Cultural Studies on *Dactuliochaeta glycines*, the Causal Agent of Red Leaf Blotch of Soybeans

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ABSTRACT

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Sclerotia of *Dactuliochaeta glycines* were isolated from leaf lesions of soybeans (*Glycine max*), a wild perennial relative (*Neonotonia wightii*) of soybeans, and soil. Recovery of sclerotia from soybean field soil ranged from three to 19 sclerotia per gram of dry soil. More than 90% of sclerotia germinated at 5 C after 18 mo, and more than 22% germinated when incubated at 100 C for 120 min. Pycnidiospore germination was optimal at 20 and 25 C. Pycnidiospores did not germinate after incubation for 12, 24, or 36 hr at 5 or 35 C. In culture, the fungus produced sclerotia on substrates with organic nitrogen sources, oat and wheat seeds, wood, and detached leaves of some legume species. Pycnidia and sclerotia were produced abundantly on media containing asparagine or casein hydrolysate, respectively. Pycnidia did not fully develop on culture media with greater than 1% sodium chloride. Inoculated leaf disks of 10 *Glycine* species and six other leguminous plants were infected, and on some species, typical lesions, pycnidia, and sclerotia developed.

Red leaf blotch of soybeans (*Glycine max* (L.) Merr.) occurs primarily in southern Africa (2,5) and is caused by *Dactuliochaeta glycines* (R. B. Stewart) Hartman & Sinclair (3). The fungus also infects a wild perennial legume, *Neonotonia wightii* (Arnott) Lackey (1,2,4,10). No other hosts have been reported.

D. glycines was first reported to be cultured in 1986 when it was shown to have a synanamorphic state, *Dactuliophora glycines* Leakey (1). In 1987, Levy (5) reported growing the fungus on eight media. He found that pH 4.5 was optimal for growth, sporulation increased with longer exposure to near-UV, and the fungus produced a vinaceous pigment. Hartman and Sinclair (3) compared nonsclerotial species of *Phoma* isolated from soybean leaves to sclerotia-producing isolates of *D. glycines* and found that they could be separated by growth rate and other morphological characteristics. A teleomorphic state associated with either the synanamorphic or anamorphic states has not been reported. The lack of information and the potential importance of *D. glycines*, especially if it spreads to other continents, makes it imperative to conduct further studies on its biology.

This study reports on the recovery of sclerotia from soil; the survival of sclerotia at low and high temperatures; the effect of light, pH, and temperature on growth; and the growth and production of pycnidia and sclerotia on a variety of substrates, including other potential hosts.

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MATERIALS AND METHODS

Source of cultures. Sclerotia were collected from leaves of infected soybean plants grown on a farm in Lusaka Province, Zambia, in March 1985 and coded Y1 and Y2 and from *N. wightii* leaves at Mt. Makulu, Zambia, and coded NW1–NW6. Sclerotia were sieved from soybean field soil from Mpongwe, Copperbelt Province, Zambia, in April 1985, and coded M1–M23. One isolate (ZW1) was obtained from Zimbabwe as a mycelial culture. Only some of the 32 isolates were used in the following experiments.

Isolation of sclerotia from soil. Soil samples were collected within 2 wk after soybean harvest with a 6-cm-diameter soil probe to a depth of 5–8 cm from two commercial fields (70 ha each) and from an experimental field trial at Mpongwe where triphenyltin acetate (0.6 kg a.i./ha) had been applied on a regular basis as a main plot and four cultivars (Oribi, Sable, Santa Rosa, and Tunia) as subplots. After maturity, five subsamples of soil collected from each 2 × 12 m plot were pooled by cultivar within each sprayed or nonsprayed treatment. In the two commercial fields, five subsamples of soil were pooled from each of four corners and center of the field. Soil was mixed and air-dried, and five 1-g samples were removed from the commercial field sample and three 1-g samples were removed from each experimental plot. Soil samples were wet-sieved separately through a 500-μm-mesh screen, followed by a 45-μm-mesh screen. Particles on the 45-μm-mesh screen were backwashed on filter paper inside of 9-cm-diameter culture dishes. Sclerotia were counted with a dissecting microscope (×100).

All of the following studies were conducted under quarantined conditions at the University of Illinois at Urbana-Champaign using 9-cm-diameter plastic culture dishes, unless stated otherwise. The photoperiod was 12-hr light/dark (fluorescent, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$) cycles with an incubation temperature of 25 C. The fungus was usually cultured on 2% Difco Bacto water agar (WA).

Survival of sclerotia. Sclerotia collected from soil samples were air-dried at ambient room temperature (24–28 C) for 4 mo and then stored in the dark at 5 C during the duration of the experiment. Three times at 6-mo intervals, 20 sclerotia were removed, surface-disinfested for 30 sec in 0.5% NaOCl, rinsed in sterile deionized distilled water, and plated on 2% WA. Germination of sclerotia was recorded at 1-day intervals for 10 days.

To increase the number of sclerotia, 7-day-old mycelial plugs (4 mm diameter) of isolate Y2 grown on WA were removed and placed in the middle of freshly prepared soybean leaf disks (16 mm diameter) that had been removed from unifoliate leaves of seedlings and placed on filter paper inside culture plates. Sclerotia were harvested from inoculated leaf disks after 5 wk and air-dried for 1–2 wk at room temperature. Five sclerotia were placed in 16-mm-diameter watch glasses and moved inside a drying oven at 100 C. Three watch

glasses (representing three replications) were removed at 30, 60, 90, and 120 min. This experiment was repeated with the addition of a 160-min treatment. Both trials had one treatment in which sclerotia were not exposed to 100 C (control). Sclerotia were plated immediately on WA and observed seven times in 10 days to determine if the sclerotia germinated by forming mycelial tufts, pycnidia, or secondary sclerotia on their surfaces. Colony diameter and number of pycnidia were recorded after 10 days.

Effect of temperature on pycnidiospore germination. Water agar was cut into 16-mm-diameter disks and moved to microscopic slides that were placed on glass rods inside of 9-cm culture plates with moistened filter paper. Disks were inoculated separately with a 10-μl drop containing approximately 1×10^3 pycnidiospores per milliliter of isolates M1, N2, Y2, and ZW1. Dishes were incubated in the dark from 5 to 35 C at 5-C intervals. Twenty-five pycnidiospores of each isolate in three replications were observed for germination at 12, 24, and 36 hr after inoculation. In a related experiment, pycnidiospores of isolate M1 were incubated for 6 hr at 20 C and examined hourly to record germination rate. Pycnidiospores were observed using a bright-field compound microscope (×125 or ×250) and counted as germinated if the germ tube was twice the length of the conidium.

Effect of light and media on colony growth and production of pycnidia. The effect of 12-hr light/dark cycles (fluorescent light, 50 $\mu E \cdot m^{-2} \cdot s^{-1}$) and no light on the colony growth rate and pycnidia production was compared on Difco Bacto and Noble water agars alone or a 50:50 mixture of each at 20 C. Media contained agar at 20 g/L, and after autoclaving, 20 ml was poured into each 9-cm culture dishes. Four-millimeter plugs from a 2-wk-old culture of isolate Y2 from WA were placed on the medium in the center of each dish. Colony diameter and pycnidial number were recorded 10 and 20 days after inoculation. There were three replications of each medium, and the experiment was repeated once.

Effect of pH on colony growth and production of pycnidia. Phosphate and citrate-phosphate buffers at 0.01 M were mixed with 20 g/L Difco Bacto agar. The pH levels of the citrate-phosphate buffer were 3, 4, 5, or 6; those of the phosphate buffer were 7, 8, or 9. All pH levels were tested with a Beckman pH meter. Two nonbuffered solutions were made with sterile, deionized, distilled water using either Difco Bacto or Noble agars and with tap water using Difco Bacto agar. The pH values of the nonbuffered solutions ranged from 6.0 to 6.7 after autoclaving. Sixteen-millimeter-diameter disks were cut from each test medium and moved to 9-cm culture dishes. A 1-mm-diameter plug was removed from the periphery of a 7-day-old colony of isolate Y2 grown on WA. The plug was placed in the center of each agar type. The number of pycnidia produced was recorded after 30 days. The experiment had three replications and was repeated once.

Growth on various substrates. The following substrates were tested: cellulose and methyl cellulose; filter paper (Whatman No. 3); oat (*Avena sativa* L.) and wheat (*Triticum vulgare* L.) seeds; 5- to 10-cm-diameter and 20-cm-long dormant twigs of Siberian elm (*Ulmus pumila* L.) and mulberry (*Morus rubra* L.); and soybean stems from field-grown plants (growth stage R7 and R8). Cellulose (10 g per 50 ml) and methyl cellulose (20 g per 40 ml) were mixed to make a slurry, autoclaved twice, and 20 ml of each was decanted into 9-cm culture dishes. Water agar disks (4 mm diameter) of 7-day-old colonies of isolates M1, N3, and Y2 were inoculated on the two celluloses, moistened filter paper, presoaked seeds, soybean stems, and wood that had previously been sterilized twice in 125-ml flasks (except filter paper, which was inside 9-cm culture dishes). There were three replications for each substrate and isolate, and observations were made several times over a 2-mo period.

Effect of carbon and nitrogen on growth and production of pycnidia and sclerotia. A basal synthetic medium of ammonium nitrate, P, K, Mg, micronutrients, vitamins (11), and 20 g/L Noble agar were mixed with one of the following carbon (C) sources: galactose, gluconic acid, glucose, methyl-D-glucosidase, soluble starch, sucrose, or xylose at 10 g of C per liter.

The basal medium (minus nitrogen [N] source), glucose (25 g/L), and 2% Noble agar were mixed with one of the following N sources: ammonium chloride, ammonium nitrate, casein hydrolysate, peptone, potassium nitrate, or urea at 0.49 g of N per liter.

The basal medium was adjusted to pH 5.5 using a 0.01 M citrate-phosphate buffer. The basal medium with or without the N sources was autoclaved, and the C sources and vitamins were filter-sterilized and added before pouring 20 ml into culture plates. The experimental controls consisted of 2% agar alone, and 2% agar with the basal medium with or without the C or N sources. For each test medium and controls, a 16-mm agar disk cut from solidified agar was moved to sterile culture plates, and the center of the agar disk was inoculated with a 1-mm-diameter plug of mycelium from either isolate Y2 or M23 that had been growing on WA for 7 days. Pycnidia and sclerotia were counted 30 days after inoculation. The experiment was conducted in three replicated blocks and repeated once. Data from the first experiment represent pycnidial production and data from the second experiment represent sclerotial production.

Effect of sodium chloride and sucrose on growth and production of pycnidia. Sodium chloride (NaCl) and sucrose at concentrations of 0, 1, 2, 3, 4, 5, and 10% were made with a 0.02 M citrate-phosphate buffer and 20 g/L Bacto agar. All solutions with or without NaCl were autoclaved. The sucrose solutions were filter-sterilized and added to autoclaved molten agar. Sixteen-millimeter-diameter agar disks of the different media were transferred to sterile culture dishes in a randomized complete block design with each type of test media and the control per plate in three replications. Ten microliters of an aqueous solution (1×10^2 pycnidiospores per milliliter) of isolate Y2 was pipetted onto the center of each test disk. After 24 hr, five germ tubes and the germination of 25 pycnidiospores on each agar disk were measured. After 10 days, the diameter of five individual colonies was measured for each replication. The number of pycnidia per agar disk was counted after 30 days.

Growth on leguminous seedlings and tree species. Seeds of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'California Blackeye'), lentil (*Lens esculenta* L.), lima bean (*Phaseolus lunatus* L.), common pea (*Pisum sativum* L.), pigeon pea (*Cajanus cajan* (L.) Huth), soybean, and winter vetch (*Vicia villosa* Roth) were planted in 10-cm-diameter pots containing a potting mix of soil. All plants were grown under alternating 12-hr fluorescent lights (75 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 25 ± 2 C. Leaf disks from 10-day-old seedlings were cut with a 16-mm cork borer and placed on autoclaved moistened filter paper in 14-cm-diameter glass culture plates. In one experiment, cowpea, lima bean, and soybean leaf disks were inoculated on the center with 7-day-old colonies of isolates M2, M23, Y2, and ZW1. There were three replications for each host and isolate. In another experiment, the other legume hosts were inoculated as described previously with isolate M23. The numbers of pycnidia and sclerotia produced were recorded weekly.

To determine the susceptibility of three tree species, leaves from newly expanded foliage were collected in July 1987 from honey locust (*Gleditsia triacanthos* L.), red bud (*Cercis canadensis* L.), and red oak (*Quercus rubra* L.). Leaflets from honey locust and leaf disks cut from red bud and red oak (16 mm in diameter) were surface-disinfested with 0.5% NaOCl and rinsed three times with sterile, deionized, distilled water. Leaf disks were inoculated with mycelial plugs of isolates M1, M8, Y2, or N3 or noninoculated (control) as previously described. The experiment was conducted twice with three replications each.

Growth on *Glycine* species and related genera. Newly developed leaves from greenhouse-grown plants of *G. argyrea*

Tind., *G. canescens* F. J. Herm., *G. clandestina* Wendl., *G. cyrtoloba* Tind., *G. falcata* Benth., *G. latrobeana* (Meissn.) Benth., *G. tabacina* (Labill.) Benth., *G. tomentella* Hayata, and *Pueraria lobata* var. *lobata* (Willd.) Ohwi were collected in March and September 1987. Leaves were surface-disinfested and rinsed as previously described. Leaflets were placed on moistened filter paper in 14-cm-diameter glass culture plates. Mycelial disks of isolates M23, Y2, and M6 were inoculated on leaflets as previously described. Plates were incubated for 2 wk at 25 C, and the percent leaf tissue covered and number of pycnidia formed were recorded. The second inoculation was conducted using leaf disks (16 mm diameter) with isolates Y2 and M23. The numbers of pycnidia and sclerotia were recorded after 4 wk.

Data analysis. Data were analyzed by analysis of variance ($P < 0.05$). Treatment means were compared by Fischer's least significant difference. Experiments that were repeated were combined if there was no significant experiment or experiment × treatment interaction. Regression analysis was used to determine the rate of conidial germination over time. In some cases, data were transformed to the $\log_{10}$ if error variances were not homogeneous.

RESULTS

Isolation of sclerotia from soil. There were significantly ($P < 0.05$) more sclerotia recovered from soil collected in nonsprayed plots than from soil collected in sprayed plots. The range of sclerotia recovered in nonsprayed plots was six to 19 per gram of air-dried soil (mean = 12), whereas in sprayed plots, the range was three to 12 (mean = six). Sclerotia in the two separate commercial production fields ranged from three to eight (mean = five) and two to five per gram of soil (mean = four).

Survival of sclerotia. More than 90% of the sclerotia germinated when stored for 18 mo at 5 C after 10 days. Sclerotial germination decreased significantly with increased exposure to 100 C (Table 1). Viability was <40% when exposed for 120 min. Regardless of the treatment, sclerotia that did germinate produced similar colony diameters and number of pycnidia after 10 days on WA. There appeared to be no relationship between duration of exposure time at 100 C to the type of sclerotial germination.

Effect of temperature on pycnidiospore germination. After 12, 24, and 36 hr, the percentage of pycnidiospore germination averaged over isolates was greatest at 20 C and then at 25 C (Fig. 1). No pycnidiospores germinated for any isolate at 5 or 35 C after 36 hr. Germination was not significantly different at 15, 20, or 25 C at 24 or 36 hr, but germination was significantly greater at 15–25 C than at 10 or 30 C. Germination was not observed at 10 C until after 36 hr. More than 90% of the pycnidiospores of isolate M1 had germinated at 20 and 25 C after 12 hr (Fig. 2). Germination at 20 and 25 C was significantly greater for each isolate than at 15 or 30 C (Fig. 2). Percentage of pycnidiospore germination (Y) of isolate M1 from 1–6 hr (X) was linear ($Y = -6.6 + 9.4X$, $R^2 = 0.85$).

Effect of light and media on colony growth and production of pycnidia. There was no significant difference between experiments, so data were combined. Colony diameter averaged 16 mm after 10 days and 23 mm after 20 days with no difference in diameter attributable to agar media. The colony diameter was significantly greater when grown under 12-hr light/dark cycles than when grown in the dark. The average number of pycnidia from both light and light/dark incubation was significantly greater on Difco Bacto agar (94 per plate) than on Noble agar (41 per plate) and Bacto/Noble agar (79 per plate) after 20 days.

Table 1. Mean percent germination of *Dactuliochaeta glycines* sclerotia on water agar after exposure to 100 C for various time intervals

Exposure time (min)	Trial 1 (%)	Trial 2 (%)
0	100[a]	100
30	67	89
60	89	67
90	78	78
120	39	22
160	—[b]	22
LSD[c]	27	39
C.V., %	19	34

[a] Means based on three replications of five sclerotia.
[b] Not tested.
[c] Least significant difference ($P < 0.05$).

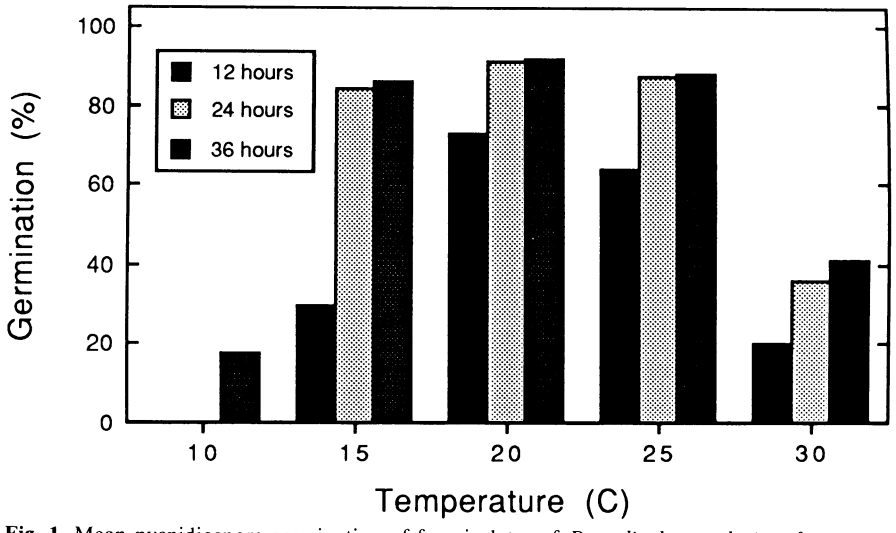

Fig. 1. Mean pycnidiospore germination of four isolates of *Dactuliochaeta glycines* from two experiments each with four replications incubated over five temperatures in the dark after 12, 24, and 36 hr.

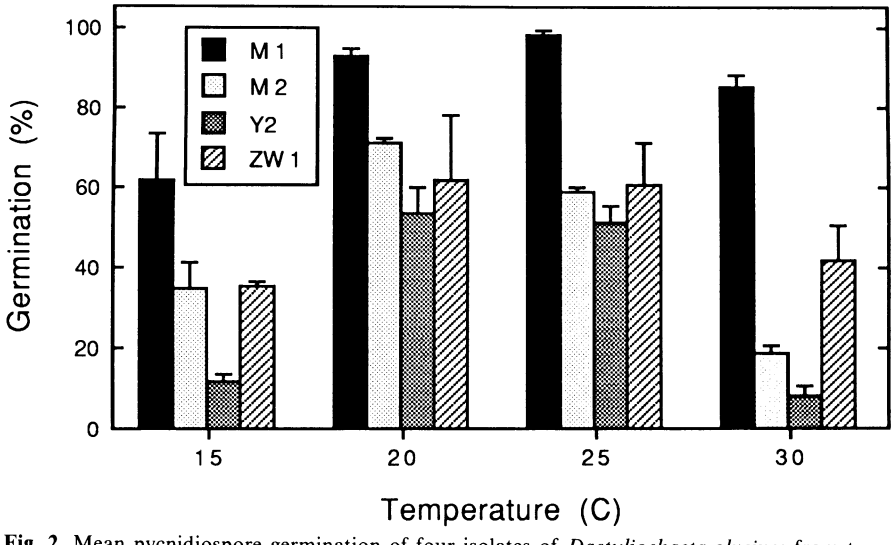

Fig. 2. Mean pycnidiospore germination of four isolates of *Dactuliochaeta glycines* from two experiments each with four replications incubated over four temperatures in the dark after 12 hr. Vertical bars represent the standard error of the mean.

Combined over media, 121 pycnidia were produced per culture dish under light/dark cycles, and 19 were produced per culture dish under continuous darkness after 20 days.

Effect of pH on colony growth and production of pycnidia. Pycnidial production was significantly greater at pH 6 and decreased gradually below pH 6 and abruptly above pH 7 (Table 2). Few pycnidia developed on Noble agar at pH > 7.

Table 2. Mean number of pycnidia produced by *Dactuliochaeta glycines* at various pH values and in nonbuffered media with Bacto water agar in tap or deionized distilled water and on Noble agar with deionized water under 12 hr light/dark photoperiod at 20 C 30 days after incubation[a]

Agar type and pH[b]	Number of pycnidia[c]
Buffered	
3	5.2
4	18.7
5	19.5
6	27.5
7	4.8
8	1.8
9	2.0
Not buffered	
Tap water	16.8
Deionized water	10.0
Noble agar	4.2
LSD[d]	8.7

[a] Based on two experiments with three replications each.
[b] pH 3–6 made with citrate-phosphate buffer; nonbuffered media pH ranged from 6.0 to 6.7; and pH 7–9 made with phosphate buffer.
[c] In a 9-cm culture dish with 20 ml of media.
[d] Least significant difference ($P < 0.05$).

Table 3. Number of pycnidia and sclerotia of *Dactuliochaeta glycines* produced on 16-mm agar disks of various carbon and nitrogen sources and controls under 12-hr light/dark photoperiod at 25 C 30 days after incubation

C or N source[a]	Pycnidia	Sclerotia
Galactose	0[b]	22
Gluconic acid	19	20
Glucose	0	0
Methyl-D-glucosidase	0	20
Soluble starch	0	25
Sucrose	0	0
Xylose	0	0
Ammonium chloride	0	0
Ammonium nitrate	0	0
Aspartic acid	38	13
Casein hydrolysate	54	40
Peptone	0	43
Potassium nitrate	99	0
Urea	118	15
Control 1 (with C and N)	0	0
Control 2 (with C, no N)	19	0
Control 3 (no C with N)	0	9
Control 4 (no C, no N)	19	0

[a] Carbon sources = 10 g/L; nitrogen sources = 0.49 g/L; all mixed with a basal synthetic medium (10).
[b] Means of three replications.

Growth on various substrates. Inoculated soybean stems (growth stage R8), oat and wheat seeds, and wood all supported growth and abundant production of pycnidia and sclerotia. Sclerotia often were in glomerate masses and frequently atypical in shape. Sclerotia were not produced on soybean stems (growth stage R7), cellulose, or methyl cellulose. Growth occurred on filter paper, but only a few pycnidia and no sclerotia formed. A teleomorphic state was not observed on any substrate.

Effect of carbon and nitrogen on growth and production of pycnidia and sclerotia. The greatest number of pycnidia was produced with urea as a N source, followed by potassium nitrate (Table 3). N sources of ammonium chloride, ammonium nitrate, or peptone had no pycnidia. On C sources, pycnidia formed on only gluconic acid and the controls without an N source. Sclerotia were produced when the fungus was grown on some C and N sources (Table 3). Sclerotia were nonpigmented on C sources and pigmented on N sources other than ammonium nitrate. Both ammonium compounds restricted growth of mycelia and sclerotial production. Sclerotia were produced more on organic sources of N than on inorganic sources. Colony characteristics such as color, density of mycelia, and morphological characteristics varied with the C and N source. Pycnidia that formed when urea was used had long setae, those on potassium nitrate had very long pycnidial necks, and those on gluconic acid were greatly reduced in size. Different C and N sources frequently caused variation in pycnidial and sclerotial shapes and proximity or clustering to each other. Data were not analyzed because many of the treatments had no pycnidia or sclerotia.

Effect of sodium chloride and sucrose on growth and production of pycnidia. Germination of pycnidiospores or lengths of germ tubes did not differ significantly between any sucrose concentration and the control. Germ tube lengths after 24 hr ranged from 4–9 μm without sucrose to 5–12 μm on medium containing 1% sucrose. At all sucrose concentrations, the colony covered the agar disks after 10 days. The mean number of pycnidia produced after 30 days was 19 and did not significantly differ by concentration. Pycnidia produced on agar with sucrose tended to be smaller than pycnidia produced on agar without sucrose.

After 24 hr, only pycnidiospores on agar amended with 0, 1, and 2% NaCl germinated. On 1% NaCl agar, germination was 74%, which was not different from the control (78%) but was significantly greater than the 32% germination on 2% NaCl agar. Colony diameter was greatest at 1% NaCl and decreased with increased concentrations of NaCl (Fig. 3). Pycnidia did not form on any concentration after 30 days, although immature pycnidia and pycnidia initials were observed on agar with 1 and 2% NaCl.

Growth on leguminous seedlings and tree species. Among the four isolates, significantly ($P < 0.05$) more pycnidia developed on soybean leaf disks than on cowpea and lima bean leaf disks, which did not differ from each other (Fig. 4A). There was a significant isolate × host interaction in the number of sclerotia produced. Isolate M23 produced significantly more sclerotia than did the

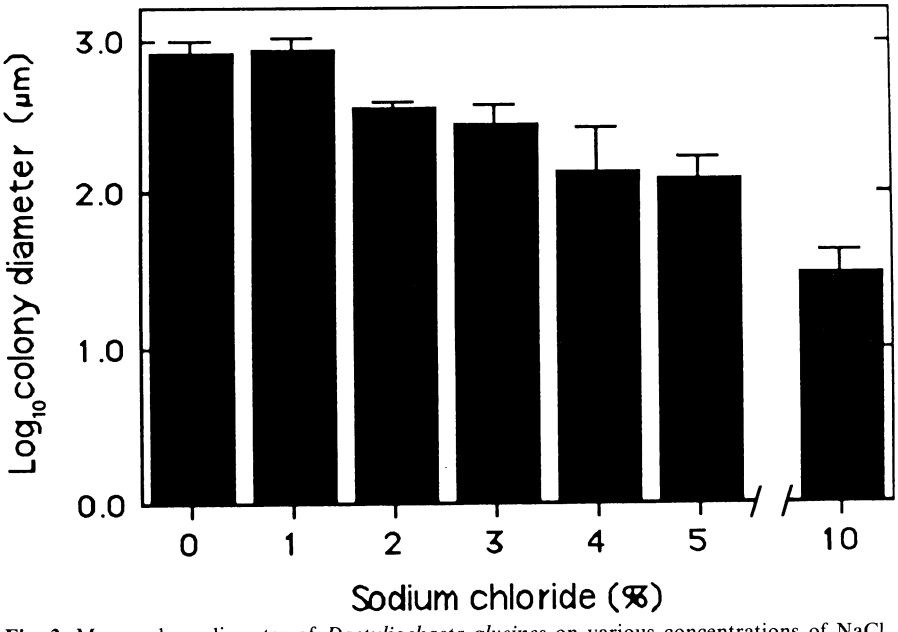

Fig. 3. Mean colony diameter of *Dactuliochaeta glycines* on various concentrations of NaCl amended in Bacto agar from five samples of three replications each at 20 C 10 days after incubation. Vertical bars represent the standard error of the mean.

other isolates (Fig. 4B). Significantly ($P < 0.05$) more sclerotia were produced among the four isolates on soybean and lima bean than on cowpea leaf disks. On soybeans, isolate M23 produced significantly ($P < 0.05$) more sclerotia than did the other three isolates but had the lowest pycnidial production.

The average number of pycnidia and sclerotia produced was 6.6 and 5.8 on lentil, 6.7 and 2.7 on pea, 12.1 and 7.1 on pigeonpea, and 10.1 and 3 on winter vetch, respectively. Symptoms were not observed on the three tree species, although mycelium occasionally grew over the leaf tissue and pycnidia formed on the cut edge of the leaf disk.

Growth on *Glycine* species and related genera. All *Glycine* species tested had symptoms with pycnidia forming on the leaf surface. The greatest number of pycnidia and sclerotia was produced on *G. clandestina*; the least was produced on *P. l. lobata* (Table 4). Significant differences probably were not detected among the *Glycine* spp. because of the variation between the replications. In the repeated experiment, a similar trend occurred and only ranges of sclerotial production are presented (Table 4).

DISCUSSION

Sclerotia of *D. glycines* were recovered from soil, which would be expected since they have been reported to occur abundantly on infected soybean leaves (2). Under natural conditions in Southern Africa, sclerotia survive in an environment that often has up to seven consecutive dry months. In our studies, sclerotia survived up to 18 mo at 5 C and more than 22% germinated when stored at 100 C for 160 min. The survival time of sclerotia in nature has not been determined, but at least under experimental conditions they are able to survive for extended periods.

Under certain conditions, sclerotia of some fungi are known to germinate in various ways. Sclerotia of *D. glycines* were reported to germinate by forming mycelium and by forming pycnidia or by forming sclerotia on their surfaces (1–3). Without nutrients, sclerotia of *Sclerotium rolfsii* Sacc. germinated indirectly in an eruptive manner on Noble agar after short periods of drying or exposure to other treatments (7,8). Wymore and Lorbeer (11) found that cold treatment was more effective than air-drying at room temperature in stimulating eruptive germination over noneruptive mycelial germination of sclerotia of *Sclerotinia minor* Jagger. Heat or cold storage did not affect the type of sclerotial germination in our study. However, studies dealing with other isolates and treatments may have detected consistencies in germination that have been reported with other sclerotia-producing fungi. An indepth understanding

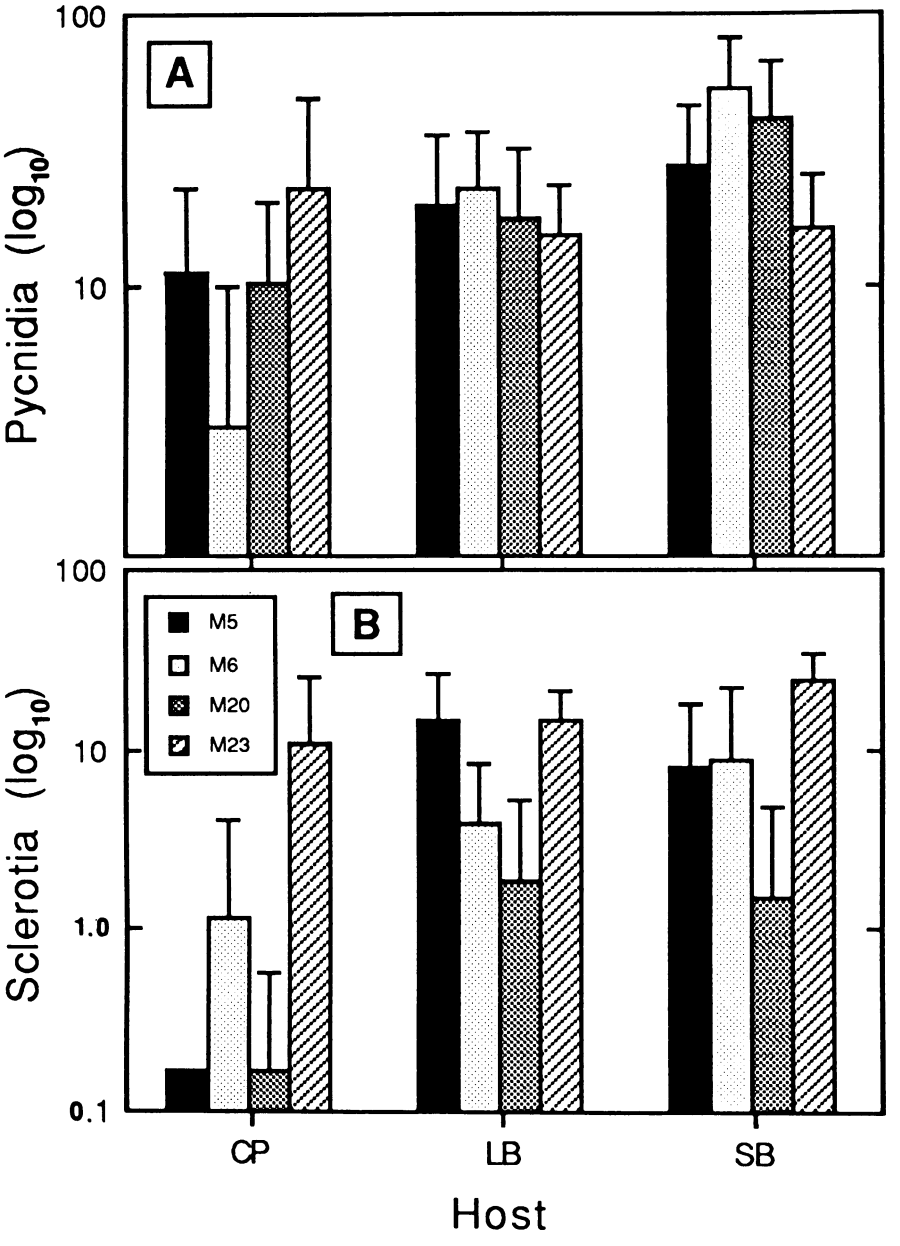

Fig. 4. Mean number of **(A)** pycnidia and **(B)** sclerotia produced by four isolates of *Dactuliochaeta glycines* on leaf disks of cowpea, lima bean, and soybean 30 days after inoculation under 12-hr light/dark cycle with three replications at 20 C. Vertical bars represent the standard error of the mean.

Table 4. Percent leaf area infected and number of pycnidia produced after 2 wk and sclerotia produced after 4 wk on either leaf disks or leaflets of legume species inoculated with *Dactuliochaeta glycines* under 16-hr day lengths using fluorescent lights at 25 ± 2 C[a]

Glycine and legume species	Leaf area infected (%)	Pycnidia[b]	Sclerotia[c]
G. argyrea	62	33	...
G. canaescens	14	3	2–9
G. clandestina	98	67	11–51
G. cyrtolaba	31	14	2–15
G. falcata	38	15	...
G. latrobeana	29	4	...
G. soja	67	16	...
G. tabacina	33	10	4–21
G. tomentella	47	28	...
Neonotonia wightii	12	1	...
Pueraria lobata var. *lobata*	2	1	...

[a] Data based on three replications of three leaf disks or leaflets each.
[b] Data recorded from experiment one.
[c] Data recorded from experiment two.

about how these sclerotia germinate may help to determine their behavior under field conditions. For example, the observation of multiple pycnidia formed on sclerotial surfaces would provide more inoculum than if sclerotia formed only mycelium.

In our studies, sclerotia did not readily form on common nutrient or WA medium, but pycnidia did. Some isolates produced sclerotia within 2–4 wk on some types of C and N sources. In other fungi, such as *Sclerotinia sclerotiorum* (Lib.) de Bary, sclerotia were produced more abundantly on ammonium phosphate than on other N sources (9). Punja (6) showed that sclerotia formed most abundantly when cultures of *S. rolfsii* were subjected to an N step-down and incubated under light. In our study, more sclerotia formed on the organic sources of N, and few mostly nonpigmented sclerotia were produced under different C sources. It is likely that the C/N ratio determines whether an isolate will more readily produce pycnidia or sclerotia. Sclerotia were not produced on green autoclaved soybean stems but were abundant on other substrates, including matured soybean stems and oat and wheat seed. In nature, sclerotia have been reported only on leaves of *N. wightii* and soybeans, although it seems likely that sclerotia could be found on other substrates considering the extensive tree legumes that occur within the geographic range of the fungus. Sclerotia produced on inoculated leaf disks in our study were similar in shape and size to those produced on lesions of field-grown soybeans or those isolated from infested soil, although on other substrates, sclerotial morphology was not always uniform. Under natural conditions on other potential hosts, the morphology of these structures could be more variable than previously reported.

The fungus grew on most hosts based on detached leaf inoculations. All grain legumes tested were susceptible. Some of the wild perennial soybeans seemed less susceptible than *G. max*. If high levels of resistance are not found in *G. max*, other *Glycine* species may show promise as resistant sources. It is important to have these and other legumes tested in the field to verify the findings on detached leaves.

The effect of substrates and the use of C and N sources had a physiological effect on *D. glycines*. More information is needed to determine what effect various light intensities, relative humidities, temperatures, and other environmental factors have on the growth and survival of pycnidiospores, pycnidia, and sclerotia. Further cultural studies would help to understand more about the basic biology of the pathogen and to determine how these environmental factors interact with the pathogen and host under field conditions.

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