Variability in Pathogenicity and Symptomatology of *Leptosphaerulina crassiasca* on Peanut

P. SUBRAHMANYAM, Visiting Research Scientist, and D. H. SMITH, Professor, Texas A&M University, Texas Agricultural Experimental Station, P.O. Box 755, Yoakum 77995

ABSTRACT

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Four of 11 isolates of *Leptosphaerulina crassiasca* obtained from various sources produced discrete, necrotic-type lesions (pepper spots) on peanut (*Arachis hypogaea*) leaflets. The other isolates also produced necrotic lesions, which enlarged rapidly and coalesced to produce irregular necrotic areas scattered over the entire leaflet (leaf scorch), depending on the environmental conditions. Wedge-shaped necrotic lesions on leaflet tips, which are often observed in the field, were not produced in inoculation tests in the glasshouse. The optimum temperature range for growth of the fungus in culture was 20–30 C. Production of ascocarps on autoclaved peanut leaf disks was abundant at 20 C.

A foliar disease of peanut (*Arachis hypogaea* L.) caused by *Leptosphaerulina crassiasca* (Sechet) C. R. Jackson & D. K. Bell occurs in Madagascar (12), Taiwan (13), the United States (4,5,9,10), Argentina (2), Mauritius (1), Malawi (7), India (6,8), South Africa (14), Senegal and Zambia (P. Subrahmanyam and D. H. Smith, *personal observations*), and Nigeria and the Peoples' Republic of China (D. McDonald, *personal communication*). The disease is characterized by two distinct symptoms. The pepper spot phase of the disease (5) consists of dark brown to black lesions, usually 0.25–1.0 mm in diameter, irregular to circular in outline, and occasionally depressed. Lesions are discrete and visible from both sides of the leaflets but are more conspicuous on the adaxial surface. In the field, lesions usually remain small. When lesions are abundant, the leaflet has a netted appearance because of coalesced lesions. These leaflets soon die, and numerous ascocarps are produced in necrotic areas of defoliated leaflets (3,11).

Present address of first author: Principal Plant Pathologist, SADCC/ICRISAT Groundnut Project, Chitedze Agricultural Research Station, P.O. Box 1096, Lilongwe, Malawi.

Present address of second author: Principal Plant Pathologist (Legumes), ICRISAT Center, Pantancheru P.O., Andhra Pradesh 502 324, India.

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In the leaf scorch phase, symptoms develop along the leaflet margins (3,8,13) or more commonly as a wedge-shaped lesion at the leaf tip (4,10,11). Wedge-shaped lesions have a bright yellow zone along the periphery of the advancing margin of the lesion (Fig. 1). Ascocarps are abundant in the necrotic tissue. The necrotic tissue becomes dark brown and frequently fragments along the leaflet margins, giving the leaflet a tattered appearance (4,11). Based on field observations of numerous genotypes, Porter et al (10) indicated that pepper spot and leaf scorch were two different diseases caused by the same pathogen; however, there is no published information on symptom development on peanut inoculated with *L. crassiasca* from leaflets with the different symptom types.

This paper reports the results of pathogenicity tests and symptom development on peanut plants inoculated with 11 isolates of *L. crassiasca* obtained from various sources. In addition, the influence of temperature on colony diameter, mycelial dry weight, and ascocarp production was studied to assess the variability among isolates.

MATERIALS AND METHODS

Fungal isolates. The state of origin, host, and symptoms associated with isolates of *L. crassiasca* used in this study are presented in Table 1. Each isolate was initially obtained by transferring a single ascospore to Difco potato-dextrose agar (PDA).

Inoculum. Inoculum was produced on PDA at 25 C in continuous light (123 $\mu E \cdot m^{-2} \cdot s^{-1}$). Ascospores were harvested by agitating 10-day-old cultures in sterile distilled water containing Tween 80 (0.1 ml/L). Ascospores trapped on the inner surface of the lid of the petri dish were collected into a beaker with a camel's-hair brush. The ascospore suspension was adjusted to a concentration of approximately 50,000 spores per milliliter with a hemacytometer.

Host. Peanut cv. Tamnut 74 plants were maintained in plastic pots (10 cm diameter) containing sandy loam soil fumigated with methyl bromide. The soil was also treated with carbofuran. Three seeds were planted in each pot, and the seedlings were later thinned to one per pot. Plants were fertilized at 10-day intervals with a commercial fertilizer (Rapidgro Corp., Dansville, NY).

Inoculum was applied with a hand-powered plastic atomizer at 40 days after planting. Leaves were sprayed until runoff. Three replicates were used for each isolate in each experiment, and three separate experiments were conducted. The inoculation method was similar in all three experiments, but plants were exposed to different postinoculation environmental conditions in each experiment.

In the first experiment, inoculated plants were placed in clear plastic bags and incubated at 20 C in a growth chamber (Model E-544, Percival Mfg. Co., Boone, IA) with a 12-hr (0600–1800 hours) light (123 $\mu E \cdot m^{-2} \cdot s^{-1}$) period. Plastic bags were removed from plants 2 days after inoculation. Twenty days after inoculation, the plants were transferred to a polyethylene chamber in the glasshouse (20–25 C). Plants were misted with distilled water for a 14-hr period (1800–0800 hours) with a humidifier (Model 500, Herrmidifier Co., Lancaster, PA) until 30 days after inoculation.

In the second experiment, the postinoculation treatment was similar to that in the first except that the plants were kept in plastic bags for 10 days after inoculation.

In the third experiment, plants of cv. Tamnut 74 were inoculated with all isolates, and plants of cv. Florunner were also included and inoculated with isolate LC8. During the postinoculation period, plants were maintained in a polyethylene chamber in the glasshouse and misted with distilled water for a 24-hr postinoculation period initially and subsequently for 14-hr (1800–0800 hours) periods with a humidifier until 30 days after inoculation. Temperature in the glasshouse ranged from 20 to 25 C during the experiment.

Symptom characterization. Disease symptoms were recorded at 5-day intervals until 30 days after inoculation in all experiments. In the third experiment,

disease efficiency and lesion diameter were also assessed 30 days after inoculation.

To quantify disease efficiency, lesions were counted in a 1-cm^2 area on each leaflet of the quadrifoliolate leaf at the middle of the main stem. A droplet-counting aid with a 1-cm^2 window was placed in the leaflet to define the counting area. Disease efficiency was expressed as number of lesions per square centimeter of leaflet area. Additionally, the diameters of five randomly selected lesions on each leaflet of the quadrifoliolate leaf at the middle of the main stem were measured with a micrometer.

Effect of temperature on growth in vitro. Seven isolates (LC2, LC4, LC5, LC7, LC8, LC9, and LC11) were grown on PDA at 25 C in continuous light (123 $\mu E \cdot m^{-2} \cdot s^{-1}$). Disks (5 mm diameter) were removed from the margins of 5-day-old cultures and transferred to petri dishes containing PDA and to the 250-ml flasks containing potato-dextrose broth. Five plates of five flasks were maintained for each isolate. Plates were wrapped in aluminum foil and incubated at 5, 10, 15, 20, 25, 30, 35, and 40 C. Flasks were also placed in the same incubators but under continuous illumination (123 $\mu E \cdot m^{-2} \cdot s^{-1}$). Colony diameter on PDA was estimated after incubation for 10 days by averaging two measurements taken at right angles. Mycelial dry weight was determined after incubation for 15 days by filtering the fungus on Whatman No. 1 tarred filter paper and drying the mycelium for 40 hr in a 30–95 C oven. The experiment on effects of temperature on colony diameter of isolates on PDA was repeated once.

Effect of temperature on ascocarp production. Leaf disks (5 cm diameter) of Tamnut 74 were autoclaved and inoculated by immersion in an ascospore suspension (approximately 10,000 spores per milliliter) of isolate LC11. Five leaf disks were placed in each of five petri dishes containing 2% water agar. The petri dishes were then incubated at 5, 10, 15, 20, 25, 30, 35, and 40 C in continuous light (123 $\mu E \cdot m^{-2} \cdot s^{-1}$). Ascocarps were counted on a 4.9-mm^2 area of the leaf disk after incubation for 7 days.

Ascocarp production. The same method, but with only one incubation temperature (20 C), was used to quantify ascocarp production by all 11 isolates of *L. crassiasca*. The experiment was repeated.

RESULTS AND DISCUSSION

All isolates of *L. crassiasca* were pathogenic to peanut, including the isolates from dead leaves of peanut and *A. glabrata* Benth. The postinoculation conditions were important in disease onset and symptom expression. Disease development was poor in the first and second experiments when plants were placed in polyethylene bags for 2 or 10 days. Some necrotic lesions were present 10 days after inoculation, but the lesions remained small 20 days after inoculation. However, when plants were transferred to polyethylene chambers and exposed to 14 hr of leaf wetness each day, disease development accelerated. This was especially evident in plants inoculated with isolates LC6, LC7, LC8, LC9, LC10, LC11, and LC12, where lesions enlarged rapidly and coalesced, to give irregular necrotic areas. However, plants inoculated with isolates LC2, LC3, LC4, and LC5 had only discrete necrotic lesions. Thus, the duration of the leaf wetness period was an important factor in disease development. With 14 hr of leaf wetness per day, necrotic lesions appeared within 5 days after inoculation with all isolates.

There was considerable variability in symptoms induced among isolates, and two distinct types of symptoms were observed (Table 2). Isolate LC3 pro-

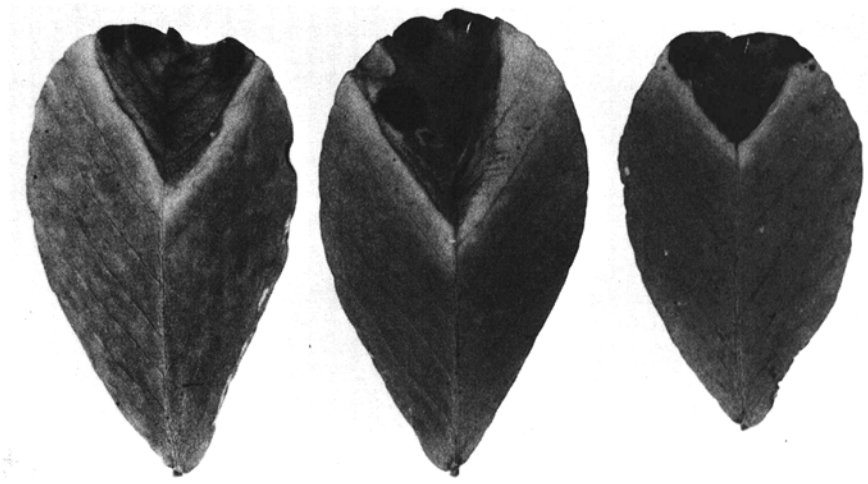

Fig. 1. Wedge-shaped scorch lesions (field symptoms) at the tip of peanut leaflets. Note the lesions of *Cercosporidium personatum* (dark circular lesions) associated with the scorch lesions.

Table 1. Sources of isolates of *Leptosphaerulina crassiasca*

Isolate	Source	Location
LC2	Dead leaves of *Arachis glabrata*	Texas
LC3	Dead leaves of *A. glabrata*	Texas
LC4	Peanut with leaf scorch symptoms	Georgia
LC5[z]	Peanut with leaf scorch symptoms	Florida
LC6[z]	Unknown	Georgia
LC7[z]	Dead peanut leaves	Georgia
LC8	Peanut with leaf scorch symptoms	Florida
LC9	Peanut with leaf scorch symptoms	Texas
LC10	Peanut with leaf scorch symptoms	Texas
LC11	Peanut with pepper spot symptoms	Texas
LC12	Peanut with pepper spot symptoms	Texas

[z] Isolates LC5, LC6, and LC7 were supplied by E. S. Luttrell, Department of Plant Pathology, University of Georgia, Athens, GA, under the accession numbers 9345, 8210, and 9389, respectively.

Table 2. Characteristics of lesions caused by 11 isolates of *Leptosphaerulina crassiasca* on peanut (cv. Tamnut 74)[y]

Isolate	Disease efficiency (lesions/cm^2)	Lesion diameter (mm)	Symptom type
LC2	6.0 c[z]	0.27 b	Pepper spot
LC3	79.2 a	0.13 b	Pepper spot
LC4	5.3 c	0.42 b	Pepper spot
LC5	4.5 c	0.42 b	Pepper spot
LC6	36.3 b	1.23 a	Leaf scorch
LC7	40.8 b	1.15 a	Leaf scorch
LC8	43.5 b	1.23 a	Leaf scorch
LC9	43.8 b	1.12 a	Leaf scorch
LC10	39.8 b	1.25 a	Leaf scorch
LC11	42.0 b	1.13 a	Leaf scorch
LC12	44.2 b	1.30 a	Leaf scorch

[y] Plants were misted with distilled water for 24 hr after inoculation and subsequently for 14 hr (1800–0800 hours) with a humidifier until 30 days after inoculation.

[z] Means followed by the same letter in a column do not differ significantly at $P = 0.05$ according to Duncan's multiple range test.

duced the highest number of lesions (79.2 lesions per square centimeter of leaf area). The lesions were small (0.13 mm diameter), black, and had no halos (Fig. 2). Lesions remained as minute spots until the end of the experiment and were prominent on the upper surface of leaflets. Lesions did not coalesce, and defoliation did not ensue. Isolates LC2, LC4, and LC5 produced small (0.27–0.42 mm in diameter) necrotic lesions but few lesions formed (4.5–6.0 lesions per square centimeter of leaf area). Brown lesions with chlorotic halos were distributed over the entire leaf surface. There was no coalescence of lesions, but leaflets became chlorotic in advanced stages of disease development and eventually abscised. Ascocarps were abundant on these fallen leaflets. These symptoms were previously described as pepper spots (5). The other isolates (LC6, LC7, LC8, LC9, LC10, LC11, and LC12) produced necrotic lesions similar to those produced by LC2, LC4, and LC5, but the lesions were large (1.13–1.30 mm in diameter) and the disease efficiency was higher (36.3–44.2 lesions per square centimeter of leaf area) (Table 2). Lesions enlarged rapidly, became pale brown with dark brown margins, and coalesced to produce irregular scorched areas over the entire leaflet surface (Fig. 3).

Ascocarps were abundant in the necrotic tissue. Isolates LC2, LC3, LC4, and LC5 were less virulent than the other isolates. Isolate LC8 produced similar symptoms on both Florunner and Tamnut 74.

The pepper spot symptoms obtained in inoculation trials in the glasshouse were similar to field symptoms. However, there was some uncertainty about the scorch symptoms. Luttrell and Boyle (5) observed scorch lesions over the entire leaflet. However, in subsequent publications by other workers (4,10,11), the occurrence of wedge-shaped lesions at the tips of the leaflets was reported as a prominent symptom of leaf scorch (Fig. 1). Porter et al (10) observed both pepper spot and leaf scorch symptoms in the field and concluded that they were two different diseases caused by the same pathogen. We are unaware of any published information where typical field symptoms of wedge-shaped scorch have been reproduced in inoculation tests in the glasshouse.

In this investigation, the isolates obtained from wedge-shaped scorch lesions (Fig. 1) produced scorch lesions over the entire leaflet (Fig. 3). None of the isolates produced only wedge-shaped scorch lesions on the leaf tips. In other studies (P. Subrahmanyam, *unpublished*), when plants of peanut (cv. Chalimbana) were inoculated with five isolates of *L. crassiasca* obtained from leaflets with pepper spots and 12 obtained from wedge-shaped scorch lesions in Malawi, all isolates were pathogenic. Under conditions unfavorable for disease development, only pepper spot symptoms appeared. However, under favorable conditions, scorch lesions developed over the entire leaflet. None of the isolates produced typical wedge-shaped scorch lesions (P. Subrahmanyam, *unpublished*).

Lesions caused by either *Cercospora arachidicola* S. Hori or *Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton are often present in association with wedge-shaped scorch lesions (11) (Fig. 1). Mercer (7) observed a close association between *C. arachidicola* lesions and the occurrence of leaf scorch in Malawi. In addition, he found *L. crassiasca* on tip-burn lesions caused by leafhoppers (*Empoasca facialis* Jacobi), thus providing circumstantial evidence that *L. crassiasca* is a secondary invader. We observed *L. crassiasca* in leaflets injured by pesticides under field conditions. *L. crassiasca* readily colonizes necrotic leaf tissue irrespective of the cause of the necrosis (P. Subrahmanyam and D. H. Smith, *unpublished*). Hence, we conclude that *L. crassiasca* is not the primary causal agent of wedge-shaped scorch lesions. *L. crassiasca* caused both pepper spots and leaf scorch; however, the type of symptoms depended on the isolates and environmental conditions. Less virulent isolates caused only pepper spots. More virulent isolates caused both pepper spots and leaf scorch, depending on environmental conditions.

Marked differences in colony characteristics, growth, and ascocarp production were noted among isolates of *L. crassiasca*. Radial growth on PDA was greatest (mean colony diameter range 4.5–6.5 cm) at 25 C for all isolates. No isolate grew at 40 C, and only LC4 grew (mean colony diameter 0.7 cm) at 5 C. The optimum temperature range for most of the isolates was 20–30 C. Growth of isolates in broth followed a similar trend. Isolate LC4 grew poorly on both media. Production of ascocarps as determined in LC11 was greatest at 20 C. Ascocarps were not produced on autoclaved peanut leaf disks at 5, 35, and 40 C; however, at 10, 15, 20, 25, and 30 C, seven, 85, 166, 101, and 83, respectively, were produced per 4.9 mm^2 of leaf tissue. Ascocarp production varied significantly among isolates (35–150 ascocarps per 4.9 mm^2 of leaf disk area). Isolate LC4 produced the lowest and LC11 the highest numbers of unit area. Yen et al (13) reported cardinal temperatures of 8, 28, and 35 C for growth of *L. crassiasca* on PDA.

In the present investigation, a temperature range of 20–30 C was optimum for all isolates. Although there were statistically significant differences in growth among isolates, the differences were not associated with apparent differences in isolate virulence. Isolates LC6, LC7, LC8, LC9, LC10, and LC11 (more virulent on peanut) produced more ascocarps than LC2, LC3, LC4, and LC5 (less virulent on peanut). These observations should not be construed as important in defining isolate virulence. It may be possible to study differences in isolate virulence by inoculating a set of genetically different hosts.

Fig. 2. Pepper spots caused by *Leptosphaerulina crassiasca* (isolate LC3) on leaflets of peanut (cv. Tamnut 74) inoculated in the glasshouse.

Fig. 3. Leaf scorch caused by *Leptosphaerulina crassiasca* (isolate LC8) on leaflets of peanut (cv. Tamnut 74) inoculated in the glasshouse.

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