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STEM ROT: A DISEASE OF COWPEAS CAUSED BY AN UNDESCRIBED SPECIES OF *PHYTOPHTHORA*

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SUMMARY.

A stem rot disease of cowpeas which is the cause of serious losses in Queensland is described.

A species of Phytophthora **is shown to be the causal agent and is described in detail. The name** Phytophthora vignae **n. sp. is proposed.**

Field observations show that the disease is soil-borne and that there is a build-up of the pathogen in the soil as a result of growing successive crops of susceptible varieties.

Sporangia formed on disease lesions are capable of producing infection above ground level. It is suggested that this may explain the rapid spread of the disease in moist weather.

Water plays an important role in the dissemination of the disease. Excessive water increases the incidence of disease on lightly infested soil but on heavily contaminated soil a high moisture level is not required for serious disease development.

It has not been possible to demonstrate seed-borne infection. No control of the disease was obtained by fungicidal treatment of seed prior to sowing in infested soil.

Sowing time has a marked influence on disease incidence, there being a fall of infection with falling temperatures under glasshouse conditions. The range of temperature over which infection can occur in the laboratory (66–82 deg. F.) is narrower than the range over which growth of the fungus can occur (52–93 deg. F.).

I. INTRODUCTION.

A stem rot condition in cowpeas was first recorded in Queensland early in 1952 (Purss 1953). In that and successive years severe outbreaks of the disease were recorded throughout most of the State. Poona and Reeves, the principal varieties grown, have both proved particularly susceptible to the disease and many growers are now refraining from planting the crop on this account.

A wilt of cowpeas caused by a species of *Phytophthora* has also been recorded from Carlingford in New South Wales (New South Wales Department of Agriculture 1950), and a similar disease was later responsible for heavy losses at Kyogle (New South Wales Department of Agriculture 1955). It is suspected that this disease is identical with the one described in this paper, as isolations from material recently supplied from New South Wales yielded a *Phytophthora* identical with the one occurring in Queensland.

A disease described as red stem canker has been reported in the United States of America (Weimer 1949). The causal organism was shown to be *Phytophthora cactorum* (Leb. & Cohn.) Schroet. The symptoms described are somewhat similar to those of the Queensland disease, with minor differences. However, the organism responsible in this State is distinct from that described by Weimer.

II. SYMPTOMS.

When the disease occurs in an area where it has never previously been encountered it is usual for the outbreak to be confined at first to isolated patches. These patches rapidly expand during favourable weather conditions. On the other hand, where the disease has been reported during previous seasons it is usual for the first infected plants to be scattered throughout most of the paddock.

Affected plants turn yellow; during moist weather they may survive for a considerable time but in dry conditions they rapidly wilt and die (Fig. 1).

Fig. 1.

A Cowpea Crop of the Poona Variety Affected with Stem Rot.

Fields varying in size from 2 acres to 30 acres in which it has been difficult to find healthy plants have been observed. The disease has been located under a variety of conditions of soil type and drainage from black alluvial flats to poor stony hillside country. Plants in all stages of growth from seedling to maturity are subject to attack. Wilting is very rapid in infected seedlings.

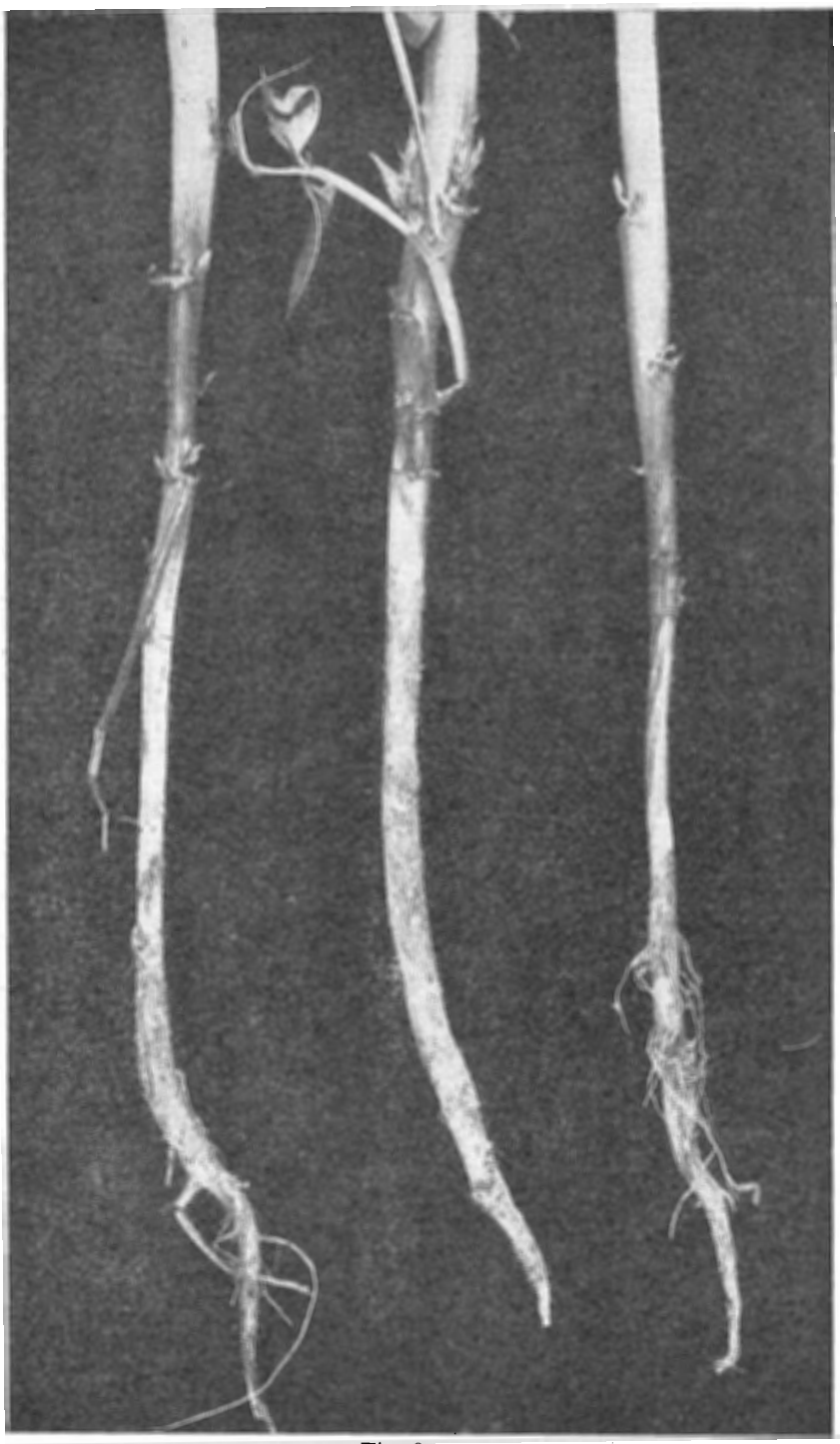

Fig. 2.

Lesion Development on Diseased Plants.

Individual plants are characterised by a brown lesion commencing at ground level and extending upwards, often reaching the top of the stem. This lesion is sunken and completely girdles the stem. The margin is usually watersoaked and during moist weather is covered by a light fungal growth in which sporangia can be distinguished. Internally, a brown discoloration extends for a short distance into the healthy tissue. The root system rapidly decays and is usually covered by a mat of white and pink fungal spores (Fig. 2).

In addition to these typical symptoms, under moist conditions it is common to find aerial lesions high up on an otherwise healthy stem (Fig. 3).

The red coloration characteristic of red stem canker is not apparent with this disease.

Fig. 3.

Stem Decay Resulting from Aerial Infection.

III. ETIOLOGY.

(1) Isolations.

Many fungi have been isolated from diseased lesions (both basal and aerial) but those that appear regularly are *Fusarium* spp., *Macrophomina phaseoli* (Maubl.) Ashby and a white phycomycete. Fruiting bodies of the first two fungi are usually abundant on the earlier affected parts. The white phycomycete can only be isolated from the advancing edge of the lesion.

(2) Pathogenicity Tests.

The species of *Fusarium* and *M. phaseoli* were inoculated on to healthy cowpea seedlings of the variety Poona by dipping the roots into a slurry formed by macerating in water a potato dextrose agar (P.D.A.) culture of the fungus being tested. No infection was obtained in either case.

The white phycomycete was tested by the method described by Weimer (1949). Cowpea seedlings of the variety Poona approximately four weeks old were raised in pots of sterilized soil. These were inoculated by placing small pieces ($\frac{1}{4}$ in. square) of a P.D.A. culture of the fungus on the uninjured stem tissue just above ground level. These pots were then well watered and covered with a bell-jar. A similar series of inoculations was done in which sterile agar was used as inoculum.

Seedlings inoculated with the phycomycete commenced to show lesion development at ground level in three days and at 10 days all seedlings had collapsed. Seedlings inoculated with sterile agar remained healthy.

Results of a typical series of pathogenicity tests are given in Table 1. These tests were repeated a number of times and on each occasion gave similar results.

Table 1.

RESULTS OF PATHOGENICITY TESTS ON COWPEAS.

Treatment.	Number of Plants Treated.	Number Affected After 10 Days.
(1) Inoculated above ground with phycomycete. Moist chamber	12	12
(2) Inoculated with sterile agar. Moist chamber	12	0
(3) Inoculated below ground with phycomycete	8	8
(4) Uninoculated	8	0
(5) Roots dipped in *Fusarium* sp. slurry	16	0
(6) Roots dipped in sterile water	14	0

The lesion produced commenced at the point of inoculation and spread both upwards and downwards. Twenty-four hours after symptoms first appeared the plants collapsed at ground level (Fig. 4). The phycomycete was reisolated from the lesion, and this isolate reproduced the disease. The phycomycete is therefore considered to be the cause of stem rot.

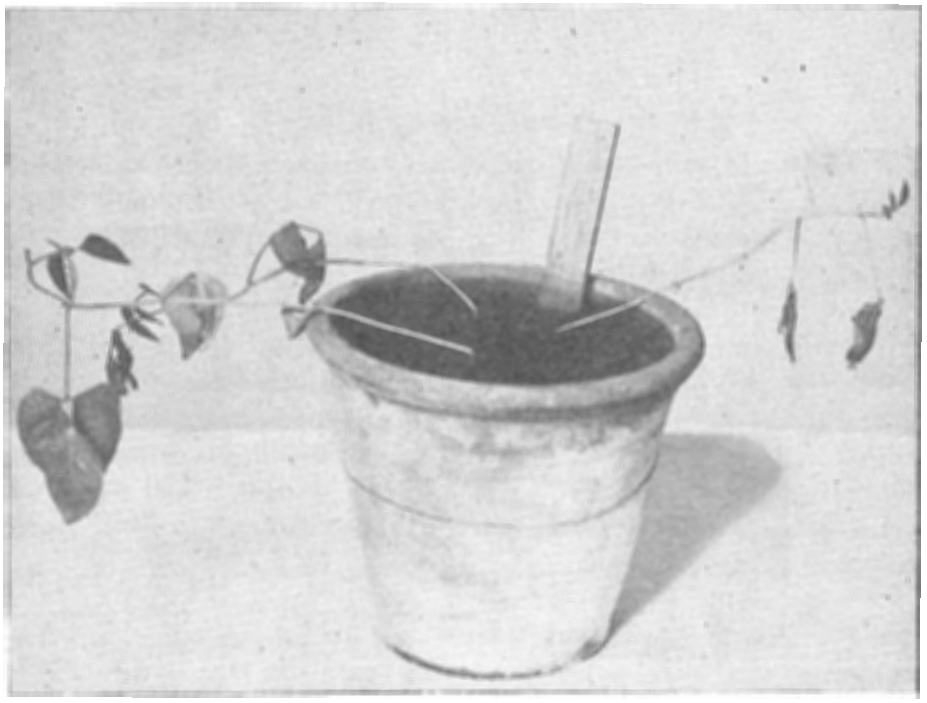

Fig. 4.

Cowpea Plants Inoculated Above Ground and Covered with a Bell-jar.

An alternative method of inoculation was tried with a view to establishing a technique which could be applied to large numbers of seedlings. This technique had been used previously by Weimer (1949). Inoculum growing on P.D.A. was cut into small cubes ($\frac{1}{4}$ in. square), which were placed against the uninjured stem just below the ground level and covered with soil. The pots were kept constantly moist. It was found that this technique yielded results equal to those obtained by inoculating above ground level and incubating in a moist chamber (Table 1 and Fig. 5). In addition, it was noted that the fungus produced a lesion which completely encircled the stem below ground level. Red stem canker, on the other hand, forms a narrow band which widens when it moves to the aboveground portion of the stem (Weimer 1949).

Other methods of inoculation were tried. In one, use was made of a culture of the fungus growing on a mixture of wheat and oats for soil inoculation. The mixture was added at the rate of 1 pint to a seedbox of sterilized soil measuring 18 in. x 15 in. x 5 in. Seed of the variety Poona was immediately sown in this soil. The fungus in this case attacked seedlings, producing a "damping-off" effect which killed all plants during the "two-leaf" stage. When in later work a resistant variety Blackeye 5 was uncovered, this technique was again used in comparing the reaction of Blackeye 5 and Poona. Although Blackeye 5 proved highly resistant under all other conditions, it damped-off to the same extent as Poona when tested in this manner. The technique was discarded for any future testing work.

Another technique tried involved the use of the fungus growing in a nutrient solution, which was poured on to the soil in which seedlings were growing. This technique, however, gave rather inconsistent results.

Fig. 5.

Cowpea Plants Inoculated Below Ground Without a Moist Chamber (left), and Uninoculated Plants (right).

IV. BIOLOGY OF THE COWPEA ORGANISM.

(1) Morphology.

Hyphae are hyaline, with occasional septa developing when old. Diameter of hyphae is very variable, with an average of 4·5 μ and with variations from 3 to 9 μ within the same hypha. Branching is mostly irregular and rather frequent.

The fungus forms both terminal and intercalary chlamydospores sparsely in distilled water, oatmeal agar (Tucker 1931) and maizemeal agar (Tucker 1931), but abundantly in Petri solution (Tucker 1931). These are variable in shape and size but mainly spherical, with an average diameter of 17 μ and a range of 12 to 21 μ.

Hyphal swellings are formed abundantly in Petri solution and oatmeal agar and rarely on maizemeal agar. These are variable in shape and size, usually roughly spherical and mostly intercalary with a few terminal.

Sporangia are never formed on solid artificial media and are produced only rarely when mycelium is transferred from pea broth (Leonian 1934) to distilled water. However, when cowpea nutrient broth (200 g. young green cowpea plants boiled in water, filtered, made up to 1,000 c.c., sterilized) is substituted for pea broth, sporangia are produced abundantly in the distilled water after 48 hours. Production of sporangia is sparse in Petri solution, but fairly abundant in non-sterile soil extract solution (Mehrlich 1935). Sporangia are produced abundantly when diseased cowpea stems are immersed in water for 24 hours.

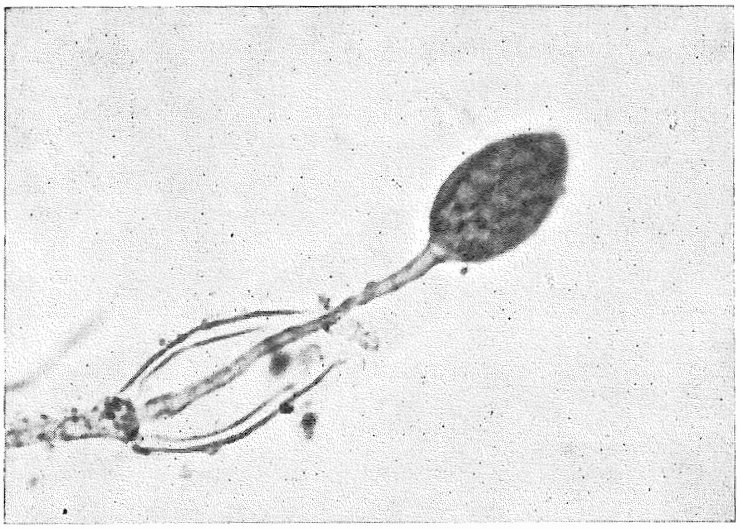

Fig. 6.

Phytophthora vignae. Nested and extended sporangium (X 367).

Sporangia growing in distilled water following growth in cowpea nutrient broth are borne mainly on simple undifferentiated sporangiophores, with a few on simple monochasial sympodia with proliferation. The latter type predominates on diseased tissue immersed in water, where an occasional compound sympodium also develops. Nested and nested and extended types have both been observed in monochasial sympodia (Fig. 6).

Table 2.

SIZE OF SPORANGIA OF THE COWPEA PHYCOMYCETE DEVELOPED ON VARIOUS SUBSTRATES.

Substrate.	Number Measured.	Range. (μ)	Average. (μ)	L/B ratio.
Diseased plants in field ..	50	24–69 x 15–39	49 x 27	1·8 : 1
Diseased plants immersed in water	100	30–72 x 18–54	58 x 32	1·8 : 1
Water following pea broth ..	50	24–60 x 15–30	37 x 22	1·7 : 1
Water following cowpea broth	50	30–57 x 15–27	42 x 21	2·0 : 1
Petri solution	20	30–61 x 21–36	40 x 30	1·3 : 1
	270	24–72 x 15–54	48 x 27	1·8 : 1

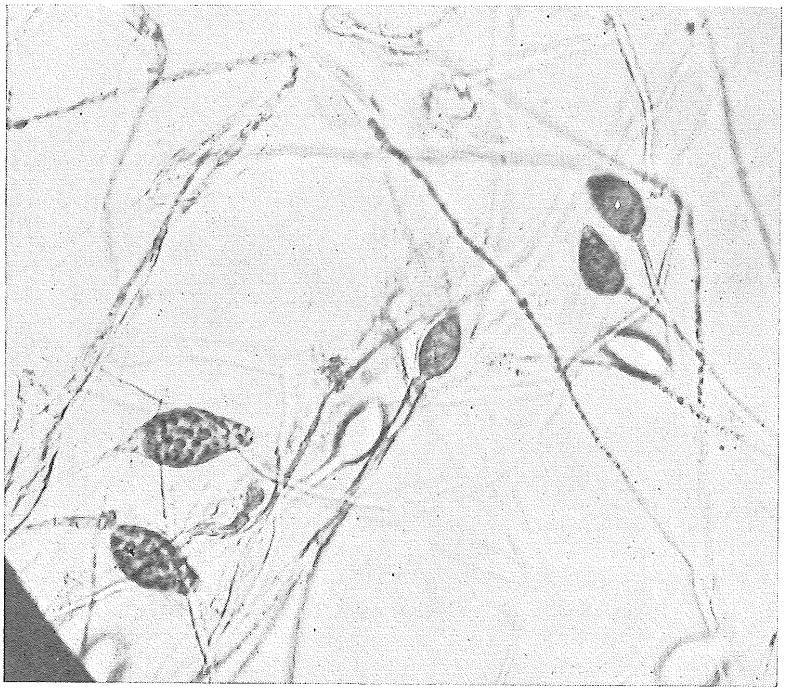

Fig. 7.

Phytophthora vignae. General View of Sporangia (X 170). Two sporangia showing zoospore differentiation.

Sporangia are very variable in shape and size but are mostly ovoid to ob-pyriform (Fig. 7). They are either non-papillate or very inconspicuously papillate. Measurements of sporangia developed on various substrates appear in Table 2.

Zoospores are differentiated within the sporangium (Fig. 7) and liberated singly through an apical pore. Up to 50 zoospores have been counted in a single sporangium. It is not uncommon to find a few zoospores trapped within the sporangial wall. On expulsion, the zoospores are reniform and very active. They become encysted after approximately half an hour. These encysted spores have an average diameter of 10 μ and germinate by a single germ tube after about two hours at room temperature.

Sexual organs are produced abundantly in host tissue (Fig. 8), oatmeal agar, maizemeal agar, and Petri solution and sterile water following growth in pea or cowpea broth. They are produced sparsely on malt extract agar (Leonian 1934), potato dextrose agar and cowpea extract agar (60 g. crushed cowpea seed, 17 g. agar, 1,000 c.c. water).

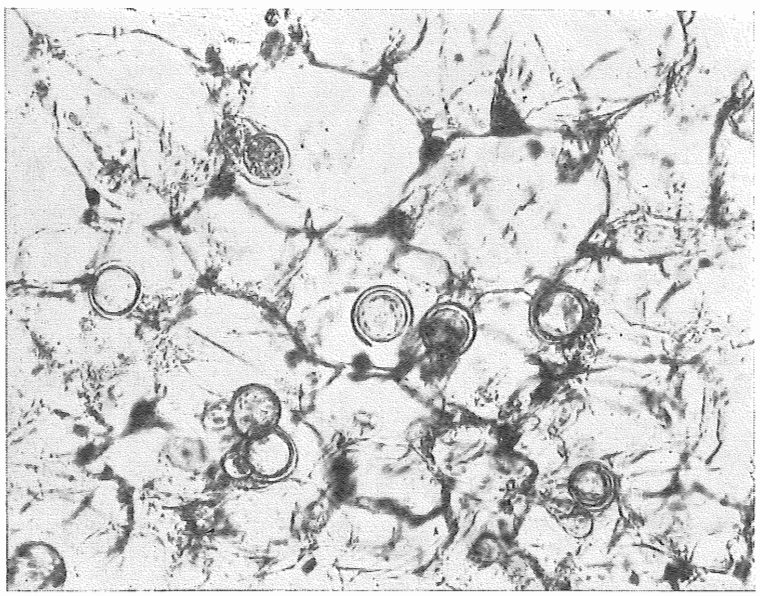

Fig. 8.

Sexual Bodies of *P. vignae* in the Host Tissue (X 180).

Antheridia are colourless, vary in shape from almost spherical to ovate, and measure 12-27 x 9-18 μ (av. of 250, 16 x 15 μ). They are almost exclusively amphigynous (Fig. 9).

Oogonia are colourless, smooth-walled and almost spherical in shape. They measure 27-42 x 24-46 μ (av. of 250, 33 x 31 μ). Oospores are colourless to light-brown, smooth-walled and spherical. They are slightly larger on artificial culture media than in host tissue, measuring 24-32 μ (av. of 175, 27 μ) on maizemeal agar and 21-30μ (av. of 148, 25μ) in diseased tissue.

The fungus is homothallic, both oogonia and antheridia being formed by hyphal tip cultures.

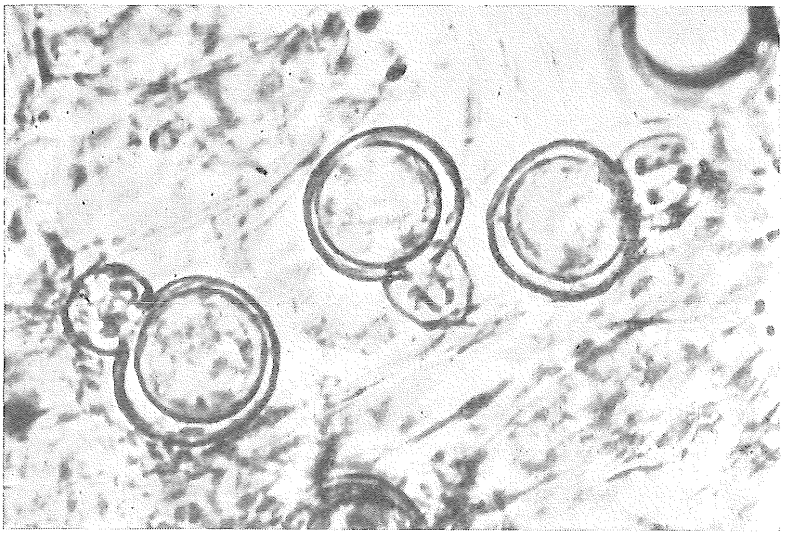

Fig. 9.

Phytophthora vignae. Oogonia, antheridia and oospores (X 533).

The oospore has a distinct outer wall, which is thin and much darker than the thick inner wall, the total width being approximately 3 μ. The oospores do not completely fill the oogonial cavity, in most cases not nearly so (Fig. 9).

(2) Histology.

The fungus is located chiefly in the parenchyma of the cortex, where it eventually causes a complete breakdown of the tissue, giving the typical sunken appearance to the diseased stem. A similar breakdown occurs in the pith. While the vascular tissue may also be invaded, there is no actual breakdown of the tissue. This may partly explain why even badly affected plants can remain green for long periods under favourable environmental conditions.

The mycelium is chiefly intercellular. Haustoria have been observed on rare occasions passing into cell tissue. Two types have been distinguished. The more common type is finger-like, being 3-5 μ long and 1·5-2·0 μ wide. The other type encountered is typically stud-shaped and measures up to 6 μ long

(Fig. 10). The haustoria are considered to be distinct from intra-cellular mycelium which is encountered frequently in young tissue and can also be seen passing through the thickest walls of the vascular vessels (Fig. 11). Where haustoria have been observed, the protoplasm of the cell has been either completely disintegrated or plasmolysed into a ball in a corner of the cell. Oospores are abundant in the diseased tissue (Fig. 8).

Fig. 10.

Stud-shaped Haustorium of *P. vignae* Passing into Host Cell (X 700).

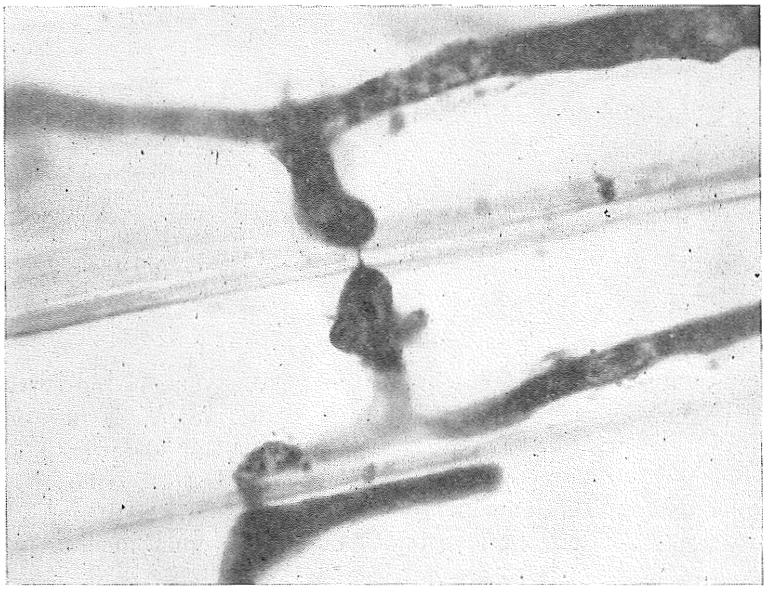

Fig. 11.

Mycelium of *P. vignae* Passing Through Thickened Walls of the Vascular Tissue (X 1260).

(3) Cultural Characteristics.

The fungus produces dense white colonies on potato dextrose agar at 27·5 deg. C., with moderate development of a low velvety mat of aerial mycelium and a definite margin. On malt extract agar, oatmeal agar and cowpea agar there is little development of aerial mycelium, and colonies are not dense. On maizemeal agar growth is rapid, with loose aerial hyphae.

Large, firm, brown lesions are produced on apple fruit (var. Granny Smith) following wound inoculations with this fungus. It is only slightly pathogenic to potato tubers.

The fungus makes good growth in a concentration of 1 in 4,000,000 malachite green but fails to grow in higher concentrations.

(4) Growth-Temperature Relationships.

The temperature relationships of this fungus were determined on maizemeal agar in a multi-temperature incubator giving a range of temperature from 0 deg. C. to approximately 40 deg. C. Uniform discs of inoculum ($\frac{1}{4}$ in. diameter) from a 5-day-old culture were centrally placed on the media in

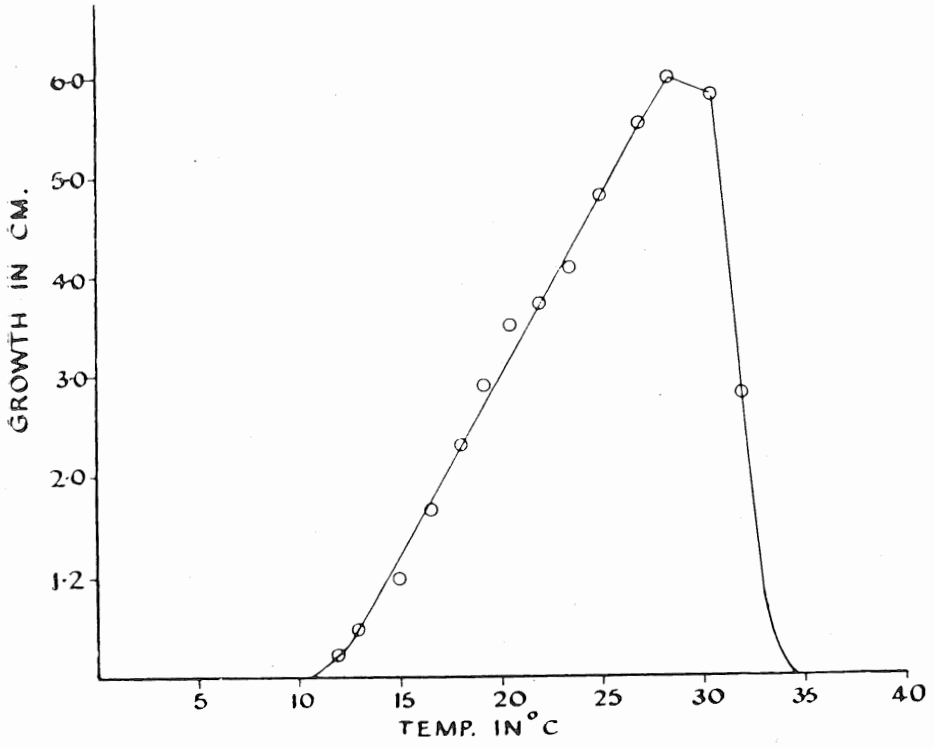

Fig. 12.

Growth-temperature Curve for *P. vignae*. 72 hours' growth on maizemeal agar.

4 oz. clear bottles and incubated for 24 hours at 27·5 deg. C. Measurement of the growth made in each bottle was recorded and three bottles were then placed in each of the 20 chambers of the multi-temperature range incubator. Growth of the colonies was measured at intervals of 24 hours. In Fig. 12 the growth made in 72 hours is expressed in graphical form.

This fungus has a minimum between 10 deg. and 12 deg. C., has an optimum from 28 deg. to 30 deg. C., and fails to grow at 34·5 deg. C. A culture of *P. cryptogea* Pethyb. & Laff. isolated from lucerne root rot and tested for comparison grew at a temperature below 5 deg. C., had an optimum of 24 deg. C., failed to grow at 34·5 deg. C., and grew only slightly at 32 deg. C.

V. IDENTITY OF THE COWPEA PHYCOMYCETE.

(1) Comparison with Other Species of Phytophthora.

General morphological characters and the nature of zoospore formation within the sporangium place this fungus in the genus *Phytophthora*.

Based on the key prepared by Tucker (1931) the fungus fits into the group of which *P. cryptogea* is the only member. It grows at 20 deg. C. on malt extract agar; has amphigynous antheridia; fails to grow at 35 deg. C. after four days on maizemeal agar; has sporangia mainly non-papillate, absent on ordinary agar media, but developing in Petri's mineral solution; has oospores that measure less than 30 μ and develop freely on solid media; and is pathogenic to wounded apple fruit.

It is, however, impossible to classify this organism as *P. cryptogea,* mainly because of the wide difference found in temperature responses. *P. cryptogea,* according to Tucker, grows well at 5 deg. C. and fails to grow at 32·5 deg. C. Results with the local isolate of the organism from lucerne agree with Tucker's observations. On the other hand, the cowpea organism makes no growth below 10·5 deg. C. and grows fairly well at 32·5 deg. C.

The cowpea isolate produces sexual organs very readily in three days on maizemeal agar and in one week on oatmeal agar. This is in direct contrast with the experience of Pethybridge and Lafferty (1919) and Tucker with *P. cryptogea,* the latter finding sexual organs only after exposure to winter temperatures at Columbia, Missouri. However, it is interesting to record that *P. cryptogea* is reported to exhibit the renewed growth of sporangiophores similar to that found in the cowpea organism and for this reason the two species are considered to be related. Tucker gave descriptions of another 19 species of *Phytophthora,* but this fungus fails to fit into any of the groups suggested by this author.

Leonian (1934) also prepared a key used for identification. In this key the organism falls into the group of which *P. boehmeriae* Saw. is the only member. The fungus makes good growth on malt extract agar at 20 deg. C., grows at 27 deg. C., has amphigynous antheridia, possesses oospores measuring less than 31 μ, and grows in the presence of 1 in 8,000,000 malachite green.

However, for two reasons it is impossible to accept this organism as *P. boehmeriae.* Firstly, *P. boehmeriae* produces sporangia readily on agar media (Tucker 1931; Freezi 1941) and the sporangia are distinctly papillate, having papilla 9-12 μ x 9-17 μ (Sawada 1927). Secondly, *P. boehmeriae* has antheridia which are nearly spherical, measuring 12-16 μ x 11-15 μ. The antheridia of the cowpea fungus are usually much larger than this. Dr. G. M. Waterhouse, who examined a culture, considered the size and shape of the antheridia to be a fairly constant feature and quite different from that of *P. boehmeriae.*

Attempts made to infect the leaves of *Boehmeriae nivea* Gaudich with this organism, using both P.D.A. culture cubes and a sporangial suspension in a moist chamber, were unsuccessful.

The close similarity in symptoms of red stem canker and stem rot necessitates a careful comparison of the causal organisms. The Queensland *Phytophthora* differs from *P. cactorum* as described by Weimer from cowpea in the following characteristics:—

——	*P. cactorum.*	Queensland *Phytophthora.*
Antheridia	Largely paragynous	Amphigynous
Oogonia	Av. 25·3 x 26·7 μ	Av. 33 x 31 μ
Oospores	Av. 20·8 μ dia.	Av. 26 μ dia.
Sporangia	Distinctly papillate	Non-papillate or very inconspicuously papillate

While the oogonial and oospore measurements of the two cowpea organisms differ considerably, they both fall within the range of variability found for *P. cactorum* by Tucker. The size difference, therefore, is not sufficient to exclude the Queensland fungus from this species. This leaves the differences in antheridia and sporangia, both of which are important from the point of view of classification. The difference in type of antheridia is of special importance, as both Tucker and Leonian regarded the presence of predominantly paragynous antheridia as a constant distinguishing feature of *P. cactorum.* As the Queensland *Phytophthora* always produces amphigynous antheridia, it is impossible to classify it as this species. Dr. G. M. Waterhouse has expressed the opinion that it is quite different from *P. cactorum.*

It is interesting to record here the similarity of temperature responses of *P. cactorum* isolated by Weimer from red stem canker and the local species of *Phytophthora.*

The organism has also been compared with descriptions of the following 17 species of *Phytophthora* described since 1931, but appears distinct from all of these.

Phytophthora primulae Tomlinson (Tomlinson 1952), *P. verrucosa* Alcock and Foister (Foister 1940), *P. macrospora* (Sacc.) Ito and Tanaka (Tanaka 1940), *P. quininea* Crandall (Crandall 1947), *P. inflata* Caroselli and Tucker (Caroselli and Tucker 1949), *P. porri* Foister (Foister 1931), *P. fagopyri* Takimoto nom. seminud (Ito and Tokunaga 1935) and *P. megasperma* Drechsler (Drechsler 1931) have either predominantly paragynous antheridia or at least a fair percentage of such antheridia. *P. quininea* also has large oospores (63 μ) and *P. megasperma* has larger oospores (av. 41·4 μ). In *P. himalayensis* Dastur (Dastur 1948) zoospores are unknown, sporangia are distinct and oospores are larger. *P. lateralis* Tucker and Milbrath (Tucker and Milbrath 1942) has large chlamydospores, sexual stages are absent, and growth is inhibited at 30 deg. C. *P. stellata* Shanor (Shanor 1938) has characteristic spiny oogonial walls. *P. formosana* Saw. (Sawada 1942a) has antheridia much smaller than those of this fungus. *P. leersiae* Saw. (Sawada 1941) has extremely large oogonia. *P. cinchonae* Saw. (Sawada 1936) and *P. murrayae* Saw. (Sawada 1942b) apparently have not been observed to form sexual spores and have papillate sporangia. *P. ricini* Saw. (Sawada 1942c) has smaller antheridia and papillate sporangia. *P. fragariae* Hickman (Hickman 1940) fails to grow at 30 deg. C.

(2) Description.

Because of the evidence presented, the fungus is considered to be a new species, for which the name *Phytophthora vignae* is accordingly proposed. English and Latin descriptions follow:

Phytophthora vignae n. sp.

Mycelium hyaline, non-septate when young, occasional septa developing when old; within host tissue mycelium mostly intercellular but some intracellular. Chlamydospores variable, mainly spherical, 12–21 μ (av. 17 μ), terminal and intercalary. Hyphal swellings in both solid and liquid media, irregular, both terminal and intercalary, single. Sporangia on host and in liquid nutrient media developed frequently on simple unbranched sporangiophores, frequently on a simple monochasial sympodial sporangiophore, rarely on a compound sympodial sporangiophore; sporangia variable in shape, mostly ovoid to ob-pyriform, 24–72 μ x 15–54 μ (av. 48 x 27 μ), non-papillate or inconspicuously papillate, germinating by zoospores; zoospores differentiated within sporangium, liberated singly, reniform when motile, spherical when encysted, average diameter of encysted zoospores 10 μ. Homothallic; sexual organs abundant in artificial media and host tissue; oogonia colourless, smooth, almost spherical, 27–42 x 24–46 μ (av. 33 x 31 μ). Antheridia amphigynous, variable in shape from almost spherical to ovate, 12–27 μ x 9–18 μ (av. 16 x 15 μ). Oospores colourless to light brown, 18–32 μ (av. 26 μ) in diameter, oogonial cavity not filled.

Habitat: In stem and root tissue of *Vigna sinensis* (L.) Endl. ex Hassk. in Queensland, causing a stem rot.

Phytophthora vignae n. sp.

Chlamydosporae variabiles, pro more sphaericae 12–21 μ (media 17 μ) terminales atque intercalares. Vesiculae hypharum in cultura atque solida atque liquida irregulares, terminales atque intercalares, solitariae. Sporangia in hospite et in cultura liquida in sporangiophoris vel simplicibus eramosisque vel monochasialibus sympodialibus proliferentibus, raro in sporangiophoro composito sympodiali enata; sporangia variabilia pro more ovoidea vel obpyriformia 27–72 μ x 15–54 μ (media 51 x 29 μ), epapillata vel subtilissime papillata; zoosporae singulae liberatae. Homothallica; genitalia plurima et in cultura artificiosa et in hospitae; oogonia sine colore, laevia, fere sphaerica 27–43 μ x 24–36 μ (media 33 x 31 μ). Antheridia amphigyna, e forma subsphaerica in ovatam variabilia 12–27 μ x 9–18 μ (media 16 x 15 μ). Oospora 18–32 μ (media 26 μ) diam. cavum oogonii haud impleta.

Habitat: In Queenslandia, intra caules radicesque *Vigna sinensis* (L.) Endl. ex Hassk.

VI. FACTORS AFFECTING DISSEMINATION AND EPIDEMIOLOGY OF THE DISEASE.

(1) Soil-borne Infection.

Numerous field observations have indicated that the disease is soil-borne. The most striking of these was on the Darling Downs during the summer of 1951-52. Half of a paddock of approximately 10 acres had been planted to cowpeas of the Poona variety during the 1950-51 season. In the 1951-52 season the farmer planted the whole paddock to the same variety. Stem rot caused a 50 per cent. loss on the land previously planted to Poona pea but only isolated loss on the other half of the paddock. In nearly every case of severe stem rot incidence the paddock has had a history of previous cowpea crops.

To test these observations experimentally, a simple pot experiment was designed in which soil collected from a badly diseased field was used. A drum of this soil was kept for 12 months in a glasshouse in which no cowpea plants had been grown. The soil was watered at fairly regular intervals and the surface soil only was allowed to dry out. At the end of the period half the soil was sterilized and placed in four sterilized pots. Another four sterilized pots were filled with the unsterilized soil. All were then sown at the rate of 10 seeds per pot with Poona cowpea seed harvested from healthy plants in a paddock free from the disease. Observations were taken; the results after 28 days appear in Table 3. All plants in the unsterilized soil showed typical symptoms of the disease within three weeks of germination (Fig. 13). All plants in the sterilized soil remained healthy.

Table 3.

RESULTS OF GROWING COWPEAS (VAR. POONA) IN SOIL FROM AN INFECTED FIELD AFTER 12 MONTHS' STORAGE.

Treatment.	Number of Plants Emerged per Pot.	Number of Plants per Pot with Stem Rot.
Sterilized	8	0
Unsterilized	8	8

Fig. 13.

Disease Development in Infected Soil Retained for One Year. Soil on the right sterilized.

This shows conclusively that the fungus can survive in moist soil for at least one season. Furthermore, field observations indicate that severe disease incidence can be expected where even two seasons have elapsed between the previous occurrence of the disease and the planting of another cowpea crop.

That the disease is soil-borne and does in fact build up in the soil as a result of growing successive crops of susceptible varieties has been brought out very strikingly by varietal trials conducted on infected soil. A plot of land at Boonah in south-eastern Queensland has been used for disease testing of cowpeas for a number of years. Table 4 indicates the incidence of disease in two varieties, Poona and Cristaudo, over a period of four years in replicated

and randomised trials during the 1952-53, 1953-54 and 1955-56 seasons. No trial was conducted during 1954-55, but the paddocks were sown to Poona cowpea with almost complete loss of plants.

Table 4.

DISEASE INCIDENCE DURING SUCCESSIVE SEASONS AT BOONAH.

Variety.	Percentage Infection Stem Rot.		
	1952–53.	1953–54.	1955–56.
Poona	60	90	100
Cristaudo	0	36	100

The figures indicate that there is a build-up of inoculum in the soil with successive plantings of susceptible varieties and that the variety Cristaudo requires a high level of inoculum in the soil to be severely attacked. This has been further demonstrated in other trials during the 1955-56 season and will be discussed in a later paper when field varietal trials are considered.

(2) Air-borne Infection.

Reference has already been made to the sporangia which frequently occur under moist conditions on disease lesions. It has been observed that the disease moves rapidly during periods of moist weather suited to the production of sporangia. It is considered that sporangia play a role in this spread, as there is a high incidence of aerial lesions during this type of weather. A simple trial was designed to test the ability of sporangia to reproduce the disease.

Diseased specimens of Poona and Reeves varieties were collected from a disease resistance trial in the Kingaroy district on Feb. 8, 1956. Clumps of sporangia on undifferentiated sporangiophores were seen emerging in great numbers from stomata on disease lesions. These were observed towards the active end of the lesion, in a zone extending downwards for a distance of up to two inches.

Two Poona cowpea plants growing in a pot sown on Jan. 6, 1956, were used for inoculation. One inch of the stem approximately half-way up each plant was marked off, using pieces of thin white thread tied loosely. This area, together with leaves attached to it, was surface sterilized by gentle application of 70 per cent. alcohol. Drops of sterile water were then placed on these areas and with the aid of a dissecting microscope sporangia were carefully picked from the diseased material and placed in the drops on one of the plants. Inoculations were carried out at 5 p.m. on Feb. 8. A bell-jar was placed over each pot. A sample of a drop on the leaf tissue was examined 16 hours later and large numbers of zoospores were seen. Some were still in

an active condition while others had germinated. A further examination of the surface of the old diseased lesions revealed many sporangia germinating directly, indicating that under conditions of high humidity the sporangia could act as spores.

The inoculated plant was examined at daily intervals. On Feb. 10 the belljar was removed. A slight water-soaking was noticed on the inoculated stem; by Feb. 13 this had developed into a typical aerial stem rot lesion (Fig. 14). *P. vignae* was isolated from the original diseased material and the inoculated stem tissue. No evidence of disease development was found on the inoculated leaf tissue and there was no lesion development on the neighbouring plant which had been sprayed with sterile water.

Sporangia are therefore capable of directly infecting stem tissue and this fact could account for the rapid spread of the disease under moist conditions. The disease has frequently been observed moving in a "front" across a paddock during moist weather (Fig. 15). It is probable that sporangia play a role in such spread.

(3) Effect of Water.

Examination of many infected fields has revealed that where plants are growing in drainage channels or areas of bad surface drainage the disease is usually much more prevalent than in the rest of the field. Detailed

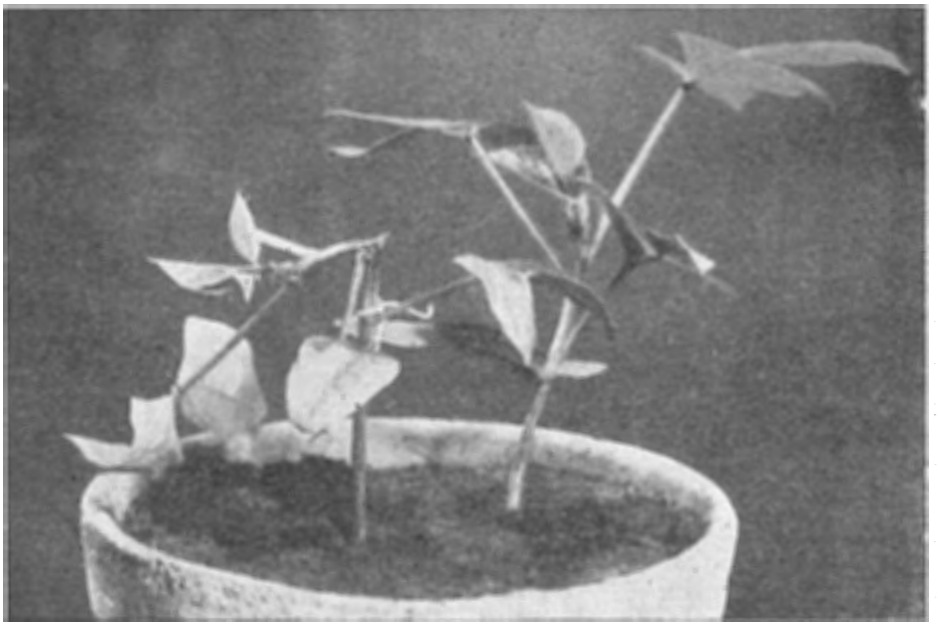

Fig. 14.

Sporangial Infection. Plant on left artificially inoculated with sporangia of *P. vignae* to produce aerial lesion.

Fig. 15.

Stem Rot Moving Along a Front Across a Paddock.

observations were recorded in a field of cowpeas on the South Coast of Queensland in which stem rot was spreading. The area was lowlying and had been artificially drained by means of furrows about a foot deep around the field. Twenty cowpea plants were scattered intermittently in these furrows, apparently as a result of the seed falling there during sowing operations. All of these plants were diseased, although stem rot was severe in only one corner of the paddock. It seems highly probable that the drainage furrow was acting as a medium for dissemination of the disease.

Another area close to Brisbane was traversed by shallow surface drains and the cowpea crop sown across the drains. Observations made in this paddock early in 1955 indicated that the drainage channels were aiding the dissemination of the disease. The plants in the channels were affected much earlier than the remaining plants and the general infection appeared to be spreading from these channels.

Numerous instances of the disease spreading from paddock to paddock down natural drainage channels have been observed.

During the 1955-56 season an opportunity to study disease incidence under varying conditions of free surface moisture was afforded in a varietal trial conducted on lightly infected soil in the South Burnett. The area had been sown to Poona cowpea during September 1955 and when examined some 10 weeks later was found to be infected with stem rot to the extent of approximately 5 per cent. of the plants. This infection was scattered lightly throughout the area.

The farmer ploughed this crop in almost immediately and a trial to assess the potentialities of 12 varieties was sown early in January 1956. Five replicated blocks were sown, each plot being a single half-chain row in which sufficient seed was sown to give approximately 100 plants. The block of land used was situated on an alluvial flat having a downward slope away from the creek bank. There was no fall at all in Block 1, a 12 in. fall in Block 2 and a 12 in. fall over the remaining three blocks. Conditions over each separate block were comparatively uniform. Heavy rains with light flooding of some of the trial occurred at intervals during the course of the experiment. Blocks 4 and 5 were swampy or lightly flooded for almost all the time after the first three weeks; Blocks 2 and 3 were swampy for short periods; but there was no excess water for any length of time on Block 1. Although full details of varietal reactions will be given in a later paper, the figures showing percentage infection of a susceptible variety (Poona) and a variety possessing some field resistance (Cristaudo) appear in Table 5. These figures have as far as possible been based on plants actually observed to have typical stem rot symptoms. However, some plants were in such an advanced diseased condition when examined that infection with *Phytophthora vignae* had to be assumed.

Table 5.

PERCENTAGE INFECTION OF TWO VARIETIES OF COWPEAS ON INFECTED SOIL IN THE SOUTH BURNETT.

Variety.	Percentage Infection.					
	Block 1.	Block 2.	Block 3.	Block 4.	Block 5.	Mean.
Poona	87	100	100	100	100	97
Cristaudo	17	57	64	100	100	68

In the planting the occurrence of waterlogging apparently played a leading part in the incidence of stem rot in the somewhat resistant variety Cristaudo. With the susceptible variety Poona, actual waterlogging of the soil was not necessary for severe attack.

While a high moisture level in the soil undoubtedly favours the spread of disease in soil previously not infested or only slightly infested, the position with soil carrying a heavy infestation is somewhat different. Under such conditions it is possible to get heavy losses from the disease under relatively

dry conditions. Such outbreaks have been observed on infertile hillside country in the Gympie and South Burnett areas. In order to measure this effect, an experiment was designed in which heavily infested soil was held at three different moisture levels and disease incidence recorded.

Soil was collected from an area that had been used as a disease testing garden about two months previously. This soil was thoroughly mixed and 5 lb. of the dry soil (calculated) was placed in each of 44 sterilized 7 in. pots. Eight of these pots of soil were then steam sterilized. Fourteen seeds of the variety Poona were sown in each pot and the pots placed on a bench in the glasshouse.

The soil was of a sandy nature and it was calculated that the addition of 16 fl. oz. of water would bring the soil to field capacity. Twelve of the pots of unsterilized soil were so treated (Group A). Twelve more had 12 oz. water added (Group B) and to 12 others 8 oz. was added (Group C). In addition, 3 pots of the sterilized soil were included in Group A, 3 in Group B and 2 in Group C for checking purpose.

Moisture levels were maintained by weighing every day and adding the required amount of water. It was noted in Group C that although germination was good subsequent growth of the plants was poor and the plants were often wilting prior to watering each day. In addition to stem rot caused by *Phytophthora vignae,* some losses were caused by a seedling foot rot (*Rhizoctonia* sp.) The trial was terminated 28 days after sowing. Relative incidence of the two diseases appears in Table 6.

Table 6.

EFFECT OF DIFFERENT MOISTURE LEVELS ON THE INCIDENCE OF TWO DISEASES OF COWPEA IN NATURALLY INFECTED SOIL.

Moisture Level.	Soil Treatment.	Number of Plants.	Disease Infection after 28 Days.	
			Percentage *Rhizoctonia.*	Percentage *Phytophthora* Stem Rot.
Field capacity	Unsterilized ..	140	6	57
	Sterilized	36	0	0
¾ Field capacity	Unsterilized ..	142	15	67
	Sterilized	35	0	0
½ Field capacity	Unsterilized ..	153	33	58
	Sterilized	26	0	0

The results show that while decreasing the moisture level increased the incidence of seedling foot rot caused by *Rhizoctonia* sp., there was little effect on the incidence of *Phytophthora* stem rot. This experiment further confirms the field observations recorded above that moisture level does not have a great bearing on disease incidence when the inoculum level in the soil is high.

(4) Seed-borne Infection.

While it is probable that spread by air and water accounts for the movement of the disease within districts, it is difficult to explain the simultaneous occurrence of the disease in relatively isolated areas on the basis of these disseminating agencies. In addition, outbreaks of the disease have been reported on virgin land and land which has never previously grown cowpeas. The possibility of seed transmission has therefore been considered. Although detailed observations have been carried out over the last three seasons, no trace of any pod infection has been found. All attempts to infect pods artificially on the plant have to date been unsuccessful. Moreover, it is uncommon for a severely affected plant, with the lesion well advanced up the stem, to set seed. Consequently the collection of any quantity of seed from such plants has been impossible and this has limited the scope of any investigation.

Attempts have been made to determine whether the disease is seed-borne in the true sense of the term and whether it is transmitted in the trash with cowpea seed.

Initial testing involved plating seed from diseased plants onto malt extract agar. Seed was selected for all experiments from plants which had a lesion up to the tip of the stem. Eighty seeds were selected at random from each of eight seed sources and these were further divided into lots of 20 seeds. Four different treatments were then given to these lots of seed:—

(1) Wetted in 95 per cent. alcohol and immersed in a 1 per cent. solution of sodium hypochlorite for 10 minutes followed by two washes of sterile water.

(2) Wetted in 95 per cent. alcohol, immersed in 0·1 per cent. mercuric chloride for five minutes and washed twice in sterile water.

(3) Washed twice in sterile water.

(4) Untreated.

The seed was then plated out onto malt extract agar, half the seed in each treatment being cut open prior to plating.

In every treatment a number of fungi were isolated. These were all of the quick-growing type, with species of *Alternaria* predominating. Other fungi isolated were *Macrophomina phaseoli* fairly consistently, *Phomopsis* sp., *Penicillium* sp., *Fusarium* sp. and a number unidentified. In no case was *Phytophthora* sp. isolated. However, the test cannot be considered conclusive because it is highly probable that any colony of *Phytophthora* would be quickly overgrown by one of the quicker-growing organisms and its identity lost.

The second series of experiments consisted of planting unsterilized seed from infected plants in sterilized soil. Seed lots from four different sources each totalling 100 seeds were planted in a separate box of sterilized soil in a

glasshouse away from any possible source of contamination. Of the 383 plants germinating only two became diseased, the disease being due to *Rhizoctonia* infection in the seedling stage.

The third experiment involved incorporating trash from diseased cowpea plants in sterilized soil prior to planting seed of a susceptible variety. Dead diseased plants stored for up to three months have been used effectively to inoculate soil for varietal testing purposes. However, for the experiment being described the trash used was obtained from the pods and pod stalks only of the infected plants from which seed was collected for the previous two experiments. This is the normal type of trash occasionally encountered with cowpea seed, which is, in the main, remarkably free from such contamination. Seed of the variety Poona was sown in the soil so contaminated and of the 110 plants which grew two developed a foot rot in the seedling stage. *Macrophomina phaseoli* was isolated from these diseased plants. No further loss of plants occurred. In view of the frequent occurrence of *M. phaseoli* on maturing pods and stalks it is not surprising that there are losses from this fungus.

In these experiments no evidence has been found to show that the disease is transmitted by seed. The possibility still remains, but the difficulty of obtaining any quantity of seed from infected plants handicaps further investigations. Further testing is, however, contemplated.

In order to determine the effect of seed treatment on the incidence of disease in infested soil, a small trial was conducted in the glasshouse. Soil was collected from a field of cowpeas badly diseased with stem rot and this was placed in earthenware pots. Four seed treatments were used, these being 50 per cent. thiram dust, Tetroc (chloronil 98 per cent.), Agrosan (1·0 per cent. Hg) and untreated. All dusts were applied at the rate of 2 oz. per bushel. Three pots were sown with each treatment, 10 seeds being used in each pot. In addition, three of the pots of soil were steam sterilized and sown with untreated seed. The pots were maintained at an even moisture level, and disease incidence was recorded at regular intervals. The results appear in Table 7.

Table 7.

EFFECT OF SEED TREATMENT ON INCIDENCE OF STEM ROT IN INFESTED SOIL.

Treatment.	Total Germination.	Total Infection.	
		After 15 Days.	After 30 Days.
Thiram dust (50% thiram)	28	18	28
Tetroc (98% chloronil)	29	21	29
Agrosan (1·0% Hg)	30	20	30
Untreated	26	23	26
Soil steam sterilized	29	0	0

Seed treatment apparently has no effect in reducing incidence of stem rot in infested soil.

Should evidence be obtained to support seed-borne infection, this subject of seed treatment may have to be further considered.

VII. TEMPERATURE AND DISEASE DEVELOPMENT.

During the course of varietal testing in the glasshouse it was noted that it was difficult to obtain infection during the cooler months of the year. In order to investigate this matter further an experiment was carried out to trace the percentage infection of cowpea plants during the autumn, winter and spring months.

Naturally infested soil was collected from the disease nursery and thoroughly mixed before placing in earthenware pots. A number of these pots were then sterilized to act as controls. Sowings commenced on Mar. 8, 1955, and were repeated at approximately fortnightly intervals until June 6. A further sowing was made on Sept. 16, 1955. On each occasion, eight unsterilized pots and three sterilized pots were sown with 10 seeds of the variety Poona. All pots before and after sowing were maintained by daily watering at a moisture level of 22·5 per cent., this being close to the field capacity of the somewhat sandy soil used.

During the course of the experiment daily recordings were made of the maximum and minimum soil temperature by means of thermometers inserted into sterilized soil through holes drilled half-way down the side of the pot. The

Table 8.

INCIDENCE OF STEM ROT (*P. vignae*) ON COWPEAS IN RELATION TO TIME OF SOWING.

Date of Sowing.	Percentage Infection After											
	1 Week.	2 Weeks.	3 Weeks.	4 Weeks.	5 Weeks.	6 Weeks.	7 Weeks.	8 Weeks.	10 Weeks.	12 Weeks.	14 Weeks.	16 Weeks.
8–3–55	5	34	70	80	88	90						
22–3–55	9	36	42	42	73	83						
4–4–55	7	13	41	..	51	55	..	61	65*			
18–4–55	4	12	22	24	26	35	39	44	56	57*		
11–5–55	7	11	16	20	25	33	34	34	39	41	41	41*
23–5–55	0	19	20	22	25	26	29	32	32	32	32*	
6–6–55	0	4	9	9	9	11	..	..	..	..	30*	
16–9–55	0	3	10	25	43	60	83					

*Surviving plants had reached maturity.

mean temperature for each week was calculated from these recordings. Similar recordings were made of air temperatures, but it was found that the variation between air temperature and soil temperature was very slight. The mean soil temperature has therefore been used in all comparisons.

Weekly recordings were made of the number of healthy and diseased plants in each treatment (Table 8) and of the growth of plants in the control pots. The results have been expressed graphically in Fig. 16.

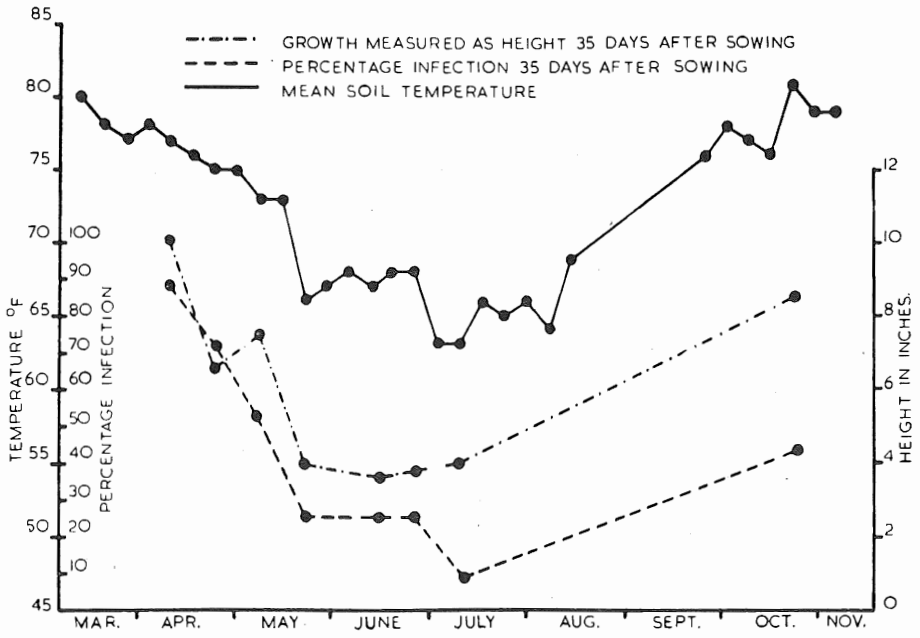

Fig. 16.

Relationship of Soil Temperature and Time of Sowing to the Incidence of Stem Rot and Rate of Growth of Cowpeas.

Stem rot caused by *P. vignae* proved the major cause of plant loss. An occasional plant died as a result of infection with *Rhizoctonia* sp., but such plants were not considered when determining the percentage infection. For the purpose of comparison a graph has been drawn to express the percentage loss 35 days after sowing in relation to the curve for the mean soil temperature. The height of plants after 35 days is also shown.

It will be seen that date of sowing has had a marked influence on both percentage infection and growth rate. That the effect has not been entirely one of delayed infection owing to slower growth of the host is demonstrated by the relatively low percentage infection at maturity for the sowings of Apr. 4 and 18, May 11 and 23 and June 6 (Table 8). Thus in these particular

sowings a greater percentage of plants survived and completed their life cycle than in the earlier sowings. The fall in infection closely follows the curve of mean soil temperature and it appears highly probable that there is a correlation between these two variables. The optimum temperature for growth of the fungus lies between 28 deg. C. and 30 deg. C. (82 deg. F. and 86 deg. F.) (Fig. 12). Although the temperature limits for growth of the fungus are comparatively wide—11–34 deg. C. (52–93 deg. F.)—it appears that the limits of its ability to function as an aggressive parasite are somewhat narrower than these.

The rise in percentage infection with the advent of warmer conditions in September and October indicates that the fungus has "overwintered" quite satisfactorily in the soil. Although there appears to be a slight initial lag in the disease incidence of the September planting, the rise in percentage infection is later particularly rapid (Table 8).

To attempt to test this effect of temperature on infection under more controlled conditions, the multi-temperature incubator used previously for determining the growth rate of *P. vignae* was employed. The 20 chambers gave a range of temperature from 0 deg. to 40 deg. C.

Nutrient agar was placed in the bottom inch of 6 in. x 1 in. flat-bottomed glass tubes and these were sterilized after plugging with cotton-wool. Two cowpea seeds of the variety Poona were then sown in each tube. The tubes were placed in sunlight until germination was complete and the plants had fully developed their seedling leaves. Five days after sowing the resulting seedlings were inoculated with *P. vignae* by placing a $\frac{1}{4}$ in. square of a P.D.A. culture on the stems just above the level of nutrient agar. Two tubes were then placed in each of the chambers of the multi-temperature incubator. Observations on lesion development were made.

Lesions appeared on the plants at 28 deg. C. on Sept. 16, and by Sept. 17 both plants had collapsed. The plants at 19·0, 21·5, 23·0, 24·5 and 26·0 deg. C. became infected and had collapsed by Sept. 21. No further lesion development was recorded. All plants above 34·0 deg. C. died, apparently as a result of high temperature conditions. The experiment was continued until Sept. 30 and by this time the remaining seedlings at all temperatures above 10 deg. C. were badly etiolated, while those below this temperature had little growth. Those results again serve to emphasise the rather narrow limits of temperatures in which aggressive parasitism of *P. vignae* can be expected—in this experiment 19–28 deg. C. (66–82 deg. F.)—compared with the range of temperature over which the fungus can grow—11–34 deg. C. (52–93 deg. F.).

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