7 days. Chickpeas had been planted 10 to 15 cm deep in these two fields; thus the low germination rate and slow seedling growth in conjunction with the two seedling pathogens could account for the poor stands in these two fields.

Fungus flora of seeds. – Storage fungi in seed can reduce seedling viability and germination rate (4, 11). Isolations on MSA failed to yield storage fungi from seedlots 1, 2, and 3. One percent of the seeds from seedlot 4 yielded an *Aspergillus* sp. (*A. niger* group), and 2% yielded *Penicillium* spp. Because few fungi were recovered, it was concluded they were not an important factor in low germination rates or poor seedling vigor.

Kendrick and Snyder (15) demonstrated that *F. oxysporum* f. sp. *phaseoli* was seedborne on *Phaseolus vulgaris*. Thus, tests were done to determine whether the chickpea *Fusarium* spp. were also seedborne. *Fusarium oxysporum* was isolated in washings from about 500 chickpea seeds but these isolates were not pathogenic. *Fusarium solani* f. sp. *pisi* was not isolated from seeds by this method. Seeds with or without surface sterilization were also plated on APDA. *Fusarium solani* f. sp. *pisi* was recovered from one seed of 250 nonsterilized seeds. More than 600 chickpea seeds were planted in vermiculite; *F. solani* f. sp. *pisi* was isolated from small black lesions on the roots of two seedlings. This low recovery rate may not indicate the true percentage of seeds carrying *F. solani* f. sp. *pisi* because it is difficult to recover from tissue and 10 to 20% of the seedlings had similar lesions from which no *F. solani* f. sp. *pisi* was recovered. Thus, there is no evidence that *F. oxysporum* f. sp. *ciceri* is seedborne. Apparently *F. solani* f. sp. *pisi* is carried on the exterior of chickpea seeds as has been reported from the fungus on the pea seed (5) and for *F. solani* f. sp. *phaseoli* on bean seed (17).

DISCUSSION. – In the central coastal area of California, fungal root rots and wilt of chickpeas are caused by at least five fungi: *F. solani* f. sp. *pisi*, *F. oxysporum* f. sp. *ciceri* *Pythium ultimum*, *Rhizoctonia solani*, and *Macrophomina phaseoli*. These fungi were consistently isolated from diseased plants and were proven to be pathogenic; they probably are important in the long-term decline in chickpea yields reported by growers. Virus diseases were not important during 1971 and 1972.

This is the first report that *F. solani* f. sp. *pisi* is an important pathogen of chickpeas in the central coastal area of California, and that it was the most prevalent pathogen in 1971 and 1972. *Pythium ultimum* was associated with disease in both old and new areas of production, while *Rhizoctonia solani* was found only in new areas. The prevalence in new areas may be due to the increase of *R. solani* on the previous crops. If *R. solani* was present in older chickpea areas, it may not have been recognized because of the predominance of *F. solani* f. sp. *pisi*. *Macrophomina phaseoli* was isolated from plants growing in dry fields, and symptoms were induced in the greenhouse only when plants were grown under a water stress regime that caused periodic wilting. This pathogen is not a problem in fields with adequate moisture, suggesting that it can be avoided by maintaining adequate soil moisture. *R. solani* was the only chickpea pathogen isolated from the plants in three areas without a known history of chickpea production in the central Sacramento Valley. *F. solani* f. sp. *phaseoli* was recovered from some of the *R. solani* lesions; however, this *Fusarium* isolate was not pathogenic to chickpeas. *Fusarium lateritium* f. sp. *ciceri* in California (9) and *F. oxysporum* in Peru (6) have been reported to cause vascular wilt of chickpea. *F. lateritium* f. sp. *ciceri* was not isolated from wilted plants in the present study. Since *F. lateritium* f. sp. *ciceri* isolates received from D. C. Erwin were not pathogenic to chickpeas, we cannot confirm this fungus as a pathogen. *F. oxysporum* f. sp. *ciceri* was isolated and was pathogenic. It is therefore regarded as the vascular wilt pathogen. *F. oxysporum* f. sp. *ciceri* was isolated from all of the plants with vascular discoloration extending above ground level, but this constituted only 6% of the wilted plants.

Factors other than pathogenic organisms contribute to stand losses in chickpea fields. Poor germination and seedling vigor may be of major importance but they can be eliminated by screening seedlots for germination rates and vigor. Soil compaction is another major problem. Seeds are planted about 15.2 cm (6 inches) deep where there is moisture. At this level, soils often have a hard "plow pan" which the roots cannot penetrate, thus they spread laterally and fail to develop normally. Burke et al. (3) reported that propagules of the pea root rot organism are mainly in the plowed areas and that obstruction of root growth by compacted soil increased Fusarium root rot of beans and peas (2). Field observations indicate that this may also occur in chickpea fields in California.

Kraft (16) suggested that chickpea roots may be susceptible to several clones of *F. solani* besides *F. solani* f. sp. *pisi*. Inoculation of chickpeas with several different isolates of *F. solani* f. sp. *phaseoli* (received from W. C. Snyder)failed to induce significant disease symptoms. However, one of the *F. solani* f. sp. *phaseoli* isolates was pathogenic not only to *Phaseolus vulgaris* 'Red Kidney'; but to *Vigna sinensis* 'Blackeye'; and *Glycine max* 'Kanrich'.

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