Occurrence, Distribution, and Pathogenicity of the Cowpea Root and Stem Rot Pathogen, *Phytophthora vignae*, in Soils of Sri Lanka

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ABSTRACT

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A *Phytophthora* sp. isolated from stem and root lesions of cowpeas (*Vigna unguiculata* subsp. *unguiculata* 'California Blackeye') grown in a mixture of riverbank and forest soils in containers under greenhouse conditions was identified as *P. vignae* based on morphological characters and pathogenicity tests. *P. vignae* was isolated from soils in three of five cowpea-growing districts of Sri Lanka by baiting with plants of the susceptible cowpea cultivar California Blackeye or by floating disks of tea leaves over soil. However, the root and stem disease was apparent at only one site, suggesting that some field soils might be suppressive. Among the 25 field soils tested, the disease potential index varied from 0 in many fields to 16 in one field. Among the cowpea cultivars grown in Sri Lanka, MI-35, ETA, Muttessa, and Sudumung were most resistant. The U.S. cultivars Mississippi Purple, Purple Hulk, and Hercules were resistant to most isolates but susceptible to three—P006, P007, and P009. The morphological characters and pathogenicity of these three isolates on the U.S. cultivars differed from those of the other isolates, suggesting they were of a different race(s). No other legume tested—*Vigna radiata* (green gram), *V. mungo* (black gram), *Phaseolus vulgaris* (cvs. Top Crop, French Bean, and Kentucky Wonder), *Glycine max* (soybean), *Cajanus cajan* (pigeon pea), *Mucuna pruriens* (velvetbean), and Lanka kadala—was susceptible to the cowpea pathogen.

Additional keywords: disease resistance, suppressive soils

A stem and root rot of cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) was first reported by Purss (17) in Queensland, Australia. In 1957, Purss (18) showed the causal agent to be a *Phytophthora* sp., which he subsequently named *P. vignae* Purss. Nirwan and Upadhyaya (14) and Kitazawa et al (10) reported this pathogen on cowpea and adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) grown in India and Japan, respectively. Disease caused by *P. vignae* in these three countries was distinct from a root rot of cowpeas caused by *Pythium myriotylum* Drechs. (2).

P. vignae has been reported only from Japan, India, Australia, South Korea, and Taiwan (5,8,10,14,17,18). It has always been host-specific, with one exception. Kitazawa et al (10) and Han et al (5) showed that adzuki (red) bean was susceptible to *P. vignae*. Unlike *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan and D.C. Erwin, which causes a similar disease in soybean (*Glycine max* (L.) Merr.) and is distributed worldwide (23), *P. vignae* is sparsely distributed. In 1991, *P. vignae* was reported for the first time in Sri Lanka by Sivakadadcham and Fernando (24) on cowpea, which is grown on approximately 400,000 ha. The disease was detected on greenhouse-grown virus-indicator plants that exhibited wilting, basal stem rot, and root rot.

The report on the existence of *P. vignae* in Sri Lanka (24) was from soils collected from the banks of the Mahaweli River and from a forest area about 1 mile from the C.A.R.I. research station in Gannoruwa. The pathogen could have moved downstream from cowpea-growing areas further up the river, or it may have been harbored by another host, possibly a wild legume in the forest. The purposes of this study were to: 1) describe the symptoms observed in the first recording of *P. vignae* in Sri Lanka and to characterize the pathogen, 2) evaluate the distribution of the pathogen in field soils, 3) determine any additional hosts, and 4) determine the pathogen's disease potential and virulence to cowpea cultivars grown in Sri Lanka.

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MATERIALS AND METHODS

Isolation and identification of the pathogen. Cowpea cv. California Blackeye seed was planted in 20 10-cm-diameter pots containing a reddish brown loam soil (pH about 6.5) in which the cowpea disease had first been observed. When symptoms appeared after 2–4 wk, stem tissue pieces 1–2 cm long were cut from the advancing edge of lesions, washed, and surface-disinfested for 30 sec in 0.1% mercuric chloride, followed by three washings in sterile distilled water. Stem pieces were cut into thin disks with a sterile scalpel in sterile distilled water. Disks were blotted dry on sterile paper towels and transferred to plates containing potato-dextrose agar (PDA) or cornmeal agar (CMA) and incubated for 7 days in the dark at room temperature (23 ± 3 C).

Mycelium growing out of the stem tissue was transferred to PDA, CMA, and cowpea agar (CPA) (crude extract of 200 g of cowpea cv. California Blackeye stems and roots plus 20 g of agar in 1,000 ml of distilled water). Plates were incubated at room temperature in alternating light and dark for 10 days and observed for mycelial growth and any sexual or asexual reproductive structures. Plugs (5 mm) of the fungus growing on PDA were transferred to Petri's mineral solution (7) in petri plates or a nonsterile garden soil extract (50 g of soil in 1,000 ml of water incubated with occasional swirling for 24 hr at room temperature and then filtered through four layers of cheesecloth and filter paper). During the first 10 days of growth, hyphae from plugs were examined for presence of sporangia.

Pathogenicity tests. Two experiments were conducted to test the pathogenicity of the isolated fungus on cowpea cv. California Blackeye. The soil used was a mixture of two parts loam soil, one part sand, and one part coir dust (pulverized coconut husk). The soil mixture was autoclaved at 120 C and 15 psi pressure for 20 min before use in 22-cm-diameter clay pots.

In the first experiment, two 1-cm-diameter plugs of the fungus grown on PDA for 7 days were placed 1 cm below the seed in the soil mix. The control consisted of noninoculated PDA plugs added to the soil mix. Five seeds were sown in each pot. There were five replicates per treatment, and the experiment was repeated once.

In the second experiment, the fungus was grown for 1 mo at room temperature in the dark on a medium containing equal volumes of loam soil and washed sand amended with cornmeal (3%, w/w). This fungal inoculum was added (3%, w/w) to a soil mixed thoroughly by hand and placed in clay pots. Five seeds were sown in each pot. The control pots received 3% (w/w) noninoculated, autoclaved cornmeal:soil:sand mixture added to autoclaved soil. There were five replicates per treatment, and the experiment was repeated once.

Plants in both experiments were fertilized twice with the recommended 5-10-10 N-P-K mixture, watered daily, and allowed to drain freely, in a greenhouse with 8 hr of daylight.

Field sites and sampling method. Five of the 10 main cowpea growing districts in Sri Lanka were selected for sampling: Kurunegala, Anuradhapura, System C, Kandy, and Matale. On the basis of annual rainfall, Anuradhapura and System C are in the dry zone, Kandy and Matale are in the wet zone, and Kurunegala is in the intermediate zone. Five sampling sites were selected randomly from each district. Cowpea was growing at each site at the time of sampling. However, the cultivars (MI-35, Arlington, Bombay) and age of plants (3–8 wk) differed.

Soil cores 15–20 cm deep were collected within rows and near plant roots at about 20-m intervals and randomly along a W-shaped path covering the entire area of each field sampled (11). One hundred soil samples were taken within a sampling site, bulked and mixed in large buckets, and then placed in polyethylene bags for transportation to the research station.

Isolation and identification of *P. vignae* from soils. Techniques for isolation of several *Phytophthora* spp. from soil have been described by Tsao (30) and Ribeiro (21), but neither report mentions isolation of *P. vignae* from soil. Isolation of *P. vignae* from cowpea-growing soils was attempted by three methods.

Plant trap method. This involved use of plants of a susceptible cowpea cultivar to trap the pathogen from soil. Soils were transferred to 12-cm-diameter pots within 24 hr after collection. Four seeds of California Blackeye cowpea were planted in each pot. There were five replicate pots for each sampled field. The control for each field was autoclaved soil. The experimental pots were placed in the greenhouse (24–28 C) under natural daylight (10 hr) conditions. The plants were watered daily and kept for 1 mo or until disease symptoms developed. If disease occurred, small pieces of stem from the advancing margin of lesions were cut and immersed in 0.1% mercuric chloride for 30 sec, washed three times in sterile distilled water, and blotted dry before being placed on CMA containing 20 μg/ml of pimaricin and 200 μg/ml of vancomycin. Plates were incubated in the dark at room temperature for 4 days. Mycelia growing out of stem tissue were transferred to CMA and CPA. Plates were incubated for 1 wk under the same conditions before examination and identification.

In vitro baiting by the double-cup method. The double-cup leaf disk trap method (13) was used for all samples from cowpea fields. After California Blackeye cowpeas were grown in soils for a month or less, small representative soil samples were collected from the five replicate pots, mixed, and used in the leaf disk trap assay. Because the bait leaves frequently became infected with *Pythium* spp., Hamm and Hansen's (4) modification of the method was used with further slight modifications. Hymexazol, which suppresses most *Pythium* spp., was added at a concentration of 35 μg/ml to distilled water, which was then added to soil at 6:1 (v/v, water:soil) (16). Disks 8 mm in diameter were cut from tea leaves and floated on the water. Ten disks were placed in each of five replicate cups. The control assay was with autoclaved soil. The leaf disks were transferred aseptically into petri plates containing a selective medium after 48 hr of incubation at room temperature under light/dark conditions. The selective medium was CMA with 10 μg/ml of pimaricin, 200 μg/ml of vancomycin, 100 μg/ml of PCNB, and 50 μg/ml of hymexazol (31). Five disks from each replicate were cut in half, and five halves were transferred to one plate. The colonies growing out of each disk were aseptically transferred to petri plates with CMA so that colony morphology and reproductive structures could be observed and *P. vignae* identified. The other halves of each of five disks were immersed in Petri's solution to induce sporangial production. After 24–48 hr, they were transferred to the selective medium and allowed to grow out.

Isolation by use of selective media. The basal component in all three selective media used was CMA. Selective medium 1 consisted of 5 μg/ml of pimaricin, 200 μg/ml of vancomycin, 100 μg/ml of penicillin, 100 μg/ml of PCNB, 2.5 μg/ml of benomyl, and 20 μg/ml of hymexazol (15). Selective medium 2 contained 10 μg/ml of pimaricin, 200 μg/ml of vancomycin, 100 μg/ml of PCNB, and 50 μg/ml of hymexazol (31). Selective medium 3 consisted of 20 μg/ml of pimaricin, 200 μg/ml of vancomycin, and 200 μg/ml of penicillin (6). The antimicrobial agents were added to CMA after autoclaving at 120 C and 15 psi for 20 min and cooling to 45 C just before pouring plates. Soil from cowpea fields was sprinkled onto three replicate plates of each medium.

Pathogen identification. The CMA and CPA plates containing the mycelial plugs transferred from any of the three isolation methods were observed under a phase contrast microscope for sporangia and/or oogonia and antheridia. If the reproductive structures of a culture were characteristic of a *Phytophthora* sp., it was further compared with the original *P. vignae* isolate from Sri Lanka (IMI316196). These cultures were grown on PDA, and eight 8-mm-diameter plugs were transferred to 500 ml of autoclaved cornmeal:soil:sand (1:1:1) (CMSS) medium in 1,000-ml Pyrex conical flasks and allowed to incubate at room temperature (approximately 26 C in the dark) for 1 mo. The *Phytophthora* inoculum was mixed in pasteurized soil at 1% (v/v), and seeds of cowpea cv. California Blackeye were planted. The control had 1% (v/v) of sterilized CMSS medium in the soil. Induction of disease symptoms on the plant confirmed that the test fungus was pathogenic, and it was further confirmed to be *P. vignae* by Koch's postulates. The cultured isolates were maintained in tube slants on PDA, CMA, and CMSS at 20 C. All isolates were recultured every month.

Quantification of *P. vignae* propagules in soil. The serial dilution end-point method (29) was modified for quantifying *P. vignae* propagule numbers, using the susceptible cowpea cv. California Blackeye as the trap plant. This method gave an estimate of the disease potential index (DPI), which reflects the inoculum potential of the pathogen in soil. Cowpea field soil was diluted twofold with sterile soil (1/2, 1/4, . . . 1/64), and seeds of California Blackeye cowpea were planted in soils of each dilution. Five replicate 12-cm-diameter pots, with four plants per pot per dilution, were used. The DPI of a given soil was calculated as the reciprocal of the highest of the dilutions that gave typical *Phytophthora* symptoms of stem lesions and wilting on susceptible cowpea cv. California Blackeye.

Additional hosts. While cowpea fields in all five districts were being sampled, weeds and legumes growing close to the fields were surveyed for stem lesions and blight symptoms. Any showing such symptoms were collected in moistened bags, and isolations were attempted as described previously. Weeds or legumes growing near soil where the pathogen had been collected for greenhouse use were also examined.

Cultivar resistance. Eight cowpea cultivars grown in Sri Lanka, five cultivars grown in the United States, and four breeder lines were compared with susceptible cv. California Blackeye. The cultivars tested in Sri Lanka were Arlington, MI-35, ETA, Bombay, Sudumung, Hawari-Mae, Polon-Mae, and Muttessa. The cultivars tested in the United States were Mississippi Purple, Mississippi Silver, Hercules, Purple Hulk, and

Cowpea Banquet, plus California Blackeye. The breeder lines tested were IT-84E-1-108, IT-83-S-818, IT-84E-124, and IT-82D-513-1.

P. vignae P001 inoculum grown in CMSS medium was used at 1% (v/v) of the soil mixture of sand and loam, pasteurized at 60 C for 30 min. Five seeds were planted in each of five replicate pots in tests in Sri Lanka. The six cultivars tested in the United States were inoculated with all isolates collected from Sri Lanka with the exception of P020 and P017, which died in transit. The inoculum was prepared in 250-ml conical flasks containing 125 ml of coarse vermiculite moistened with 75 ml of dilute V8 juice (200 ml of V8 juice concentrate, 800 ml of distilled water, and 2 g of $CaCO_3$) autoclaved twice over a period of 24 hr for 1 hr each. The inoculum was grown for 1 mo, observed under the microscope, and then added to the pasteurized soil at 1% (v/v) in 10-cm-diameter pots. Control pots contained autoclaved vermiculite moistened with V8 juice added to pasteurized soil. Four seeds were planted in each of five replicate pots. Occurrence of disease symptoms was observed over the next 2 mo.

Pathogenicity on other legumes. Nine leguminous crops grown in Sri Lanka were tested and compared with susceptible cowpea cv. California Blackeye. These were soybean, black gram (*V. mungo* (L.) Hepper), green gram (*V. radiata* (L.) R. Wilcz.), pigeon pea (*Cajanus cajan* (L.) Huth), Lanka kadala, velvetbean (*Mucuna pruriens* (L.) DC.), and *Phaseolus vulgaris* L. 'French Bean', 'Top Crop', and 'Kentucky Wonder'. Five replicate 12-cm-diameter pots of each legume with five seeds in each pot were planted in infested soil (same inoculation procedures as in the cultivar resistance trial). Disease was evaluated after 2 mo.

Data analysis. All experiments were arranged in a completely randomized design with five replicates. All greenhouse experiments were repeated at least once. Percentage data of the cowpea cultivar resistance experiments were arcsine-transformed (28) and analyzed with an analysis of variance and mean separation test suitable to the experimental design ($P < 0.05$). The variance of repeated trials was compared with a Bartlett's test of homogeneity of variance. There were no differences in variance between trials (36). The pathogenicity of different isolates of *P. vignae* on different cowpea cultivars was tested by average linkage cluster analysis (25). Results reported are from the first experiment. Data were analyzed using an SAS program (22).

RESULTS

Disease symptoms and pathogen identification. When the disease was first discovered in experimental greenhouses in Sri Lanka, symptoms of cowpea stem and root rot consisted of wilting when leaves were still green and shrinking of the basal stem at or near the soil surface or a few centimeters above it. The first or second internodal stem was generally affected. Stem lesions initially occurred on one side of the stem and were pale or gray-green but not water-soaked. Brownish streaks appeared subsequently on the affected part of the stem. A stem girdling at this region (Fig. 1A) resulted in permanent wilting and death of the plant. Roots of plants showing stem rot were poorly developed and necrotic.

A slow-growing fungus with aseptate hyphae was recovered from surface-sterilized stem lesions placed on PDA and CMA. Mycelia subcultured on PDA and CMA for 7 days produced no reproductive structures. Subcultures on CPA, however, produced abundant oogonia and antheridia (Fig. 1B). The mycelia produced on CPA were sparse, so reproductive structures were easily observed microscopically (400×). Sporangia were produced within 24–48 hr from mycelium on cowpea roots transferred to either Petri's mineral solution or soil extract (Fig. 1C). Sporangia developed on simple nonbranched sporangiophores and on simple monochasial sporangiophores. Sporangia were mostly ovoid to obpyriform and nonpapillate or inconspicuously papillate and germinated on cowpea broth by release of zoospores or directly by germ tubes through the exit pore plug. These features matched the description of *P. vignae* (36).

Oospores were observed in host tissue (400×) and were abundant in cultures on CPA. Oogonia were spherical and at first hyaline, becoming light brown with age. Antheridia were all amphigynous. Oospores were aplerotic. The fungus had all the morphological features of *P. vignae* as described by Purss (18). The identification as *P. vignae* was confirmed by the Commonwealth Mycological Institute, London, and the culture was deposited in the IMI culture collection under the accession number IMI316196.

Pathogenicity tests. In the first pathogenicity tests using agar plugs of inoculum, only about 10% of the plants showed typical root and stem rot symptoms; all control plants were healthy. In the second experiment, in which cornmeal:soil:sand inoculum was used, 100% of the plants became infected and stem girdling and leaf wilting symptoms appeared within 10–15 days after planting; plants in the control pots remained healthy. The causal agent of the stem lesions was reisolated and identified as *P. vignae*, fulfilling Koch's postulates.

Isolation from soil and DPI. Of the three methods used for isolating *P. vignae* from cowpea field soil, the plant trap and leaf disk bait methods were successful. Neither *P. vignae* nor any other *Phytophthora* spp. were isolated

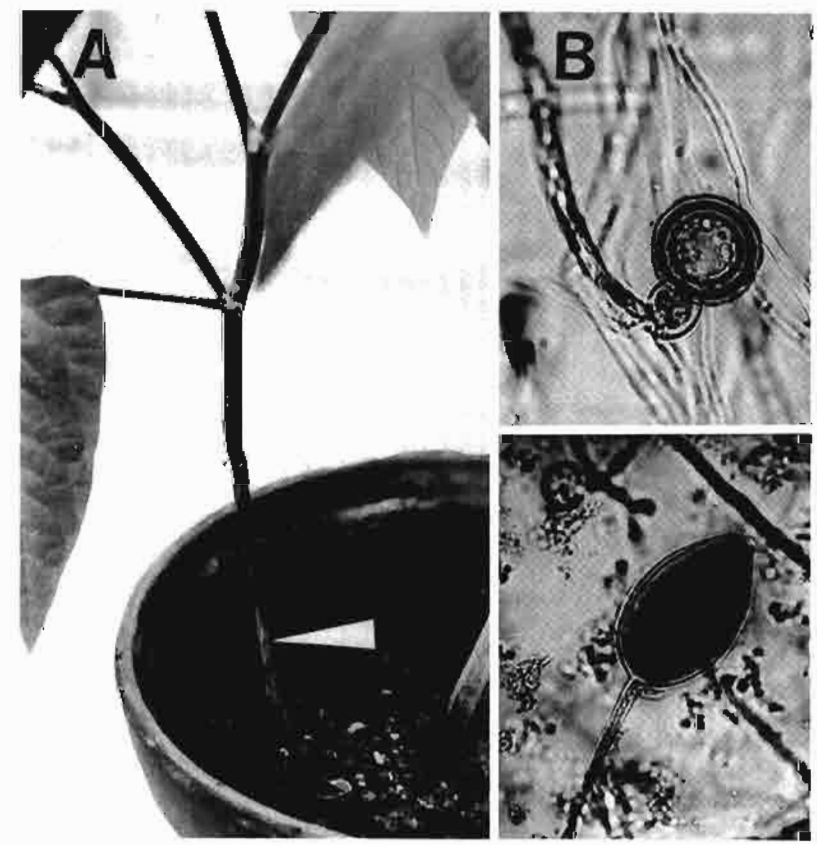

Fig. 1. Phytophthora root and stem rot disease of cowpea: **(A)** Stem lesion (arrow) on symptomatic cowpea, **(B)** oospore of the incitant, *P. vignae*, produced after 10 days on cowpea agar, and **(C)** sporangium of *P. vignae* produced within 24–48 hr in Petri's mineral solution.

directly from soils using selective media.

Sporangia characteristic of *P. vignae* developed from the tea leaf half-disks from the leaf disk bait method that were placed in Petri's solution. Hymexazol in baiting water or selective media inhibited *Pythium* growth. *P. vignae* was isolated from the Kandy, Anuradhapura, and System C districts. Of the five sites sampled in each district, three in Kandy, two in Anuradhapura, and three in System C contained the pathogen (Table 1). The isolates grew out well on V8 agar, CMA, PDA, and CPA. On CPA and CMA, the fungus formed oospores. The mycelial morphology of the isolates was similar, but isolates P006, P007, and P009 from Anuradhapura grew slowly and had slightly smaller sporangia than the other isolates. The trap plant assay also recovered *P. vignae* (Table 1); 10 isolates of the pathogen were recovered by this method. Fourteen isolates were obtained by the leaf disk bait method and confirmed to be *P. vignae* by microscopic examinations and their pathogenicity on cowpea. In one sampling site (Ku-1, in Kandy), *P. vignae* was recovered with the leaf disk bait method but not with the plant trap method. At least three isolations from three diseased plants per sample site were attempted. The roots and stem parts of collected plants were thoroughly examined for disease symptoms. Koch's postulates were fulfilled with isolates from these plants by the induction of stem and root rot symptoms on cowpea and reisolation. Other *Phytophthora* spp. were isolated by the baiting methods but did not cause disease symptoms on cowpea.

In the serial dilution end-point experiment, the greatest DPI was 16, in soil from site MI-3, in Anuradhapura. All other soils had smaller DPIs or zero (Table 1).

Additional hosts. Repeated attempts were made to isolate *P. vignae* from weeds and leguminous plants, other than cowpea, growing in the area and exhibiting some possible root or stem symptoms. Since *P. vignae* was never recovered, no attempt was made to identify any of the plants.

Of the cowpea cultivars grown in Sri Lanka, Muttessa and the four breeder lines (which came from the parent Muttessa) were the most resistant (Table 2). Compared with the other cultivars, ETA, MI-35, and Sudumung were moderately resistant to isolate P001. All other cultivars had below 50% survival rates when *P. vignae* was present at 1% (v/v) inoculum:soil. Among the cultivars grown in the United States, Mississippi Purple, Hercules, and Purple Hulk were resistant to all isolates except P006, P007, and P009 (Table 3). These three isolates were more virulent on U.S. cultivars than all other isolates. There was no difference in virulence among the three isolates on any cultivar, except on Mississippi Purple. There were two distinct groups of *P. vignae* pathogens separated on the basis of average linkage cluster analysis. Isolates P006, P007, and P009 (cluster 1) were distinct from the rest of the isolates (cluster 2) in their pathogenicity reactions on different cowpea cultivars (Fig. 2). The average distance between these clusters was about 1.9. In cluster 2, the most distant branches were between P018 and other isolates (average distance around 0.5), which is about four times smaller than for the first two main clusters. We used several other cluster analysis procedures as well and found no clearer delineations among isolates in the second cluster. For example, P019 and P013 were linked closely to each other; the only difference was in their pathogenicity on cultivar Mississippi Silver, i.e., one more plant survived after inoculation with P019 than after inoculation with P013. Within cluster 2, there was one main subcluster (with 11 isolates) and a series of very small subclusters with one or two isolates that apparently did not differ in distance from the main subcluster in cluster 2. Results indicated that U.S. cultivars are completely resistant, moderately resistant, or highly susceptible to isolates P001 and P006 in a greenhouse pot environment.

Table 1. *Phytophthora vignae* isolates obtained from soil from five cowpea-growing districts of Sri Lanka, percent mortality of cowpea cv. California Blackeye plants grown in test soils, and calculated disease potential index (DPI)

District	Site	Isolates obtained by: Plant trap	Isolates obtained by: Leaf disc bait	Percent dead plants	DPI[y]
Anuradhapura	MI-1			0.0	0
	MI-2	P010	P001, P003	32.5	8
	MI-3[z]	P007, P009	P005, P006	37.5	16
	PPD-2			0.0	0
	PPD-3			0.0	0
System C	DK-1	P021	P012, P002	30.0	8
	DK-2	P019	P004, P008	27.5	4
	SE-1	P020, P022	P016	37.5	8
	SA-1			0.0	0
	NA-1			5.0	4
Kurunegala	NI-1			0.0	0
	MAK-1			0.0	0
	MAW-2			0.0	0
	PA-1			0.0	0
	PA-2			0.0	0
Kandy	GN-1	P014, P015	P011	12.5	1
	HA-1	P018	P013, P017	20.0	2
	PI-1			0.0	0
	KU-1		P023, P024	0.0	0
	KU-2			0.0	0
Matale	MA-1			0.0	1
	SI-1			0.0	0
	SI-2			0.0	0
	DA-2			0.0	0
	DA-3			0.0	0

[y] Values based on reciprocal of last twofold dilution with a positive disease reaction. Five pots each with four plants per pot were used to provide means for percent dead plants and DPI.
[z] Only site where disease was observed in the field.

Table 2. Responses of cowpea cultivars and breeder lines to *Phytophthora vignae* isolates P001 and P006 2 mo after inoculation under greenhouse conditions[y]

Cultivars and breeder lines	Percent plant survival P001	P006
Arlington	20 de[z]	...
MI-35	60 bc	...
ETA	70 b	...
Bombay	0 e	...
Sudumung	60 bc	...
Hawari-Mae	30 de	...
Polon-Mae	40 cd	...
Muttessa	95 a	...
Mississippi Purple	100 a	45 a
Mississippi Silver	25 de	20 b
Hercules	100 a	0 c
Purple Hulk	100 a	0 c
Cowpea Banquet	0 e	0 c
California Blackeye	0 e	0 c
Breeder lines		
IT-84E-1-108	100 a	...
IT-83-S-818	90 a	...
IT-84E-124	100 a	...
IT-82D-513-1	100 a	...

[y] Five pots per treatment with four plants per pot were used. Plants in control pots (noninoculated pasteurized soil) were completely disease-free.
[z] Values followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls test. Values were arcsine-transformed before analysis.

Table 3. Pathogenicity of *Phytophthora vignae* isolates from Sri Lankan soils on U.S. cowpea cultivars[y]

Isolate	Number of infected plants per cultivar[z]					
	CBE	MP	MS	CB	PH	H
P001	20	1	20	17	0	0
P003	20	1	17	20	0	0
P010	20	0	18	18	0	0
P005	20	0	20	19	0	0
P006	20	10	20	20	19	20
P009	20	9	18	20	20	20
P007	20	9	18	19	20	20
P012	20	0	15	16	5	1
P002	20	0	17	18	0	0
P021	20	0	18	18	0	0
P019	20	0	14	20	0	5
P004	20	0	19	20	4	0
P008	20	0	20	20	0	0
P016	20	0	15	18	5	0
P022	20	0	14	19	0	0
P011	20	1	19	20	0	0
P014	20	0	19	20	1	0
P015	20	0	15	20	0	0
P013	20	0	15	20	0	5
P018	20	0	20	19	5	5
P023	20	1	20	18	0	0
P024	20	0	20	18	0	0

[y] Five pots per treatment with four plants per pot were used. Average linkage cluster analysis was applied to differentiate isolates that produced different reactions.
[z] CBE = California Blackeye, MP = Mississippi Purple, MS = Mississippi Silver, CB = Cowpea Banquet, PH = Purple Hulk, H = Hercules.

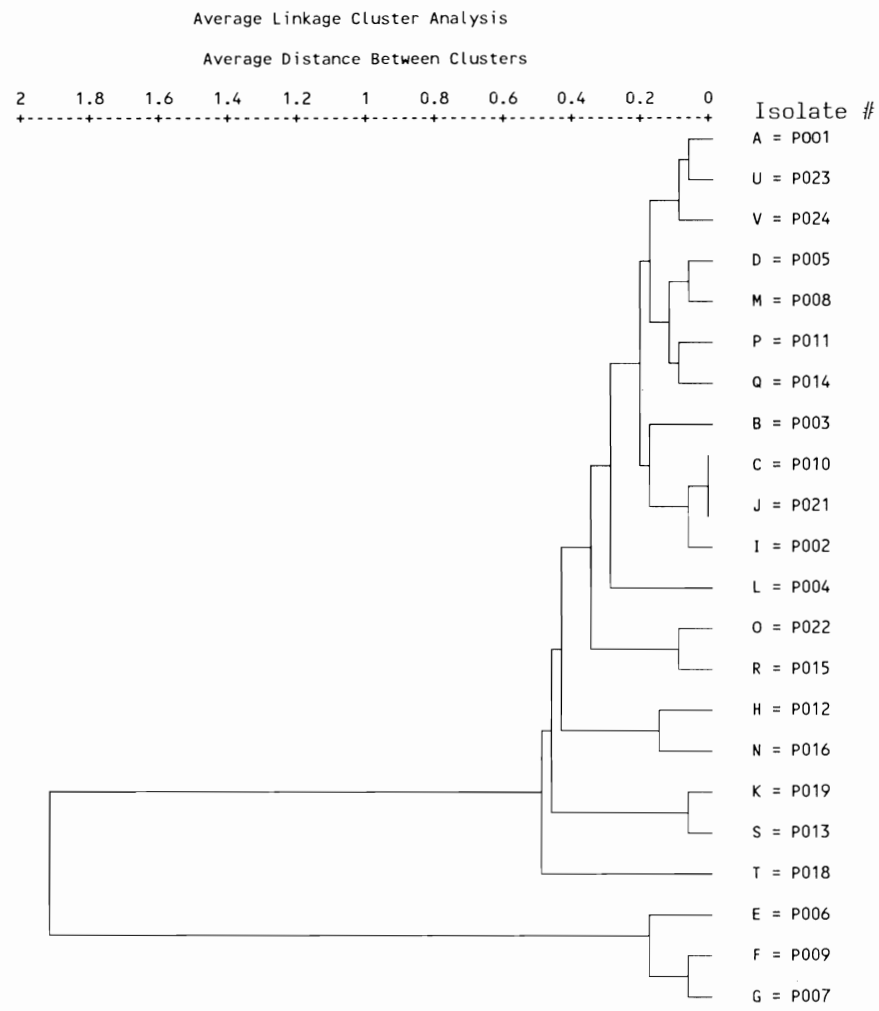

Fig. 2. A dendrogram, drawn from a cluster analysis, on pathogenicity of 22 isolates of *Phytophthora vignae* on cowpea cultivars California Blackeye, Mississippi Purple, Mississippi Silver, Cowpea Banquet, Purple Hulk, and Hercules. On the basis of the cluster hierarchy, two main clusters are distinct.

In pathogenicity tests with nine other legumes grown in Sri Lanka, all were resistant to *P. vignae*.

DISCUSSION

P. vignae was shown to be the causal agent of stem and root rot of cowpea in Sri Lanka. It is placed under key group VI in the genus *Phytophthora* (27), along with some broad host range *Phytophthora* spp. such as *P. cambivora* and *P. cinnamomi*.

Our success in isolating *P. vignae* from cowpea field soils in Sri Lanka can be attributed largely to the use of the susceptible cowpea cultivar California Blackeye. Growing the susceptible cowpea cultivar in soil allowed *P. vignae* to cause lesions on which the pathogen could multiply. Heavy watering also created environmental conditions conducive for the disease to occur in the greenhouse.

The leaf disk bait assay to isolate *P. vignae* was also successful, possibly because the soil used had been heavily irrigated and planted with a susceptible host grown under conducive greenhouse conditions. These conditions appeared to activate the pathogen propagules. Canaday and Schmitthenner (1) reported that keeping the soil moistened for 2 wk prior to baiting increased the chances of isolating *P. m. glycinea* from soil. Our method went a step further by using a susceptible host plant to enhance the production of sporangial inoculum in situ before baiting. Using hymexazol in both the bait water and the selective medium helped to minimize colonization of the baits by *Pythium* spp. This is important in baiting, especially when the pathogen propagule number in soil is low (31). We also used a high water-to-soil ratio to encourage sporangial production and to reduce fungistatic effects by soil dilution.

The serial dilution end-point technique was used successfully to determine DPI values of different soils. All the fields sampled generally had a low DPI. Only with soil from one field where disease was apparent was the DPI as high as 16, and that field was watered daily and soil remained very moist. But even that DPI was low compared with the DPIs of 128 reported by Greenhalgh (3) for fields examined for *P. cinnamomi*. The Kandy district had the lowest DPI values of the areas from which the pathogen was isolated, possibly because of milder temperatures (21–26 C) than in Anuradhapura and System C (30–35 C). One site in the Matale district had a DPI of 1, but *P. vignae* was not isolated from that region. Also, *P. vignae* was isolated from the Ku-1 site in the Kandy district by the leaf disk bait method, but the DPI was 0. These results suggest that the soils sampled had different potentials to cause disease, even under conducive conditions. For soils with a DPI of 0 and from

which the pathogen was not isolated, the pathogen probably was not present or propagule levels were below the detection limit. Soils with the pathogen present and at detectable levels, however, may be suppressive to the disease because of physical, chemical, or biological properties.

This study established the presence of *P. vignae* in at least three different districts of Sri Lanka, even though the disease was observed in the field at only one site in the Anuradhapura district. That site was more heavily watered than the other sites, as it was managed by the agricultural research station at Maha Illuppallama. All other sites in that district had well-drained, drier soils with high soil temperatures. Disease occurs in fields where the pathogen is present only if susceptible cultivars are planted. Antagonistic microorganisms in soils of those fields may restrict the pathogen from causing disease (12). Alternatively, symptoms or signs of the disease may have been overlooked. For example, low yield could easily have been attributed to environmental factors rather than to root disease. Somasiri (26) stated that the performance of cowpea in poorly drained soils was unsatisfactory but that response could have been due to the presence of *Phytophthora* spp. causing disease.

The fact that we did not isolate the pathogen from any weeds or legumes does not rule out the possibility of additional hosts. We examined only weeds exhibiting stem lesions or wilting near cowpea fields. An additional host could harbor the pathogen without exhibiting visible aboveground disease symptoms.

The cultivar Muttessa and the four breeder lines were highly resistant to the pathogen isolate P001, and the cultivars ETA, Sudumung, and MI-35 showed a moderate resistance. MI-35 and ETA are the most common cultivars grown in the districts sampled. Breeding for these cultivars was done in the Anuradhapura district, and breeders may have unknowingly selected for resistance to *P. vignae*. These cultivars also have higher yields and command a higher market price because of their better texture and flavor. The higher yields could be related to the resistance to stem and root rot. We have not made any yield comparisons to confirm this, however.

Three U.S. cultivars—Purple Hulk, Mississippi Purple, and Hercules—were completely resistant to isolate P001. However, all were susceptible to isolates P006, P007, and P009. This was confirmed by average linkage cluster analysis. These three isolates were in one cluster and were distinct from the rest. These results suggest the occurrence of two races of *P. vignae* in Sri Lanka, with P006, P007, and P009 belonging to one race (race A) and all other isolates belonging to the other (race B). It is interesting that race A was found only at the MI-3 site in the Anuradhapura district. Purss (20) described four races according to cultivar resistance observed in Australia. Tsuchiya et al (34) proposed three races of *P. vignae* f. sp. *adzukicola* based on pathogenicity of isolates in Japan.

We feel that the race designated A is a new race in Sri Lanka that is pathogenic on all six U.S. cultivars tested. It would be interesting to test both races on Sri Lankan cultivars of cowpea and also to compare both races with races found in Australia. In a field situation, some of these cultivars showed "field resistance," except in the Maha Illuppallama area, where soils were heavy and moist. Purss (19) also observed field resistance of certain cowpea cultivars that broke down when conditions favored the pathogen.

Our work showed that, depending on the race (isolate) or the inoculum level of the pathogen, immunity or high resistance could be broken down, as encountered with isolate P006. We would therefore be cautious in describing the nature of resistance of cultivars, as some cultivars appear to have a general type of resistance while others have a more specific type. These may be analogous to horizontal and vertical resistance, respectively (35).

All legumes other than cowpea that we inoculated showed complete resistance. Purss (19) showed similar results with some legumes, and our work adds to his susceptibility/resistance list of different legumes and legume cultivars. Adzuki bean is the only legume other than cowpea known to be susceptible to *P. vignae* (9,10,33). Kitazawa et al (9) further tested the four races of *P. vignae* from Australia on adzuki beans and cowpeas, along with the Japanese isolates of *P. vignae* that were pathogenic on adzuki bean. Tsuchiya et al (34) suggested designating formae speciales for isolates of *P. vignae* differentiated on the basis of their pathogenicity to adzuki bean and cowpeas. They named *P. vignae* f. sp. *adzukicola* and *P. vignae* f. sp. *vignae* for the isolates pathogenic on adzuki bean and cowpea, respectively.

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