This dissertation has been microfilmed exactly as received

BHELWA, Peter Wales, 1926–
SEED DECAY, SEEDLING BLIGHT, AND ROOT ROT OF CICER ARIETINUM CAUSED BY PHYTOPIITHORA CRYPTOGEA.

The Ohio State University, Ph.D., 1962
Agriculture, plant pathology

University Microfilms, Inc., Ann Arbor, Michigan
SEED DECAY, SEEDLING BLIGHT, AND ROOT ROT OF CICER
ARIETINUM CAUSED BY PHYTOPHTHORA CRYPTOGEA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

PETER WALES BHELWA, B.Sc., M.Sc.

The Ohio State University

1962

Approved by

O. C. Allison
Adviser
Department of Botany and
Plant Pathology
The writer wishes to express his sincere thankfulness and gratitude to Dr. C. C. Allison, his adviser, for his advice, his patience, his encouragement and reassurance, his helpful suggestions and his criticism during the course of this work. He also wishes to thank Dr. C. W. Ellett, who advised the candidate while Dr. Allison was in Southern Rhodesia as a visiting professor for a year. Thanks are also due to Dr. A. F. Schmitthenner for supplying the writer with the isolates and for his suggestions and advice on several occasions.

He would like to thank the members of the Department of Botany and Plant Pathology, both staff and graduate students, for their advice, suggestions, and criticisms during this investigation. He is deeply indebted to the Edward W. Hazen Foundation, New Haven, Connecticut, for the fellowship which enabled him to come to the United States of America for higher studies; and to the Danforth Foundation, St. Louis, Missouri, for a 'Special Study Grant' which helped him in the completion of the requirements for the degree Doctor of Philosophy.

Appreciation and thankfulness are also extended to the Principal, Dr. D. G. Moses, and to the Board of Direction, Hislop College, Nagpur (M.S.), India, for granting the writer leave from the
college for three years. Finally, the writer wishes to thank the
members of his family for their patience, understanding, and en-
couragement during the course of this research.
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>9</td>
</tr>
<tr>
<td>EXPERIMENTAL RESULTS</td>
<td>26</td>
</tr>
<tr>
<td>Inoculum production</td>
<td>26</td>
</tr>
<tr>
<td>Physiology of isolates</td>
<td>27</td>
</tr>
<tr>
<td>Utilization of carbon and nitrogen</td>
<td>27</td>
</tr>
<tr>
<td>Mycelial growth of <em>P. cryptogea</em> at different temperatures and pH values</td>
<td>33</td>
</tr>
<tr>
<td>Factors affecting the formation of sporangia, oogonia, and antheridia</td>
<td>38</td>
</tr>
<tr>
<td>Pathogenicity of single zoospore isolates of <em>P. cryptogea</em> on chick pea</td>
<td>42</td>
</tr>
<tr>
<td>Susceptibility of chick pea to different species of <em>Phytophthora</em></td>
<td>45</td>
</tr>
<tr>
<td>Symptoms of the disease</td>
<td>46</td>
</tr>
<tr>
<td>Effect of three soils on pathogenicity of <em>P. cryptogea</em> and on disease development</td>
<td>47</td>
</tr>
<tr>
<td>Host range of the pathogen</td>
<td>50</td>
</tr>
<tr>
<td>Relation of age of plants to susceptibility</td>
<td>54</td>
</tr>
<tr>
<td>Inoculum concentration in relation to disease development</td>
<td>55</td>
</tr>
<tr>
<td>Effect of <em>Fusarium</em> on the incidence of disease caused by <em>P. cryptogea</em></td>
<td>56</td>
</tr>
<tr>
<td>Hydrogen ion concentrations of soil and disease development</td>
<td>58</td>
</tr>
<tr>
<td>Factors influencing the prevalence and severity of the disease</td>
<td>58</td>
</tr>
<tr>
<td>Location of inoculum</td>
<td>59</td>
</tr>
<tr>
<td>Control measures</td>
<td>61</td>
</tr>
<tr>
<td>Seed treatment</td>
<td>61</td>
</tr>
<tr>
<td>Soil fungicides</td>
<td>53</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>68</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>78</td>
</tr>
<tr>
<td>LITERATURE CITED</td>
<td>92</td>
</tr>
<tr>
<td>AUTOBIOGRAPHY</td>
<td>85</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Mean dry weight of <em>P. cryptogea</em> on 12 different media</td>
</tr>
<tr>
<td>2</td>
<td>Analysis of variance for the data of Table 1</td>
</tr>
<tr>
<td>3</td>
<td>Mean diameter of <em>P. cryptogea</em> colonies on 3 solid media</td>
</tr>
<tr>
<td>4</td>
<td>Dry weights of <em>P. cryptogea</em> isolates 1301 and 1322 on different carbon sources</td>
</tr>
<tr>
<td>5</td>
<td>Analysis of variance for the data of Table 4</td>
</tr>
<tr>
<td>6</td>
<td>Dry weights of <em>P. cryptogea</em> isolates 1301 and 1322 on different nitrogen sources</td>
</tr>
<tr>
<td>7</td>
<td>Analysis of variance for the data of Table 6</td>
</tr>
<tr>
<td>8</td>
<td>Dry weights of <em>P. cryptogea</em> on different concentrations of sucrose in diurnal light and in complete darkness</td>
</tr>
<tr>
<td>9</td>
<td>Analysis of variance for the data of Table 8</td>
</tr>
<tr>
<td>10</td>
<td>Dry weights of <em>P. cryptogea</em> isolates 1301 and 1322 maintained at different temperatures for 14 days</td>
</tr>
<tr>
<td>11</td>
<td>Analysis of variance for the data of Table 10</td>
</tr>
<tr>
<td>12</td>
<td>Dry weights of <em>P. cryptogea</em> isolates 1301 and 1322 at different hydrogen ion concentrations</td>
</tr>
<tr>
<td>13</td>
<td>Analysis of variance for the data of Table 13</td>
</tr>
<tr>
<td>14</td>
<td>The mean and range of diameters and lengths (in microns) of oogonia and sporangia of <em>P. cryptogea</em></td>
</tr>
<tr>
<td>15</td>
<td>Pathogenicity of 85 single zoospore isolates of <em>P. cryptogea</em> on chick pea. Isolates 1281-1305 were from APS 14 (Wayne County, Ohio); isolates 1318-1348 were from APS 13 (California); and isolates 1352-1383 from APS 15 (Wood County, Ohio)</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>16</td>
<td>Susceptibility of chick pea to six species of <em>Phytophthora</em>. LBA discs with mycelium were used for infesting the soil</td>
</tr>
<tr>
<td>17</td>
<td>Disease development in different soils (loam, sand, and a mixture of 1 part of sand, 1 part of peat moss, and 2 parts of loam) infested with <em>P. cryptogea</em> isolate APS 13</td>
</tr>
<tr>
<td>18</td>
<td>Relation of soil types to disease development as measured by plant height and number of plants killed</td>
</tr>
<tr>
<td>19</td>
<td>Germination percentages of seeds of various plant species in steamed and in Phytophthora-infested soil</td>
</tr>
<tr>
<td>20</td>
<td>Number of chick pea plants killed at five different ages of plants (one week apart) for each of four weeks after inoculation</td>
</tr>
<tr>
<td>21</td>
<td>The relationship of inoculum concentration and disease incidence in infested soils using different concentrations of inoculum</td>
</tr>
<tr>
<td>22</td>
<td>Number of plants surviving 30 days after planting seeds in soil infested with <em>P. cryptogea</em> and a species of <em>Fusarium</em> at four different concentrations of inoculum</td>
</tr>
<tr>
<td>23</td>
<td>Analysis of variance for the data of Table 22</td>
</tr>
<tr>
<td>24</td>
<td>Germination percentages of seeds planted at different pH values (soil) in infested and non-infested soils. Initial pH of the soil was 7.8</td>
</tr>
<tr>
<td>25</td>
<td>Number of plants killed in relation to location of inoculum in soil</td>
</tr>
<tr>
<td>26</td>
<td>Effect of seed treatments at different inoculum concentrations</td>
</tr>
<tr>
<td>27</td>
<td>Dry weight of mycelium of <em>P. cryptogea</em> (isolate 1322) after growing on V-8 medium containing concentrations of SD 345 and SD 4741</td>
</tr>
<tr>
<td>28</td>
<td>Number of plants surviving 27 days after planting seeds in soil infested with <em>P. cryptogea</em> and treated with soil fungicides</td>
</tr>
</tbody>
</table>
TABLES--(Continued)

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>Number of plants surviving 28 days after planting seeds in treated and non-treated soil infested with <em>P. cryptogea</em>. One hundred ppm of SD 345 was used for treating the soil</td>
<td>65</td>
</tr>
<tr>
<td>30</td>
<td>Number of plants killed 30 days after soaking the roots in SD 345 and SD 4741 water solutions and planting the seedlings in soil infested with <em>P. cryptogea</em></td>
<td>66</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>Rate of mycelial growth (dry weight) of two isolates of <em>P. cryptogea</em> at different temperatures</td>
<td>35</td>
</tr>
<tr>
<td>2</td>
<td>Rate of mycelial growth (dry weight) of two isolates of <em>P. cryptogea</em> at different hydrogen ion concentrations</td>
<td>37</td>
</tr>
<tr>
<td>3</td>
<td>Morphology of <em>P. cryptogea</em> on liquid LBA medium. A. Non-papillate sporangium. B. Zoospores</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>A. Hyphal swellings, empty sporangia, and discharged zoospores (at the center of the photograph). B. Oogonium and amphigynous antheridium</td>
<td>41</td>
</tr>
<tr>
<td>5</td>
<td>A. Pre-emergence damping-off and seed decay of chick pea in soil infested with <em>P. cryptogea</em>. B. Chick pea plants at a later stage of the Phytophthora root rot. The diseased plants are stunted, necrotic, and dry.</td>
<td>48</td>
</tr>
<tr>
<td>6</td>
<td>Root rot (extensive) and crown rot of chick pea caused by <em>P. cryptogea</em></td>
<td>49</td>
</tr>
<tr>
<td>7</td>
<td>Root rot of chick pea in sterile culture</td>
<td>60</td>
</tr>
</tbody>
</table>
INTRODUCTION

Erwin and Kennedy (11) isolated Phytophthora cryptogea from rotted roots of alfalfa and in the course of checking the host range of this pathogen found Cicer arietinum L. susceptible. Since this was the only report of susceptibility of C. arietinum to P. cryptogea, Dr. A. F. Schmitthenner, Department of Botany and Plant Pathology, the Ohio Agricultural Experiment Station, Wooster, Ohio, suggested that further information on the pathogenicity and destructiveness of this pathogen on chick pea was desirable. Freszi (16) reported that P. citrophthora and P. megasperma were isolated from rot ted roots of C. arietinum. Whether or not these organisms were causal agents was not established.

Cicer arietinum, commonly known as garbanzo bean, chick pea, or gram, is indigenous to Southern Europe, where it is still extensively cultivated. It is an important food plant also in many parts of Asia, Africa, and South and Central America. The plant is a branching bushy annual which matures in about 90 days. The chick pea is one of the best legumes for human consumption, since the protein content of the seeds is about 20 to 21 per cent (9).

Phytophthora cryptogea has a wide host range, is widely distributed, and has been reported from many areas where chick pea is cultivated but chick pea has not yet been reported as a susceptible in India. On the basis of the present research the potential of the
fungal species is great, and it is quite probable that in India a very destructive disease could be caused by this fungus under conditions favorable for its establishment in a host-pathogen relationship. Consequently it is deemed desirable to obtain as much information as possible on the physiology of the fungus, the factors influencing disease development, and possible control measures.
According to Tucker (34), a species of Phytophthora isolated by Robinson in 1915 from aster was identified by Pethybridge and Lafferty as Phytophthora cryptogea on the basis of its non-papillate sporangia, scanty development of reproductive organs and its host range. Pethybridge and Lafferty (29), in 1919, recorded P. cryptogea for the first time on tomato stems. They observed non-papillate sporangia of P. cryptogea and a few in cultures, 24-50 x 17-30 μ (average 40 x 27μ), and oogonia averaging 30 μ and oospores 25μ in diameter on oat meal cultures. The growth of sporangiophores through empty sporangia and development of new sporangia within or beyond the old were observed. Ashby (1) reported on three strains of P. cryptogea which produced non-papillate sporangia and after some time oogonia in pure culture. In mixed culture with P. cinnamomi, oogonia were abundant after 9-10 days; their distribution in the growth zone of P. cryptogea indicated their production by this species; the oogonia were 24-34 μ (average 28.3μ) and oospores 19-29 μ (average 27.3μ) in diameter. Sporangia produced on a flower stalk of a Tulipa species in water averaged 43.9 x 29.8 μ and those of pure culture in water 32.3 x 22.8 μ. Pethybridge and Lafferty, and Ashby reported amphigynous antheridia.

Tucker (34) reported that his culture of P. cryptogea grew in all media that he used. No sporangia developed on solid media,
and swollen vesicles in the hyphae, resembling those sometimes formed by *P. cambivora* and very commonly by *P. cinnamomi*, were found in oatmeal agar cultures after exposure to winter temperatures at Columbia, Missouri. The oogonia averaged 25.8 μ and oospores 22.8 μ in diameter. The antheridia were amphigynous. In Petri's mineral solution, *P. cryptogea* produced non-papillate sporangia, 25-49 x 16-29 μ (average 36.7 x 21.1 μ), and sporangiophores growing through bases of evacuated sporangia.

Tucker (34) regarded *P. cryptogea* as a member of the group containing *P. cambivora*, *P. cinnamomi*, *P. erythroseptica*, *P. richardiae*, and *P. drechsleri*. He reported that *P. cryptogea* grew more rapidly at 5°C than any other isolate studied. According to Tucker, optimum growth was at 25°C and the fungus did not grow at 32.5°C. According to reports of Tucker, *P. cryptogea* differs from *P. cambivora* in temperature relations, in pathogenicity, and in size of the sexual organs; from *P. cinnamomi* in rare development of vesicles in cultures, in growth characters, and in pathogenicity; from *P. erythroseptica* in size of oospores and in temperature relations; from *P. richardiae* in pathogenicity and in temperature relations; and from *P. drechsleri* in temperature relations.

*Phytophthora cryptogea* has a wide host range and is reported from several countries mostly in temperate regions (19). Pethybridge and Lafferty (29) reported *P. cryptogea* is pathogenic to petunia, tomato, aster, wallflower, *Gilia tricolor*, potato, and *Fagus sylvatica*.

According to Middleton, Tucker, and Tompkins (25), *P. cryptogea* causes a disease of many species of the family Gesneriaceae
such as gloxinia, Achimenes cardinalis, A. grandiflora, A. longiflora, Aeschynanthus lobbibalis, A. speciosus, Alloplectus schlimii, Episcia cuprea, Gesneria cardinalis, Isoloma amabile, I. hirsutum, Naegelia cinnabarina, N. multiflora, N. sebrina, Saintpaulia ionantha, and Streptocarpus kewensis. They reported that annual stock, cineraria and Transvaal daisy are more susceptible to P. cryptogea than China- aster, cockscomb, gloxinia, godetia, and slipperwort. The fungus was isolated from Prunus amygdalus, Cotoneaster lactus, Dianthus caryophyllus, and Ceanothus prostratus. They reported the minimum temperature for mycelial growth of P. cryptogea isolated from gloxinia was below 1°C, the optimum between 22° and 25°, and the maximum between 31° and 34°C.

According to Hickman (19), P. cryptogea has been recorded on plants in 25 genera (including 9 genera of the Compositae) in 11 families of flowering plants.

Tucker (34) reported that P. cryptogea is highly pathogenic to potato tubers, tomato and eggplant fruits, and tomato seedlings and is only weakly pathogenic to apple fruits.

Erwin (10), in 1954, gave the first expanded account of Phytophthora root rot of alfalfa and identified the fungus as Phytophthora cryptogea Pethybridge and Lafferty. The disease was found in several fields in five counties in California. According to him, the cardinal temperatures for the Phytophthora species from alfalfa were 8°, 25°, and 30°-33°C. He found that the fruits of citron melon and cucumber were susceptible. Although this was the first expanded account of Phytophthora root rot of alfalfa, Erwin was of the opinion
that the disease probably was not new. Other workers in the past
had noted plants with similar symptoms but could not ascertain the
cause because of difficulty in isolation of the pathogen (10).

Bushong and Gerdemann (6), in 1959, reported a highly de-
structive foot rot disease of alfalfa in Illinois. They compared
the five isolates of Phytophthora from alfalfa in Illinois with an
isolate of P. cryptogea obtained from alfalfa in California. They
found four of the Illinois isolates to be nearly identical with the
California isolate in both morphology and pathogenicity, and one to
be different morphologically and in pathogenicity.

Erwin and Kennedy (11) reported that root rot of alfalfa
seedlings in California, due to P. cryptogea, was maximum in green-
house experiments at soil temperatures of 22° and 27°C, very little
occurred at 17°C and none at 32°C. They found that Cicer arisitum
and a species of Sesbania were also susceptible to P. cryptogea.

White and Hamilton (39), in 1935, reported that Rhododendron
maximum, R. ostawbiense, and R. carolineanum were susceptible to P.
cryptogea. In 1937, Pirone (30) reported for the first time in
New York a disease of marigold caused by the fungus.

In England, P. cryptogea has been reported to cause damping-
off and foot rot of tomatoes, asters, and dahlia; 'shanking' of
tulip; and a 'black neck' disease of Chrysanthemum frutescens (2, 3,
14, 40). In 1933, Cavisn and Muscle (7) reported pink rot of potato
caused by P. cryptogea in Ireland.

Moreau and Moreau (26), in 1957, reported a severe wilt of
cinerarias in a nursery in the Saone-et-Loir Department in France
which was caused by \textit{P. cryptogea}. In Austria, Vukovits (35), in 1956, reported a stem and leaf rot of gloxinia caused by \textit{P. parasitica} and \textit{P. cryptogea}. Weber (38) reported foot rot of tomato caused by \textit{P. cryptogea} in Denmark. According to Nicklovo-Navratilova (28), asters were also susceptible to \textit{P. cryptogea} in Czechoslovakia.

\textit{Phytophthora cryptogea} has also been reported from Australia. Brittlebank and Fish (5), in 1927, reported a wilt of tomatoes and Iceland poppies caused by the fungus. Purss (32) reported a root rot of lucerne caused by \textit{P. cryptogea} and \textit{P. parasitica}. According to Newhook (27), \textit{P. cryptogea} is one of the several \textit{Phytophthora} spp. that causes a disease of \textit{Pinus radiata} and other conifers. Potato and French marigold have also been reported susceptible to \textit{P. cryptogea} in Australia. In 1939, Brien (4) reported for the first time \textit{P. cryptogea} causing the foot rot of tomatoes in New Zealand. According to Wade (36), a damping-off of tomatoes is caused by \textit{P. cryptogea} in Tasmania.

As far as known to the writer, species of \textit{Phytophthora} have been isolated from rotted roots of \textit{Cicer aritinum} from Argentina only. Frezzi (16), in 1950, reported that \textit{P. citrophthora} and \textit{P. megasperma} were isolated from rotted roots of \textit{Cicer aritinum}. Whether or not these organisms were causal agents was not established. Pontis and Feldman (31), in 1959, reported for the first time the occurrence of a destructive crown and root rot of China-aster caused by \textit{P. cryptogea} in Argentina.

Erwin and Katzenelson (13) recently reported that \textit{P. cryptogea} grew well over a wide range of pH values (5.6-7.2) on a synthetic
medium containing sucrose as the carbon source. The fungus grew well on glucose and soluble starch, L-asparagine was the most favorable source of nitrogen, and low concentrations of CaCl$_2$.2H$_2$O promoted growth of the fungus. According to them, thiamine is necessary for the growth of *P. cryptogea* and the minimal that the fungus required was between 12 and 25 mg/liter.

Cultural practices, such as drainage and rotation of crops, have been recommended to control root rot and damping-off of several species caused by *P. cryptogea* (2, 32, 40). Steam sterilised soil is recommended wherever it is practicable. The use of Cheshunt compound (a mixture of two parts of copper sulfate and 11 parts of ammonium) as a soil fungicide has been highly recommended (3, 5, 35). Other soil fungicides that gave fairly satisfactory control of foot rot of tomatoes caused by the fungus were 1 to 2% Cuprox, 0.063% Phygon, 0.25% Formalin, 0.063% Puratized, and 3 to 4 ppm Chloropicrin (20, 21).
MATERIALS AND METHODS

Sources of isolates

Isolates of *Phytophthora cryptogea* were obtained from Dr. A. F. Schmitthenner, Department of Botany and Plant Pathology, the Ohio Agricultural Experiment Station, Wooster, Ohio. The original isolates were from alfalfa; from Erwin (10), Citrus Experiment Station, California (APS 13), from Wayne County, Ohio (APS 14), and from Wood County, Ohio (APS 15). The stock cultures were maintained on lima bean agar at 10°C. A single zoospore isolate (1322) was used in all the greenhouse experiments excepting a few in which the California isolate was used. For physiological studies isolates 1301 and 1322 were used, the former was found to be the least and the latter one of the most pathogenic.

Media used

Several media were evaluated to select a medium which would yield maximum amounts of inoculum in the shortest time. The formulae for the media are given below.

<table>
<thead>
<tr>
<th>Yeast extract</th>
<th>Nutrient broth</th>
</tr>
</thead>
<tbody>
<tr>
<td>yeast extract (Difco)</td>
<td>nutrient broth (Difco)</td>
</tr>
<tr>
<td>1.00 g</td>
<td>8.00 g</td>
</tr>
<tr>
<td>dextrose</td>
<td>dextrose</td>
</tr>
<tr>
<td>3.00 g</td>
<td>8.00 g</td>
</tr>
<tr>
<td>potassium dibasic phosphate</td>
<td>distilled water</td>
</tr>
<tr>
<td>0.50 g</td>
<td>1000 ml</td>
</tr>
<tr>
<td>distilled water</td>
<td></td>
</tr>
<tr>
<td>1000 ml</td>
<td></td>
</tr>
</tbody>
</table>
Potato dextrose broth

dextrose 20.00 g
potato 200.00 g
distilled water 1000 ml

Corn meal extract

(A) corn meal 20.0 g
 distilled water 1000 ml

(B) corn meal 20.0 g
 peptone 20.0 g
dextrose 20.0 g
distilled water 1000 ml

Oat meal extract

oat meal extract 100.0 g
distilled water 1000 ml

(The corn meal and oat meal were steeped at 60°C for one hour in 500 ml of distilled water, filtered through cheese cloth and made up to volume.)

Pea extract

dried peas 400.0 g
distilled water 1000 ml

Lima bean extract

dried lima beans 400.0 g
distilled water 1000 ml

Chick pea extract

dried chick peas 400.0 g
distilled water 1000 ml

(Peas, potatoes, lima beans, and chick peas were boiled in distilled water for about one-half hour, filtered through cheese cloth and made up to volume.)

V-8 juice broth

(A) V-8 juice 50.0 ml
distilled water 950 ml

(B) V-juice 50.0 ml
sea water (17) 950 ml

Lima bean agar medium

lima bean agar (Difco) 1.50 g
distilled water 1000 ml
The following synthetic media were tried for physiological studies.

**Richard's solution (modified)**
- distilled water: 1000 ml
- potassium nitrate: 10.0 g
- potassium dihydrogen phosphate: 5.0 g
- magnesium sulfate: 2.5 g
- ferric chloride: 0.02 g
- sucrose: 50.0 g
- thiamin: 0.02 g
- trace elements: 1.0 ml

**Raulin's solution (modified)**
- distilled water: 1500 ml
- dextrose: 70.00 g
- tartaric acid: 4.00 g
- ammonium nitrate: 4.00 g
- potassium dibasic phosphate: 0.60 g
- magnesium sulfate: 0.40 g
- ammonium sulfate: 0.25 g
- ferrous sulfate: 0.07 g
- thiamin: 0.03 g
- zinc sulfate: 0.07 g

**Csapek's sucrose nitrate solution (modified)**
- distilled water: 1000 ml
- sodium nitrate: 2.00 g
- potassium dibasic phosphate: 1.00 g
- potassium chloride: 0.50 g
- magnesium sulfate: 0.50 g
- ferrous sulfate: 0.01 g
- sucrose: 30.0 g
- thiamin: 0.02 g
- trace elements: 1.00 g

**Lopatecki's and Newton's solution**
- distilled water: 1000 ml
- dextrose: 50.00 g
- potassium monobasic phosphate: 1.00 g
- thiamin: 0.02 g
- magnesium sulfate: 0.10 g
- calcium sulfate: 0.10 g
- asparagine: 4.00 g
- ferrous sulfate: 0.001 g
- trace elements: 1.00 ml
Lopatecki's and Newton's solution was modified by incorporating sucrose in place of dextrose.

(Trace elements solution contained 1 ppm zinc and 0.02 ppm molybdenum, copper, and manganese.)

Kennedy's and Erwin's medium

- potassium monobasic phosphate 0.001 M
- potassium nitrate 0.005 M
- calcium nitrate, 4 H₂O 0.005 M
- magnesium sulfate, 7 H₂O 0.002 M

Phytophthora reisolation medium

- endomycin 10.00 mg
- chloromycetin 5.00 mg
- neomycin 50.00 mg
- yeast extract (Difco) 1.00 g
- sucrose 5.00 g
- agar 20.00 g
- V-8 juice 20.00 ml
- distilled water 1000 ml

Difco yeast extract agar

(A) potassium monobasic phosphate 5.00 g
- yeast extract 1.00 g
- dextrose 20.00 g
- agar 20.00 g
- distilled water 1000 ml

(B) potassium monobasic phosphate 5.0 g
- yeast extract 7.0 g
- dextrose 20.0 g
- agar 20.0 g
- distilled water 1000 ml

Difco lima bean agar

- lima bean agar 5.0 g
- agar 15.0 g
- distilled water 1000 ml

Measurement of growth

Fifty ml of each medium were placed in 125 ml Erlenmeyer flasks, and autoclaved at 250°F at 15 pounds pressure for 20 minutes. Discs of LBA inoculum (8-day old) of 4 mm in diameter and equal
thickness were cut with a cork borer and transferred into the media in the flasks, and kept at room temperature. At the end of eight days, the contents of each flask were filtered using Buchner's porcelain filter and Whatman's # 1 filter paper; dry weights of the mycelium were recorded after the fungus was dried in an oven at 50°C for 24 hours.

In another set of experiments, diameter of colonies of the pathogen were measured. Discs of agar, 4 mm in diameter with fungal mycelium, were transferred to the petri plates containing solid media. At the end of five days, the diameter of each colony was measured.

Infestation of soil and inoculation methods

*Phytophthora cryptogea* was increased either in a liquid medium containing 1.5 g of lima bean agar in a liter of distilled water or in a V-8 juice medium containing 50 ml of V-8 juice and 950 ml of distilled water. Fifty ml of the medium in 125 ml Erlenmeyer flasks were autoclaved for 20 minutes at 15 pounds pressure. A small piece of agar with mycelium from the stock cultures was transferred to each of the flasks and kept for eight days at room temperature.

Usually, unless otherwise specified, 50 ml of an eight-day old liquid culture were either evenly distributed over the seeds in a 4-inch pot containing about 450 ml of soil or thoroughly mixed by the hands with the same quantity of soil and seeds planted. The seeds were then covered with approximately 50 ml of soil. In a few experiments, the inoculum was poured directly over the roots, and the roots were covered with soil. Checks were treated in a similar way except
that sterile culture media were used. The clay pots had been washed and autoclaved for 12 hours at 20 pounds pressure.

Soil

Except in one experiment, soil used throughout this study was a mixture of one part of peat moss, one part of sand, and two parts of loam. In each experiment soil was autoclaved at 250°F for 12 hours at 20 pounds pressure.

Seeds

Seeds of Cicer arietinum are difficult to find in the United States. No specific variety of seeds could be obtained. Seeds used in most of the research were obtained from the Hi-Lane Market (grocery store), 2153 N. High, Columbus, Ohio. The writer thinks that the seeds used were not of any particular variety but a mixture of several varieties. The percentage of germination of seeds in all experiments was above 85.

Seeds used in the host range experiment were supplied by the personnel of the Ohio Agricultural Experiment Station, Wooster, Ohio, and the Capitol Seed and Garden Center, Columbus, Ohio.

Reisolation of the pathogen

In all greenhouse experiments the pathogen was reisolated from diseased plants and identified under the microscope. In the beginning the writer encountered difficulty in reisolating the pathogen, for the reisolations were most inconsistent because other fungi and bacteria grew much faster than the pathogen on the media used.
Dr. Schmitthenner's Phytophthora reisolation medium when used gave very good results (oral conversation).

Diseased portions of the plants were cut in one to two-inch segments and washed with detergent under running tap water for several hours. The segments were then cut in shorter pieces with a sterile razor blade and transferred to the medium.

Pathogenicity of single zoospore isolates of P. cryptogea on chick pea

Eighty-five single zoospore isolates of Phytophthora cryptogea were obtained from Dr. Schmitthenner who numbered the isolates as follows:

# 1281 - 1305 (22 isolates from AFS 14)
AFS 14 - P. cryptogea from alfalfa, Wayne County, Ohio.

# 1318 - 1348 (31 isolates from AFS 13)
AFS 13 - P. cryptogea obtained from D. C. Erwin, Citrus Experiment Station, California.

# 1352 - 1383 (32 isolates from AFS 15)
AFS 15 - P. cryptogea from alfalfa, Wood County, Ohio.

Eight-day old cultures (liquid lima bean) of the 85 single zoospore isolates were used as inoculum, and for each single zoospore isolate, three four-inch pots were used with five seeds in each. Approximately 450 ml of soil were placed in each pot, seeds were planted and 50 ml of the fungus culture were poured over the seeds which were then covered with about 50 ml of soil. Newspapers were used to cover the pots until seedlings emerged to prevent, as far as possible, any isolate from being splashed or disseminated. The pots were randomized as to position from time to time. Ten pots with five seeds each served as checks.
After three weeks when the final counts were recorded, seeds (five seeds per pot) were again planted in the pots used in the previous experiment just at the surface of the soil so as to check if the depth of the planting of seeds was related to the severity of disease.

Susceptibility of chick pea to different Phytophthora species

The susceptibility of chick pea to Phytophthora was investigated using P. parasitica from tomato, P. parasitica from peperomia, P. parasitica from gloxinia, P. megasperma var. sojae from soybean, P. cactorum from Rome Beauty apple, P. erythroseptica from potato tuber, P. cactorum from lilac, P. palmivora and P. cryptogea (isolate 1383).

The inoculum was increased on a medium of 23 g of lima bean agar and a liter of distilled water, autoclaved at 15 pounds pressure for 20 minutes. Twenty ml of this medium were transferred by means of a syringe to petri plates, and a small piece of agar with mycelium from stock culture was then transferred to the medium. The species were allowed to grow for 10 days on this medium.

Ten 4-inch pots were used for each species, and in each pot 250 ml of soil were placed and watered. Agar discs with mycelium from petri plates were placed over the soil in the pots at the rate of one disc per pot, covered with 150 ml of soil, seeds planted (five seeds per pot) and watered again. Finally, the seeds were covered with 50 ml of dry soil.
Effects of three soils on pathogenicity of
P. cryptogea and on disease development

Different soils consisting of loam, sand, and a mixture (2
parts of loam, 1 part of sand, and 1 part of peat moss) were used in
this experiment. The California isolate was increased in a liquid
lima bean medium for 15 days at which time 15 ml of the fungus slurry
were thoroughly mixed with approximately 500 ml of each kind of soil
per 4-inch pot. Ten 4-inch pots, six for treatment and four for
checks, were used for each kind of soil. One seed was planted per
pot.

In the second experiment, the same quantity of inoculum as
the first was added when the seedlings were eight days old and about
four inches tall. The number of pots for each type of soil was 10,
with one plant in each.

Host range of the pathogen

The host range of P. cryptogea was tested on 47 different
varieties and species of plants including soybean (Henry, Blackhawk,
Lincoln, and Harosoy), hairy vetch, crown vetch, alsike clover, White
Dutch clover, ladino clover, Kenland red clover, crimson clover,
yellow sweet clover, alfalfa (Atlantic, Vernal, and Dupuits), birds-
foot trefoil (Empire, Viking), tomato (Bonny Best, WR 3, and WR 7),
snapdragon, asters, castor bean, dahlia, hollyhock, lupine, sun-
flower, sweet pea, zinnia, bean, bush lima bean, beets, cabbage,
carrot, cauliflower, cucumber, egg plant, watermelon, onion, pea
(Laxtonian and Wando), peanuts (Sweet Spanish and Improved Large
Virginia), pepper, radish, pumpkin, and turnip.
Ten seeds of each variety of plant were planted in soil in 4-inch pots replicated three times. The isolates used in this experiment were single zoospore isolates 1301 and 1322. They were cultured on liquid lima bean agar for eight days. For infesting the soil, 50 ml of the culture were poured over the seeds which were then covered with about 50 ml of soil. Pots were randomized on the bench by the Random Table.

Final counts were made after six weeks from the start of the experiment. Roots of 10 plants of some of the varieties were examined and compared with the checks. Roots of all the varieties tested could not be examined because Karathane was used in the greenhouse without the knowledge of the investigator and caused, in some cases, severe foliar injury of several varieties.

The relation of age of plants to susceptibility

To investigate the relation of age of plants to susceptibility, plants of five ages, each one week apart, were used. Every week on the same day, 100 chick pea seeds were planted in 20 4-inch pots at the rate of five seeds per pot. At the end of the fifth week the pathogen was incorporated in the soil.

About 150 ml of soil were placed in a 4-inch pot, five plants were transplanted, and 50 ml of 2-week old fungus culture were poured over the roots. Finally, 250 ml of soil were added to each pot. Seven such pots were used for each age of plants and three pots were kept as checks. In the treatment in which seeds were planted at the time of infesting the soil, five seeds per pot were used.
**Inoculum concentration in relation to disease development**

Inoculum of *P. cryptogea* was increased on V-8 medium juice, with 100 ml in each 250 ml Erlenmeyer flask. Two and one-half liters of the medium were used to increase the inoculum. The cultures were incubated at room temperature for eight days. This inoculum was strained free of the medium using cheese cloth, washed with tap water, blended for 15 seconds in a Waring blender and resuspended in 2,000 ml of tap water. Portions of the 2,000 ml were diluted serially to 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256 concentrations using tap water. Concentration 1 consisted of the undiluted inoculum from the resuspended 2,000 ml. Optical density of different concentrations of the inoculum was determined using Klett-Summerson photoelectric calorimeter. Dry weight of the mycelium per 100 ml of inoculum at different concentrations was also determined.

Eight 4-inch pots were used for each concentration of inoculum and five seeds were planted in each pot. One hundred ml of the inoculum were poured over the seeds, and the seeds were covered with 50 ml of soil. In checks only inoculum medium was used.

**Effect of Fusarium on the incidence of disease caused by P. cryptogea**

Other organisms, particularly *Fusarium* species, were found associated with the pathogen in the rotted tissues of the host. A species of *Fusarium* was selected to find out whether the presence of any other organism in the soil reduces or enhances the incidence of the disease caused by *P. cryptogea*. 
Nine hundred ml of 7-day old cultures of *Fusarium* and *P. cryptogea* on V-8 juice medium were filtered through cheese cloth, the mycelial mats removed and blended in a Waring blender for 15 seconds, and resuspended in a liter of tap water. Four different concentrations of inoculum, 1, 1/2, 1/4, and 1/8, of the two fungi were used.

Fifty ml of each concentration of inoculum were used to infest the soil in each pot and five seeds planted. In one set of treatments, 50 ml of the same concentration of inoculum of *Fusarium* and *Phytophthora* species were used for infesting the soil. In all cases five pots were used for each concentration of inoculum.

**Hydrogen ion concentrations of soil and disease development**

**Determination of pH values of soil.** Fifty ml of the steam-sterilized soil were placed in a 250 ml beaker and 50 ml of distilled water were added to it. This mixture was stirred thoroughly with a glass rod, and the pH of the soil slurry was taken by a Beckman pH meter after five minutes.

**Adjustment of the pH of soil.** Saturated solutions of calcium hydroxide and dilute sulfuric acid were used to adjust the pH of soil. To the 100 ml of the soil slurry was added a certain quantity of either Ca(OH)$_2$ or H$_2$SO$_4$, and the pH of the soil adjusted. On the basis of this, the pH of 1000 ml of soil was calculated. To the 1000 ml of steam-sterilized soil was added the calculated quantity of either Ca(OH)$_2$ or H$_2$SO$_4$ and mixed thoroughly by hands, and the pH of this soil was re-checked. Eight 6-inch pots were used for each pH,
1000 ml of the soil were put in each pot, and the pots were left on a bench in the greenhouse for four weeks before further treatments.

**Infestation of soil with the pathogen.** The pH was taken again after four weeks at the time of infesting the soil. One hundred ml of 8-day old fungus culture (V-8 juice medium) were mixed thoroughly with the soil of each pot. Four pots of soil of each pH were infested, and four left as checks. They were then left for a week on the bench before planting the seeds (10 seeds per pot). All through this experiment distilled water was used for watering the plants. At the end of this experiment, the pH of the soil was re-checked.

**Factors influencing the prevalence and severity of the disease**

The ability of the pathogen to rot and kill the host in the absence of other organisms, the effect of nitrogen fertilizers on disease development, and the effect of location of inoculum on the occurrence of disease were investigated.

**Sterile culture of chick pea on agar.** Eight 500 ml wide-mouth Erlenmeyer flasks with 200 ml of *Phytophthora reisolation medium* in each were autoclaved for 20 minutes at 15 pounds pressure and 250°F. A small piece of agar with mycelium from the stock culture #1322 was transferred to the medium in six of the flasks, and two flasks were left as checks.

After eight days when the surface of the agar of the flasks was covered by the mycelium of the pathogen, one square inch water agar was placed aseptically over the mycelial mat in each flask.
Chick peas were surface sterilized with 1:4 Chlorox for two minutes and transferred to 25 ml of distilled water contained in 250 ml Erlenmeyer flask. Two days later seedlings were aseptically transferred to the wide-mouth flasks and placed over the water agar blocks at the rate of one seedling per block.

**Effect of nitrogen fertilizers on disease incidence.** In checking on the effect of nitrogen fertilizers on disease development, the inoculum was increased in 2,000 ml V-8 juice using 50 ml of the medium in 250 ml Erlenmeyer flask in which fungus was incubated for one week at room temperature. The inoculum was strained free of the medium using cheese cloth, washed with tap water, blended for 15 seconds in a Waring blender and resuspended in 2,000 ml of tap water. Fifty ml of this suspension were used per pot as inoculum.

One thousand ml of the soil were taken, the nitrogen compounds added at the rate of 100 ppm of N (200 lbs. of N per acre) (18) and thoroughly mixed by hands. Two hundred fifty ml of this soil were put in each paper cup (16 oz.), and 50 ml of inoculum were mixed. Two hundred fifty ml of the soil from the original 1000 ml containing the nitrogen fertilizer were further added to the paper cup. One day later five 8-day old chick pea seedlings were transplanted in each cup.

For each treatment, there were six cups—four treated with pathogen and nitrogen compound and two as checks with nitrogen compound alone. In addition, there was another set of checks with no treatment of any kind. The nitrogen compounds used were ammonium nitrate, ammonium sulfate, potassium nitrate, and sodium nitrate.
Location of inoculum. Tall paper cups (24 oz.) were filled with sterilized soil. Holes were made in the bottom for drainage. Eight-day old chick pea seedlings were transplanted into the cups at the rate of five seedlings per cup. Seven days after transplantation, when the roots had reached the bottoms of the cups, inoculum (on V-8 medium) was injected into the soil through the cups with a syringe at either of the two locations: one inch from the top of the cups or one inch from the bottom of the cups.

Inoculum was blended in a Waring blender for 15 seconds and resuspended in 1000 ml of tap water. Fifty ml of this suspended inoculum were injected into the soil in each paper cup. The punctures were sealed with adhesive tape. In the checks, culture medium was injected instead of the fungus resuspension.

Control measures

Seed treatment. Chick pea seeds were treated by mixing them thoroughly with individual fungicides and planted in loam soil in 4-inch pots. Fifteen pots with five seeds in each were used for each fungicide. Ten-day old inoculum of three different concentrations, 1, 1/2, and 1/4, was used for infesting the soil at the rate of five pots per concentration of inoculum. In another set, treatments consisted of the same concentrations of inoculum but the seeds were not treated with the fungicides. The soil was infested at the time of planting the seeds. The fungicides used were Ceresan, Semesan, thiram, chloranil, and captan.
Soil fungicides. Two experimental soil fungicides—SD 345 and SD 4741 were obtained from the Shell Development Company, Agricultural Research Division, Modesto, California. The chemical name of SD 345 is 2-propene-1,1-diol diacetate (allyldiene diacetate) and that of SD 4741 is O,O,O-trimethyl phosphorothionate.

SD 345 was water dispersible and contained 1.04 gram/ml of toxicant. To dissolve, the required amount of formulation was premixed in 10-20 times this amount of water. After this mixture was in solution, it was added to the remaining volume of water. SD 4741 was formulated as an emulsifiable concentrate containing 0.93 gram/ml of the toxicant. This material was readily diluted with water. Three experiments were designed to test the effectiveness of these two soil fungicides against *P. cryptogea*, one in vitro and the other two in the greenhouse.

**In vitro.** Fifty, 100, 150, 200 ppm of SD 345 and SD 4741 were incorporated in V-8 juice medium. Four mm discs of LBA with mycelium were transferred to 50 ml of the medium in 250 ml Erlenmeyer flasks. Dry weight of the mycelium was determined at the end of 14 days.

**Greenhouse experiments.** The soil was infested at the time of planting the seeds and 50, 100, 150, and 200 ppm solutions of the fungicides were poured in the pots at the rate of 100 ml of solution per pot. Eight-day old cultures of the pathogen were allowed to grow in 2500 ml of V-8 juice medium. They were then filtered through cheese cloth, the mycelial mat blended in a Waring blender for 15 seconds and resuspended in 2500 ml of tap water. Fifty ml of this fungus suspension were used as inoculum per pot.
For each concentration of the chemicals, there were two treatments with five pots each. Treatment one consisted of infesting the soil, planting the seeds and watering with 100 ml solution of the chemical. Treatment two was like treatment one but without the infestation of soil. In addition, there were two sets of checks—one consisted of infested soil without the chemical and the other with no infestation and no chemical treatment. Soil applications of water solutions (100 ml per pot) of SD 345 and SD 4741 containing 50-200 ppm were made weekly till the end of this experiment.

In another experiment, eight-day old inoculum, resuspended in 2000 ml of tap water, was used after it was standardized as in the previous experiment. Roots of eight-day old seedlings of chickpea were allowed to remain in water solutions of 50, 100, 150, and 200 ppm of SD 345 and SD 4741 for 18 hours. Six 4-inch pots were used for each treatment; four of them were infested with 50 ml of inoculum suspension and two kept as checks. Five seedlings were transplanted in each pot. In addition, seedlings, which were allowed to remain in water for 18 hours, were transplanted into three pots containing infested soil and three containing non-infested soil.
EXPERIMENTAL RESULTS

Inoculum production

Of 15 media checked, liquid lima bean agar (1.5 g LBA to a liter of distilled water) was preferred for use as the standard medium for inoculum production. Solid lima bean agar medium as such, excepting once during the course of this investigation, was not used. A comparison of the mean dry weights of mycelium on 12 liquid media and that of the diameter of the fungus colonies on three media are given in Tables 1 and 3 respectively.

Table 1. Mean dry weight of *P. cryptogea* on 12 different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Dry weight of mycelium* in gms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difco yeast extract</td>
<td>0.0100b</td>
</tr>
<tr>
<td>Difco nutrient broth</td>
<td>0.0060</td>
</tr>
<tr>
<td>Potato dextrose broth</td>
<td>0.0336</td>
</tr>
<tr>
<td>Corn meal extract (A)</td>
<td>0.0111</td>
</tr>
<tr>
<td>Corn meal extract (B)</td>
<td>0.0600</td>
</tr>
<tr>
<td>Oat meal extract</td>
<td>0.0210</td>
</tr>
<tr>
<td>Pea extract</td>
<td>0.0273</td>
</tr>
<tr>
<td>Lima bean extract</td>
<td>0.0213</td>
</tr>
<tr>
<td>Chick pea extract</td>
<td>0.0433</td>
</tr>
<tr>
<td>Liquid lima bean agar</td>
<td>0.0217</td>
</tr>
<tr>
<td>V-8 juice (A)</td>
<td>0.0351</td>
</tr>
<tr>
<td>V-8 juice (B)</td>
<td>0.0632</td>
</tr>
</tbody>
</table>

L. S. D. at 5% level = 0.0101 g

*a* Cultures maintained at room temperature for 7 days.

*b* Each value is an average of 5 replicates.
Table 2. Analysis of variance for the data of Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>22809.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replicates</td>
<td>4</td>
<td>968.07</td>
<td>242.02</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>11</td>
<td>18774.57</td>
<td>1706.78</td>
<td>24.49**</td>
</tr>
<tr>
<td>Error</td>
<td>44</td>
<td>3067.10</td>
<td>69.70</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at the 1 per cent level of probability.

Table 3. Mean diameter of \textit{P. cryptogea} colonies on 3 solid media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Diameter of colony$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difco yeast extract (A)</td>
<td>20.0 mm$^b$</td>
</tr>
<tr>
<td>Difco yeast extract (B)</td>
<td>18.1 mm</td>
</tr>
<tr>
<td>Lima bean agar</td>
<td>64.7 mm</td>
</tr>
</tbody>
</table>

$^a$Maintained at room temperature for 5 days.
$^b$Each value is an average of 5 replicates.

Of the four synthetic media used, \textit{P. cryptogea} grew only on Lopatecki's and Newton's medium and therefore this medium was used for all physiological studies.

**Physiology of isolates**

**Utilization of carbon and nitrogen.** The utilization of carbon and nitrogen by isolates 1301 and 1322 of \textit{P. cryptogea} was studied in relation to carbon and nitrogen sources and the inter-relationship of carbon supply and light.
Dextrose, sucrose, soluble starch, mannose, d-mannitol, d (+) lactose, d (+) galactose, d (-) levulose, and glucono-delta lactone were substituted for dextrose in Lopatecki's and Newton's solution for studying carbon sources; and L (+) asparagine, L arginine, DL histidine, DL tryptophan, ammonium nitrate, potassium nitrate, sodium nitrate, and yeast extract were substituted for asparagine for studying different nitrogen sources in Lopatecki's and Newton's solution (50 ml per 125 ml Erlenmeyer flask). This medium was modified slightly in the nitrogen utilization study. In this case, 50 g of sucrose was added to the medium instead of 50 g of dextrose because, in the carbon utilization study, the former was found to be the better carbon source. In both the studies, two sets of blanks were kept, one without a carbon source and the other without a nitrogen source (checks). All media were adjusted to a pH of 6.5, autoclaved and cooled, and the isolates were transferred to the media in the flasks and left at room temperature. At the end of 14 days, the contents of each flask were filtered and the pH of the filtrates and dry weights of the mycelium were determined (Tables 4 and 6).

The carbon source as well as the nitrogen source did influence the growth of the two isolates as measured by dry weights of mycelia. In the carbon utilization studies, isolate 1322 grew best with starch and second best with sucrose. Good mycelial growth also occurred on dextrose, mannose, d-mannitol, d (+) galactose, and d (+) lactose. Isolate 1301 grew best with starch, mannose, sucrose, and d (+) galactose (no significant difference) and did not grow at
Table 4. Dry weights of *P. cryptogea* isolates 1301 and 1322 on different carbon sources

<table>
<thead>
<tr>
<th>Carbon sources</th>
<th>pH before auto-claving</th>
<th>pH after auto-claving</th>
<th>pH of filtrate</th>
<th>Dry weight in gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>iso-</td>
<td>late</td>
<td>iso-</td>
<td>late</td>
</tr>
<tr>
<td>Dextrose</td>
<td>6.5</td>
<td>6.0</td>
<td>4.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>6.5</td>
<td>6.3</td>
<td>4.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Starch</td>
<td>6.5</td>
<td>6.3</td>
<td>4.6</td>
<td>5.0</td>
</tr>
<tr>
<td>Mannose</td>
<td>6.5</td>
<td>6.6</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>d- Mannitol</td>
<td>6.5</td>
<td>6.2</td>
<td>7.7</td>
<td>7.8</td>
</tr>
<tr>
<td>d (+) Lactose</td>
<td>6.5</td>
<td>6.0</td>
<td>7.3</td>
<td>6.0</td>
</tr>
<tr>
<td>d (+) Galactose</td>
<td>6.5</td>
<td>6.0</td>
<td>5.4</td>
<td>4.9</td>
</tr>
<tr>
<td>d (-) Levulose</td>
<td>6.5</td>
<td>5.6</td>
<td>4.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Glucono-delta</td>
<td>6.5</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Lactone</td>
<td>6.5</td>
<td>6.3</td>
<td>7.4</td>
<td>8.0</td>
</tr>
</tbody>
</table>

*carbon x isolates* L. S. D. at 5% level = 0.0285 g

*Maintained at room temperature for 14 days.*

*Each value is an average of 5 replicates.*

Table 5. Analysis of variance for the data of Table 4

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>99</td>
<td>257603.41</td>
<td>16027.74</td>
<td>31.75**</td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>26178.06</td>
<td>6544.50</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>9</td>
<td>144249.62</td>
<td>16027.74</td>
<td>31.75**</td>
</tr>
<tr>
<td>Isolates</td>
<td>1</td>
<td>31318.38</td>
<td>38318.38</td>
<td>62.03**</td>
</tr>
<tr>
<td>Carbon x Isolates</td>
<td>9</td>
<td>17487.93</td>
<td>1943.10</td>
<td>3.85**</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>38369.42</td>
<td>504.86</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the 1 per cent level of probability.*
all with d (-) levulose. No growth of either occurred with glucono-delta lactone, probably due to a low pH. On all carbon sources the growth of isolate 1322 was greater than the other.

In the nitrogen utilization studies, the two isolates differed not only in the ability to utilize different nitrogen compounds, but also in the amounts utilized. Using dry weights of mycelium as a criterion, isolate 1301 utilized yeast extract the best and L (+) asparagine the second best whereas isolate 1322 utilized L (+) asparagine and yeast extract almost equally and ammonium nitrate the least. The growth of isolate 1301 was greater than that of isolate 1322 on all nitrogen sources except tryptophan.

The effect of light upon the isolates was investigated using different amounts of sucrose as a carbon source. Sucrose was incorporated in 50 ml of Lopatecki's and Newton's medium in 125 ml Erlenmeyer flasks at the rate of 125 mg, 250 mg, 500 mg, 750 mg, 1000 mg, and 1500 mg. The pH was adjusted to 6.5 and isolates were transferred to the media in these flasks. One set was maintained in continuous darkness at room temperature, and the other set was exposed to alternate day and night illuminations at room temperature. After 14 days, dry weights of the mycelia and the pH of the filtrates were determined. During the experiment it was discovered that most of the cultures of isolate 1301 were contaminated, therefore dry weights of mycelium of isolate 1322 alone were obtained (Table 8).

In the study of the effects of light and different concentrations of sucrose on isolate 1322, the dry weights of the fungus increased as the amount of sucrose increased. The growth of the
Table 6. Dry weights of *P. cryptogea* isolates 1301 and 1322 on different nitrogen sources

<table>
<thead>
<tr>
<th>Nitrogen sources</th>
<th>pH before auto-claving</th>
<th>pH after auto-claving</th>
<th>pH of filtrate</th>
<th>Dry weight in gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>iso-</td>
<td>iso-</td>
<td>iso-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>late</td>
<td>late</td>
<td>late</td>
</tr>
<tr>
<td>L (+) Asparagine</td>
<td>6.5</td>
<td>6.0</td>
<td>5.2</td>
<td>5.0</td>
</tr>
<tr>
<td>L Arginine</td>
<td>6.5</td>
<td>5.8</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>DL Histidine</td>
<td>6.5</td>
<td>6.5</td>
<td>5.0</td>
<td>5.2</td>
</tr>
<tr>
<td>DL Tryptophan</td>
<td>6.5</td>
<td>6.2</td>
<td>4.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Ammonium nitrate</td>
<td>6.5</td>
<td>6.1</td>
<td>4.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>6.5</td>
<td>6.4</td>
<td>4.3</td>
<td>4.9</td>
</tr>
<tr>
<td>Sodium nitrate</td>
<td>6.5</td>
<td>6.5</td>
<td>6.3</td>
<td>6.2</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.5</td>
<td>6.3</td>
<td>4.7</td>
<td>4.7</td>
</tr>
<tr>
<td>No nitrogen</td>
<td>6.5</td>
<td>6.2</td>
<td>6.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

*nitrogen x isolates* L. S. D. at 5% level = 0.0337 g

aMaintained at room temperature for 14 days.
bEach value is an average of 5 replicates.

Table 7. Analysis of variance for the data of Table 6

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>89</td>
<td>60539.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>3176.90</td>
<td>794.22</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>8</td>
<td>22160.25</td>
<td>2770.03</td>
<td>3.89***</td>
</tr>
<tr>
<td>Isolates</td>
<td>1</td>
<td>14467.88</td>
<td>14467.88</td>
<td>20.30**</td>
</tr>
<tr>
<td>Nitrogen x isolates</td>
<td>8</td>
<td>15887.09</td>
<td>1985.88</td>
<td>2.79*</td>
</tr>
<tr>
<td>Error</td>
<td>68</td>
<td>4847.63</td>
<td>712.89</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at the 5 per cent level of probability.

**Significant at the 1 per cent level of probability.
Table 8. Dry weights of *P. cryptogea* on different concentrations of sucrose in diurnal light and in complete darkness

<table>
<thead>
<tr>
<th>Sucrose in mg/50 ml</th>
<th>pH before autoclaving</th>
<th>pH after autoclaving</th>
<th>pH of filtrate</th>
<th>Dry weight in gms.ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>light</td>
<td>dark</td>
<td>light</td>
<td>dark</td>
</tr>
<tr>
<td>125</td>
<td>6.5</td>
<td>6.0</td>
<td>5.3</td>
<td>6.6</td>
</tr>
<tr>
<td>250</td>
<td>6.5</td>
<td>6.0</td>
<td>5.5</td>
<td>4.6</td>
</tr>
<tr>
<td>500</td>
<td>6.5</td>
<td>6.0</td>
<td>4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>750</td>
<td>6.5</td>
<td>5.9</td>
<td>5.2</td>
<td>4.6</td>
</tr>
<tr>
<td>1000</td>
<td>6.5</td>
<td>5.9</td>
<td>5.0</td>
<td>4.6</td>
</tr>
<tr>
<td>1500</td>
<td>6.5</td>
<td>5.9</td>
<td>5.0</td>
<td>4.4</td>
</tr>
</tbody>
</table>

L. S. D. at 5% level = 0.0127 g

ᵃCultures maintained at room temperature for 15 days.

ᵇEach value is an average of 5 replicates.

Table 9. Analysis of variance for the data of Table 8

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>55438.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>1406.10</td>
<td>351.53</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>5</td>
<td>7633.75</td>
<td>1526.75</td>
<td>15.14**</td>
</tr>
<tr>
<td>Isolate x Light</td>
<td>1</td>
<td>41785.85</td>
<td>41785.85</td>
<td>414.45**</td>
</tr>
<tr>
<td>Carbon x Isolate</td>
<td>5</td>
<td>176.79</td>
<td>35.36</td>
<td>0.35</td>
</tr>
<tr>
<td>Error</td>
<td>44</td>
<td>4436.23</td>
<td>100.82</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at the 1 per cent level of probability.
isolate was more in darkness than in light. In this investigation no attempt was made to determine the basis of this difference.

**Mycelial growth of P. cryptogea at different temperatures and pH values.** Effect of temperature and pH on the mycelial growth of the two isolates was studied using Lopatecki's and Newton's medium (modified). The isolates were transferred to the medium (50 ml per 125 ml Erlenmeyer flask) and incubated at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, 35°, and 40°C. After 14 days, the contents of each flask were filtered, and the pH of the filtrate and the dry weight of the mycelium were recorded (Table 10).

**Table 10. Dry weights of P. cryptogea isolates 1301 and 1322 maintained at different temperatures for 14 days**

<table>
<thead>
<tr>
<th>Temperature in °C</th>
<th>pH before autoclaving</th>
<th>pH after autoclaving</th>
<th>pH of filtrate</th>
<th>Dry weight in gms.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>isolate 1301</td>
<td>isolate 1322</td>
<td>isolate 1301</td>
<td>isolate 1322</td>
</tr>
<tr>
<td>5</td>
<td>6.5</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>10</td>
<td>6.5</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>15</td>
<td>6.5</td>
<td>6.0</td>
<td>5.4</td>
<td>5.3</td>
</tr>
<tr>
<td>20</td>
<td>6.5</td>
<td>6.0</td>
<td>4.8</td>
<td>5.0</td>
</tr>
<tr>
<td>25</td>
<td>6.5</td>
<td>6.0</td>
<td>4.9</td>
<td>4.5</td>
</tr>
<tr>
<td>30</td>
<td>6.5</td>
<td>6.0</td>
<td>4.7</td>
<td>5.3</td>
</tr>
<tr>
<td>35</td>
<td>6.5</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>40</td>
<td>6.5</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature x isolate</th>
<th>L.S.D. at 5% level = 0.0258 g</th>
</tr>
</thead>
</table>

*a*Each value is an average of 5 replicates.

*No growth.*
Table 11. Analysis of variance for the data of Table 10

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>79</td>
<td>462178.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>5579.56</td>
<td>1394.89</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>7</td>
<td>376848.43</td>
<td>53835.49</td>
<td>129.19**</td>
</tr>
<tr>
<td>Isolates</td>
<td>1</td>
<td>18147.25</td>
<td>18147.25</td>
<td>43.55**</td>
</tr>
<tr>
<td>Temperatures x Isolates</td>
<td>7</td>
<td>36600.80</td>
<td>5228.69</td>
<td>12.55**</td>
</tr>
<tr>
<td>Error</td>
<td>60</td>
<td>25002.77</td>
<td>416.71</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at the 1 per cent level of probability.

The isolates grew between 10° and 30°C and their dry weights were significantly different between 10°-20° and 25°-30°C. However, there was no significant difference in dry weights of the two isolates at 20° and 25°C, which suggests that the optimum temperature for both might be 20° or 25°C. Neither isolate grew at 5°, 35°, and 40°C.

In the study of the effect of hydrogen ion concentration on mycelial growth of the two isolates, ten 500 ml aliquots of medium were adjusted to pH 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 with either HCl or NaOH. Fifty ml aliquots of medium of each pH were placed in ten 250 ml Erlenmeyer flasks and autoclaved. After autoclaving, the pH of the medium was again determined and single agar discs (4 mm in diameter) of the two isolates were transferred to individual flasks.

The pH range of the isolates was from 5 to 8 with an optimum at pH 7. Isolate 1322 grew more than isolate 1301 at pH 6 and 7, though they reacted in the same manner.
Fig. 1. Rate of mycelial growth (dry weight) of two isolates of *I. cryptogea* at different temperatures.
Table 12. Dry weights of *P. cryptogea* isolates 1301 and 1322 at different hydrogen ion concentrations

<table>
<thead>
<tr>
<th>pH before autoclaving</th>
<th>pH after autoclaving</th>
<th>pH of filtrate isolate 1301</th>
<th>pH of filtrate isolate 1322</th>
<th>Dry weight in gms. isolate 1301</th>
<th>Dry weight in gms. isolate 1322</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>4.1</td>
<td>4.3</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>5.2</td>
<td>4.6</td>
<td>4.5</td>
<td>0.040</td>
<td>0.0514</td>
</tr>
<tr>
<td>6</td>
<td>5.7</td>
<td>4.6</td>
<td>4.7</td>
<td>0.0805</td>
<td>0.1142</td>
</tr>
<tr>
<td>7</td>
<td>7.0</td>
<td>4.9</td>
<td>4.9</td>
<td>0.1082</td>
<td>0.1684</td>
</tr>
<tr>
<td>8</td>
<td>8.0</td>
<td>7.6</td>
<td>7.8</td>
<td>0.0367</td>
<td>0.0389</td>
</tr>
<tr>
<td>9</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>9.7</td>
<td>10.0</td>
<td>10.0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*isolate x pH L. S. D. at 5% level = 0.0154 g*

*aMaintained at room temperature for 14 days.*

*bEach value is an average of 5 replicates.*

*No growth.*

Table 13. Analysis of variance for the data of Table 12

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>99</td>
<td>245912.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>2951.39</td>
<td>737.85</td>
<td></td>
</tr>
<tr>
<td>Treatments</td>
<td>9</td>
<td>220417.27</td>
<td>24490.80</td>
<td>164.74**</td>
</tr>
<tr>
<td>Isolates</td>
<td>1</td>
<td>3891.21</td>
<td>3891.31</td>
<td>26.18**</td>
</tr>
<tr>
<td>Isolates x pH</td>
<td>9</td>
<td>7354.10</td>
<td>817.12</td>
<td>5.49**</td>
</tr>
<tr>
<td>Error</td>
<td>76</td>
<td>11298.16</td>
<td>148.66</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at the 1 per cent level of probability.
Fig. 2. Rate of mycelial growth (dry weight) of two isolates of *F. cryptogea* at different hydrogen ion concentrations.
Factors affecting the formation of sporangia, oogonia, and antheridia of *P. cryptogea* (isolate 1322)

Kennedy’s and Erwin’s (23) medium was used to study the factors influencing the formation of sporangia, oogonia, and antheridia of *P. cryptogea*. A thousand ml aliquot of this medium was divided into two 500 ml aliquots, and in one was incorporated $8.9 \times 10^{-5}$ M of ethylenediamine tetraacetic acid (EDTA). Fifty ml of both solutions were transferred to 125 ml Erlenmeyer flasks and autoclaved for 20 minutes at 15 pounds pressure and 250°F. Discs, one-half inch in diameter, were transferred from V-8-CaCO$_3$ and V-8-NaOH agar cultures separately to the two solutions. The V-8 agar medium consisted of 50 ml of V-8 juice, 20 g of agar and 950 ml of distilled water. The two sets of flasks were kept at 20° and 25°C on shakers.

No sporangia were observed even after 48 hours at 20°C. Due to some mechanical disorder, the temperature of the room, where the cultures were kept at 25°C, rose to 28°C and the cultures remained at this temperature for 22 hours before they were transferred to an incubator set at 25°C. These cultures were examined every 24 hours for three days but no sporangia were seen. There were many (15/100 X field) globose oogonia and amphigynous antheridia after 72 hours on the V-8-CaCO$_3$ discs, few on V-8-NaOH discs, but none in the presence of EDTA. The diameter of oogonia on CaCO$_3$ discs ranged from 32 to 38.4 with an average of 35.2.

On repeating this experiment later at 25°C and making microscope examinations after every 24 hours, many sporangia and a few oogonia were observed on CaCO$_3$ discs. A few sporangia were also
noticed on NaOH discs and on CaCO$_3$ discs kept in solution with EDTA. The average diameters and lengths are the means of 10 sporangia and 10 oogonia (Table 14).

Table 14. The mean and range of diameters and lengths (in microns) of oogonia and sporangia of P. cryptogea

<table>
<thead>
<tr>
<th>Discs with pH adjusted by</th>
<th>Size</th>
<th>Oogonia diameter</th>
<th>Sporangia</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>smallest</td>
<td>38.4 x 16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>48.0 x 17.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>largest</td>
<td>57.6 x 18.2</td>
<td></td>
</tr>
<tr>
<td>CaCO$_3$</td>
<td>smallest</td>
<td>32.0</td>
<td>48.0 x 37.5</td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>35.2</td>
<td>64.0 x 38.0</td>
</tr>
<tr>
<td></td>
<td>largest</td>
<td>38.4</td>
<td>75.0 x 38.4</td>
</tr>
<tr>
<td>CaCO$_3$ *</td>
<td>smallest</td>
<td>51.2 x 30.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medium</td>
<td>67.5 x 33.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>largest</td>
<td>82.8 x 36.8</td>
<td></td>
</tr>
</tbody>
</table>

*Basic solution plus EDTA.

According to Tucker (34), sporangia of P. cryptogea ranged from 25-49 x 16-29 $\mu$m. Middleton et al. (25) reported that the oogonia of P. cryptogea from gloxinia had a mean diameter of approximately 25 $\mu$m and sporangia with an average of 35 x 20 $\mu$m. According to Erwin (10), sporangia of P. cryptogea isolated from alfalfa ranged from 27 to 63 $\mu$m in length and from 23 to 38 $\mu$m in width; and oogonia ranged from 23 to 39 $\mu$m in diameter. Waterhouse and Blackwell (37) gave the following range of measurements: sporangia 24 to 50 by 17 to 30 $\mu$m, and oogonia 30 $\mu$m.

The sizes of sporangia and oogonia observed by the writer were similar to those of Erwin's except on discs of V-8 juice-CaCO$_3$ agar where sporangia were found to be larger.
Fig. 3. Morphology of *Phytophthora cryptogea* on liquid LBA medium. A. Non-papillate sporangium (approx. 350X). B. Zoospores (approx. 350X).
Fig. 4. A. Hyphal swellings, empty sporangia, and discharged zoospores (at the center of the photograph). B. Oogonium and amphigynous antheridium (approx. 700X). The pathogen was cultured on discs of V-8 juice CaCO₃ (pH 6.5).
Pathogenicity of single zoospore isolates of *P. cryptogea* on chick pea

Eight-day old cultures of 85 single zoospore isolates of *P. cryptogea* were used separately for infesting the soil. Seeds, at the rate of five in each pot, were planted at the time of infesting the soil. The results were recorded after three weeks from the date of planting and seeds were again planted, and the results recorded after four weeks. These data are given in Table 15.

Table 15. Pathogenicity of 85 single zoospore isolates of *P. cryptogea* on chick pea. Isolates 1281-1305 were from APS 14 (Wayne County, Ohio); isolates 1318-1348 were from APS 13 (California); and isolates 1352-1383 from APS 15 (Wood County, Ohio)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1281</td>
<td>0a</td>
<td>-</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>1283</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>1284</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1285</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>1286</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>1287</td>
<td>0</td>
<td>-</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>1288</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>1289</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>1290</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>1291</td>
<td>0</td>
<td>-</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>1292</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1293</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1294</td>
<td>0</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1295</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1296</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1297</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>1298</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>1299</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>1300</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1301*</td>
<td>6</td>
<td>0</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>1302</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>1305</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>1318</td>
<td>3</td>
<td>2</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>1319</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 15. (Continued)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
<th>No. of plants emerged</th>
<th>Replanting</th>
<th>No. of plants killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1320</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1321</td>
<td>0</td>
<td>-</td>
<td>13</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1322*</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1323</td>
<td>0</td>
<td>-</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1324</td>
<td>0</td>
<td>-</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1325</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1326</td>
<td>0</td>
<td>-</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1327</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1328</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1329</td>
<td>0</td>
<td>-</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1330</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1331</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1332</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1333</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1334</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1335</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1336</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1337</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1338</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1339</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1340</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1341</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1342</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1343</td>
<td>0</td>
<td>-</td>
<td>12</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1344</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>1345</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1346</td>
<td>1</td>
<td>1</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1347</td>
<td>0</td>
<td>-</td>
<td>13</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1348</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>1352</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1353</td>
<td>0</td>
<td>-</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1354</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1355</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>1356</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>1357</td>
<td>1</td>
<td>1</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>1358</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1359</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1360</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1361</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>1362</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>1363</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>1364</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>1365</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>
Table 15. (Continued)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1366</td>
<td>0</td>
<td>-</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>1367</td>
<td>0</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>1368</td>
<td>0</td>
<td>-</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>1369</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>1370</td>
<td>1</td>
<td>1</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>1371</td>
<td>0</td>
<td>-</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>1372</td>
<td>0</td>
<td>-</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>1373</td>
<td>0</td>
<td>-</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>1374</td>
<td>0</td>
<td>-</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>1375</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1376</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>1377</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1378</td>
<td>0</td>
<td>-</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>1379</td>
<td>0</td>
<td>-</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>1380</td>
<td>0</td>
<td>-</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>1381</td>
<td>0</td>
<td>-</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>1382</td>
<td>0</td>
<td>-</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1383</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>check</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>0</td>
</tr>
</tbody>
</table>

*aBased on 15 seeds per treatment.

*Isolates used in the physiological studies.

Pre-emergence damping-off was reduced in the second experiment when the seeds were planted just at the surface of the soil and the moisture content of the soil was regulated. However, post-emergence damping-off was severe. Most of the isolates were highly virulent, but two isolates (1301 and 1321) were less virulent than the rest. For studying the physiology of the pathogen, isolates 1301 and 1322 were selected, the former from the least and the latter from the most pathogenic group.
Susceptibility of chick pea to different species of Phytophthora

In this experiment, the susceptibility of chick pea to six different species of Phytophthora was investigated. Ten-day old LBA cultures were used for infesting the soil. Ten 4-inch pots at the rate of five seeds per pot were used for each species. Results were recorded after 36 days (Table 16).

Table 16. Susceptibility of chick pea to six species of Phytophthora. LBA discs with mycelium were used for infesting the soil.

<table>
<thead>
<tr>
<th>Phytophthora species</th>
<th>Source</th>
<th>Percentages of plants emerged</th>
<th>killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. paracitica</td>
<td>tomato</td>
<td>56a</td>
<td>6a</td>
</tr>
<tr>
<td>P. paracitica</td>
<td>peperomia</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>P. paracitica</td>
<td>gloxinia</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>P. megasperma</td>
<td>soybean</td>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>P. cactorum var. sojae</td>
<td>Rome Beauty apple</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>P. cactorum</td>
<td>lemon</td>
<td>96</td>
<td>4</td>
</tr>
<tr>
<td>P. cactorum</td>
<td>lilac</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>P. erythroseptica</td>
<td>potato tuber</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>P. palmivora</td>
<td>-</td>
<td>84</td>
<td>0</td>
</tr>
<tr>
<td>P. cryptogea</td>
<td>alfalfa (#1383)</td>
<td>80</td>
<td>74</td>
</tr>
<tr>
<td>Check</td>
<td></td>
<td>92</td>
<td>0</td>
</tr>
</tbody>
</table>

*Based on 50 seeds per treatment.

From the results it is inferred that in addition to P. cryptogea, P. paracitica was also pathogenic on chick pea. P. paracitica (tomato isolate) was reisolated from the diseased plants, cultured for 10 days on liquid LBA medium, and was used for infesting
steam-sterilized soil. Ten 4-inch pots with five chick pea seeds in each were used. At the end of 14 days, 82 per cent pre-emergence damping-off was recorded.

**Symptoms of the disease**

There are three phases of the disease of *Cicer arietinum* caused by *P. cryptogea*—pre-emergence damping-off and seed decay, post-emergence damping-off, and root and stem rot associated with varying degrees of foliage chlorosis and wilting.

**Pre-emergence damping-off and seed decay.** In the greenhouse experiments, pre-emergence damping-off and seed decay was evident by fewer seedlings in infested soil than in non-infested soil. The pathogen seems to cause a rapid killing of cells and disintegration of the tissue. The damage may be complete before emergence of the seedling above ground, or the disease may continue to develop after emergence, in which case it would be a post-emergence damping-off.

**Post-emergence damping-off.** The post-emergence damping-off phase of the disease results in the death of seedlings after the seedlings emerge above the surface of soil. The leaves do not completely open and seedlings are water-soaked and discolored. Desiccation of the seedlings occurs in 2 to 4 days and they turn brown and shrink conspicuously. Young, diseased seedlings are best to use in isolating the pathogen. If older plants are used, considerable difficulty is encountered because of the rapid growth of secondary organisms, especially species of *Fusarium*.

**Root and collar rot.** Root rot, collar rot and wilting are symptoms of those plants that survive the early seedling stages of
the disease. The first symptom is marked stunting, associated with chlorosis of the foliage and wilting of the plants. Chlorosis occurs first in the lower leaves and the patches of yellow tissue are evident at the periphery of the leaflets. Gradually, the entire leaflet becomes chlorotic, dry and brittle, but does not abscise.

Most of the secondary roots as well as the tap root are destroyed. The roots are so severely decayed that most break when plants are pulled from the soil. Collar rot is very conspicuous at the soil level, the portion of the stem at this level becomes dark, a canker develops and the plant falls. Dark lesions on the stem do not usually extend more than 1 or 2 inches above the soil level because of the rapid death of the plant. The symptoms are illustrated in Figures 5 and 6.

Effect of three soils on pathogenicity of P. oryptogea and on disease development

The pathogenicity of P. cryptogea was tested on chick pea using three different soils. The results of the first experiment, in which seeds were planted at the time of infesting the soils, were recorded three weeks after planting (Table 17). Six pots with one plant in each were used for each soil. No non-inoculated check plants were diseased.

In another experiment, the roots were inoculated with the pathogen when the plants growing in the three different soils were approximately four inches tall and eight days old. Heights of the dead as well as the check plants were recorded three weeks after inoculation. Average heights and the number of the plants killed are presented in Table 18. No non-inoculated check plants were diseased.
Fig. 5. A. Pre-emergence damping-off and seed decay of chick pea in soil infested with *P. cryptogea*. Check plants at the back row. B. Chick pea plants at a later stage of the Phytophthora root rot. The diseased plants are stunted, necrotic, and dry. Check plants at the back row.
Fig. 6. Root rot (extensive) and crown rot of chick pea caused by *P. cryptogea*. Check plant on the left.
Table 17. Disease development in different soils (loam, sand, and a mixture of 1 part of sand, 1 part of peat moss, and 2 parts of loam) infested with *P. cryptogea* isolate APS 13

<table>
<thead>
<tr>
<th>Soil</th>
<th>No. of plants emerged</th>
<th>No. of plants killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sand</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Mixture</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

*Six seeds per treatment.*

Table 18. Relation of soil types to disease development as measured by plant height and number of plants killed

<table>
<thead>
<tr>
<th>Soil</th>
<th>Height* (mean)</th>
<th>No. of plants measured</th>
<th>No. of plants killed</th>
<th>Height* (mean)</th>
<th>No. of plants measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam</td>
<td>6.5**</td>
<td>8</td>
<td>10</td>
<td>11.0</td>
<td>5</td>
</tr>
<tr>
<td>Sand</td>
<td>7.2</td>
<td>9</td>
<td>9</td>
<td>11.2</td>
<td>5</td>
</tr>
<tr>
<td>Mixture</td>
<td>8.8</td>
<td>10</td>
<td>10</td>
<td>15.4</td>
<td>6</td>
</tr>
</tbody>
</table>

*In inches.

**Ten plants in each type of soil.

From the results of these two experiments at the inoculum level used, *P. cryptogea* appears to be highly pathogenic causing pre- or post-emergence damping-off or influencing height of *Cicer arietinum* even in different soils.

**Host range of the pathogen**

Host range of *P. cryptogea* was tested on 47 varieties and species of plants using isolates 1301 and 1322. Fifty ml of 8-day old cultures were used as inoculum per pot. Six weeks after planting
the seeds the plants were carefully pulled, the roots examined and observations recorded. It may be mentioned here that the plants were accidently sprayed with Karathane without the knowledge of the investigator. Consequently, several plants had foliar injury and because of this roots were not examined.

Table 19. Germination percentages of seeds of various plant species in steamed and in Phytophthora-infested soil

<table>
<thead>
<tr>
<th>Plant Variety</th>
<th>Germination % of seeds planted</th>
<th>Root examination based on 10 plants/isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steamed soil</td>
<td>Infested soil</td>
</tr>
</tbody>
</table>
|               | isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate isolate iso...
Table 19. (Continued)

<table>
<thead>
<tr>
<th>Plant Variety</th>
<th>Steamed soil</th>
<th>Infested soil</th>
<th>Root examination based on 10 plants/isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>isolate 1301</td>
<td>isolate 1322</td>
</tr>
<tr>
<td></td>
<td></td>
<td>isolate 1301</td>
<td>isolate 1322</td>
</tr>
<tr>
<td>tomato, Bonny Best</td>
<td>96.6</td>
<td>100.0</td>
<td>none</td>
</tr>
<tr>
<td>WR 3</td>
<td>86.6</td>
<td>100.0</td>
<td>none</td>
</tr>
<tr>
<td>WR 7</td>
<td>96.6</td>
<td>86.6</td>
<td>none</td>
</tr>
<tr>
<td>snapdragon, New Rocket Snap</td>
<td>60.0</td>
<td>63.3</td>
<td>none</td>
</tr>
<tr>
<td>asters, Semple's</td>
<td>53.3</td>
<td>46.6</td>
<td>none</td>
</tr>
<tr>
<td>Giant Branching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>castor bean, Finest</td>
<td>62.3</td>
<td>70.0</td>
<td>none</td>
</tr>
<tr>
<td>Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dahlia, Urwin's</td>
<td>60.0</td>
<td>60.0</td>
<td>none</td>
</tr>
<tr>
<td>Dwarf Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hollyhock, Charter's</td>
<td>56.6</td>
<td>70.0</td>
<td>none</td>
</tr>
<tr>
<td>Double</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lupine, Russell</td>
<td>46.6</td>
<td>60.0</td>
<td>none</td>
</tr>
<tr>
<td>Prize Mixed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sunflower, Sungold</td>
<td>96.6</td>
<td>93.3</td>
<td>none</td>
</tr>
<tr>
<td>sweet pea, Mixed Colors</td>
<td>73.3</td>
<td>70.0</td>
<td>none</td>
</tr>
<tr>
<td>zinnia, Pink Lady</td>
<td>76.6</td>
<td>80.0</td>
<td>none</td>
</tr>
<tr>
<td>bean, Tenderpod</td>
<td>83.3</td>
<td>80.0</td>
<td>none</td>
</tr>
<tr>
<td>bush lima bean, Burpee's Imp.</td>
<td>80.0</td>
<td>90.0</td>
<td>none</td>
</tr>
<tr>
<td>beets, Crosby's Imp.</td>
<td>83.3</td>
<td>100.0</td>
<td>none</td>
</tr>
<tr>
<td>Egyptian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cabbage, Marion</td>
<td>93.3</td>
<td>83.3</td>
<td>none</td>
</tr>
<tr>
<td>Market</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carrot, Red Color</td>
<td>76.6</td>
<td>80.0</td>
<td>none</td>
</tr>
<tr>
<td>Denver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cauliflower, Super Snow Ball</td>
<td>86.6</td>
<td>96.6</td>
<td>none</td>
</tr>
<tr>
<td>cucumber, Ohio MR 17</td>
<td>93.3</td>
<td>80.0</td>
<td>none</td>
</tr>
<tr>
<td>egg plant, Black Beauty</td>
<td>80.0</td>
<td>70.0</td>
<td>none</td>
</tr>
<tr>
<td>watermelon, Dixie Queen</td>
<td>83.3</td>
<td>86.6</td>
<td>none</td>
</tr>
<tr>
<td>onion, Evergreen White Bunching</td>
<td>86.6</td>
<td>56.6</td>
<td>50.0</td>
</tr>
<tr>
<td>pea, Laxtonian</td>
<td>100.0</td>
<td>76.6</td>
<td>20.0</td>
</tr>
<tr>
<td>Wando</td>
<td>100.0</td>
<td>100.0</td>
<td>83.3</td>
</tr>
</tbody>
</table>

Canker at collar region in 4 plants in 4 out of 5 plants
### Table 19. (Continued)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Variety</th>
<th>Steamed soil</th>
<th>Infested soil isolate 1301</th>
<th>isolate 1322</th>
<th>Root examination based on 10 plants/isolate isolate 1301</th>
<th>isolate 1322</th>
</tr>
</thead>
<tbody>
<tr>
<td>peanuts, Sweet Spanish</td>
<td>Improved Large Virginia</td>
<td>90.0</td>
<td>86.6</td>
<td>86.6</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>pepper, Small Cayenne</td>
<td>radish, Cincinnati Market</td>
<td>63.3</td>
<td>76.6</td>
<td>53.3</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>pumpkin, Small Sweet</td>
<td>turnip, Purple Top White Globe</td>
<td>93.3</td>
<td>100.0</td>
<td>93.3</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96.6</td>
<td>93.3</td>
<td>100.0</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

*No root examination.*

*Based on 30 seeds per plant.*

If seed decay and pre-emergence damping-off are used as criteria, host range of isolates 1301 and 1322 was tested on 47 varieties and species of plants. The percentages of germination of seeds of soybean (Blackhawk), hairy vetch, ladino clover, Kenland red clover, yellow sweet clover, Empire birdsfoot, and onion (Evergreen White Bunching) were low in soils infested with the isolates. The percentages of germination of seeds of crimson clover, alfalfa (Atlantic and Dupuits), sweet pea (Mixed Colors), and pea (Laxtonian and Wando) were low in the soil infested with isolate 1322. Alfalfa (Atlantic and Dupuits) and pea (Laxtonian) seem quite susceptible to isolate 1322.
The relation of age of plants to susceptibility

The relation of age of plants to susceptibility was investigated using plants of five ages, each one week apart. Five plants were transplanted to each 4-inch pot. Ten pots were used for each planting date, with three used as checks. At the end of the fourth week, the plants were inoculated by pouring 50 ml of 2-week old culture of isolate 1322 of P. cryptogea over the roots. The number of plants killed was counted each week for four weeks after the soil was infested (Table 20).

Table 20. Number of chick pea plants killed at five different ages of plants (one week apart) for each of four weeks after inoculation

<table>
<thead>
<tr>
<th>Weeks after inoculation</th>
<th>Age of plants at the time of inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0(^a)</td>
</tr>
<tr>
<td>1</td>
<td>27(^b)</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>Check</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\)Infestation of soil at the time of planting the seeds.

\(^b\)Based on 35 plants for each age.

The pathogen caused not only a severe seed decay and pre-emergence damping-off but also killed one, two, and three-week old plants. The killing of plants was less in older plants in the first two weeks after inoculation, but as the time of inoculation increased, the number of plants killed among older plants became high also. The
incidence of disease was least in the oldest plants which were four weeks old at the time of inoculation.

Inoculum concentration in relation to disease development

The relationship of inoculum concentration and disease incidence was investigated using soils with different concentrations of inoculum. One hundred ml of inoculum suspension were used for each pot. Eight 4-inch pots with five seeds in each were used for each concentration of inoculum. Results were recorded 38 days after infesting the soil and planting the seeds (Table 21).

Table 21. The relationship of inoculum concentration and disease incidence in infested soils using different concentrations of inoculum

<table>
<thead>
<tr>
<th>Concentration</th>
<th>mg of inoculum per pot</th>
<th>Klett reading</th>
<th>Percentages of plants emerged</th>
<th>Percentages of plants killed (post-emergence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>230.0</td>
<td>130.0</td>
<td>35.0a</td>
<td>32.5a</td>
</tr>
<tr>
<td>1/2</td>
<td>115.0</td>
<td>65.0</td>
<td>55.0</td>
<td>47.5</td>
</tr>
<tr>
<td>1/4</td>
<td>57.0</td>
<td>32.0</td>
<td>82.5</td>
<td>42.5</td>
</tr>
<tr>
<td>1/8</td>
<td>28.0</td>
<td>16.0</td>
<td>87.5</td>
<td>0.0</td>
</tr>
<tr>
<td>1/16</td>
<td>14.0</td>
<td>8.0</td>
<td>90.0</td>
<td>0.0</td>
</tr>
<tr>
<td>1/32</td>
<td>7.0</td>
<td>4.0</td>
<td>92.5</td>
<td>0.0</td>
</tr>
<tr>
<td>1/64</td>
<td>3.5</td>
<td>2.0</td>
<td>92.5</td>
<td>0.0</td>
</tr>
<tr>
<td>1/128</td>
<td>1.7</td>
<td>1.0</td>
<td>82.5</td>
<td>2.5</td>
</tr>
<tr>
<td>1/256</td>
<td>1.0</td>
<td>0.5</td>
<td>77.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Check</td>
<td>0.0</td>
<td>0.0</td>
<td>95.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Based on 40 seeds for each concentration.

As was expected, the severity of disease is definitely dependent upon the inoculum level. Disease incidence was greatest at 1, 1/2, and 1/4 concentrations of inoculum. The percentage of plants
killed after emergence at lower concentrations of inoculum was either very low or zero. This might have been due to the fact that this experiment was during summer (August 11 through September 19) and high temperature in the greenhouse on several days (100°F) might have checked the growth of the pathogen.

Effect of Fusarium on the incidence of disease caused by *P. cryptogea*

*Phytophthora cryptogea* and a species of *Fusarium* were used for infesting the soil at four concentrations (1, 1/2, 1/4, and 1/8). In one set, equal quantities of inoculum suspension (50 ml per pot) of different concentrations of *P. cryptogea* and *Fusarium* were used and in the other two sets of the experiment, both fungi were used separately for infesting the soil at different concentrations. Results of this experiment were recorded 30 days after infesting the soil and planting the seeds (Table 22). No plants were diseased in non-infested soil.

Both, *P. cryptogea* and *Fusarium*, were reisolated from diseased plants. The species of *Fusarium* used in this experiment was not identified. The symptoms of the disease caused by the *Fusarium* were: wilting and vascular discoloration. *Fusarium lateritium* f. *ciceri* has been reported to cause a wilt disease of *Cicer arietinum* in the United States (12).

The object of this experiment was to find out if the disease complex was in any way influenced by the presence of other organisms in the soil. The severity of the disease was high when both the fungi were in the soil, which might have been due to the fact that
Table 22. Number of plants surviving 30 days after planting seeds in soil infested with *P. oryptogea* and a species of *Fusarium* at four different concentrations of inoculum

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Number of plants surviving at concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em>Phytophthora</em> +</td>
<td>11*</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td></td>
</tr>
<tr>
<td><em>Phytophthora</em></td>
<td>14</td>
</tr>
<tr>
<td><em>Fusarium</em></td>
<td>14</td>
</tr>
<tr>
<td>Checks</td>
<td>25</td>
</tr>
</tbody>
</table>

L. S. D. at 5% level = 1.38

*a*Based on 25 seeds per concentration.

Table 23. Analysis of variance for the data of Table 22

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Sums of squares</th>
<th>Means of squares</th>
<th>Calculated F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>59</td>
<td>102.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replications</td>
<td>4</td>
<td>8.10</td>
<td>2.02</td>
<td></td>
</tr>
<tr>
<td>Isolates</td>
<td>2</td>
<td>3.64</td>
<td>1.82</td>
<td>1.50</td>
</tr>
<tr>
<td>Concentrations</td>
<td>3</td>
<td>23.60</td>
<td>7.86</td>
<td>6.55**</td>
</tr>
<tr>
<td>Concentrations x Isolates</td>
<td>6</td>
<td>14.10</td>
<td>2.32</td>
<td>1.91</td>
</tr>
<tr>
<td>Error</td>
<td>44</td>
<td>53.50</td>
<td>1.21</td>
<td></td>
</tr>
</tbody>
</table>

**Significant at the 1 per cent level of probability.
both were pathogenic on the host. Less development of the disease in Phytophthora-infested soil might have been due to the high temperature in the greenhouse during the summer.

Hydrogen ion concentrations of soil and disease development

Steam-sterilized soil was adjusted to five different pH values with either sulfuric acid or calcium hydroxide. The pots with the soils at the different pH values were left on a bench in the greenhouse for four weeks before infesting the soils with the pathogen, at which time the pH of the soils was again determined. Ten chick pea seeds per pot were planted one week after infesting the soils. At the end of the experiment, the pH of the soils was rechecked. The results were recorded 21 days after planting the seeds and are given in Table 2. Number of pots used for each pH value was eight, four with infested and four with non-infested soil.

Apparently, the adjusted pH values of the soil could not be maintained. However, the pH-range at the time of infesting the soil was fairly wide, which suggests that the pathogen is capable of growing and causing disease in a soil of wide pH range.

Factors influencing the prevalence and severity of the disease

The sterile culture technique was used to study the invasion, colonization and growth of the pathogen in the environment of the host. Invasion by the pathogen seemed to occur directly as soon as the root-tip of the host came in contact with the former resulting
Table 24. Germination percentages of seeds planted at different pH values (soil) in infested and non-infested soils. Initial pH of the soil was 7.8

<table>
<thead>
<tr>
<th>pH after adjusting</th>
<th>pH at the time of infesting</th>
<th>pH at the end of the experiment</th>
<th>Germination Percentages of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Infested</td>
<td>Non-infested</td>
</tr>
<tr>
<td>2.3</td>
<td>4.0</td>
<td>6.9</td>
<td>6.4</td>
</tr>
<tr>
<td>4.5</td>
<td>6.3</td>
<td>7.3</td>
<td>7.1</td>
</tr>
<tr>
<td>5.6</td>
<td>7.2</td>
<td>7.6</td>
<td>7.3</td>
</tr>
<tr>
<td>10.3</td>
<td>8.4</td>
<td>8.2</td>
<td>8.2</td>
</tr>
<tr>
<td>12.1</td>
<td>8.7</td>
<td>8.3</td>
<td>8.5</td>
</tr>
<tr>
<td>7.8 (check) 7.8</td>
<td></td>
<td>7.8</td>
<td>7.4</td>
</tr>
</tbody>
</table>

\(^a\)Based on 40 seeds per treatment.

in the rotting of the root-tip. The rotting progressed as the pathogen advanced in the host, and the mycelial growth of the pathogen was most in the immediate surroundings of the root-tip (Figure 7).

The differences in the severity of disease in relation to nitrogen were not significant. The incidence of disease was high in all cases. The ammonium compounds were used because they have been reported to be toxic to several Phytophthora species, such as *P. infestans* (15) and *P. phaseoli* (35).

Location of inoculum. To determine if the location of inoculum is an important factor in the development of the disease, 50 ml of the inoculum suspension were injected into the soil in tall paper cups at the top and at the bottom when the roots of the plants had reached the bottom. Ten cups were used for each location, seven with inoculum and three as checks with five plants in each. These
Fig. 7. Root rot of chick pea in sterile culture. Note the growth of the pathogen around the root tip and the rotting caused by it.
data were recorded 40 days after injecting the inoculum into the soil and are given in Table 25.

Table 25. Number of plants killed in relation to location of inoculum in soil. Number of plants per cup was five

<table>
<thead>
<tr>
<th>Top (1 inch below soil level)</th>
<th>Bottom (6 inches below soil level)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Check</td>
<td>0</td>
</tr>
</tbody>
</table>

Average 2.29 0.85

't' value significant at 1% level

*Based on 35 plants.

More plants were killed when the inoculum was located at the crown level of the plants which suggests that unless rotting occurs at this level, plants are not killed. The development of the disease was less than expected at both locations (1 inch and 6 inches below soil level) which could have been due to high temperatures in the greenhouse during summer.

Control measures

Seed treatment. Chick pea seeds were treated with Ceresan, Semesan, thiram, chloranil, and captan. Three levels of inoculum (1, 1/2, and 1/4) were used for infesting the soil. Seeds treated
with each of these fungicides were planted at the rate of five seeds per pot and the number of pots for each level of inoculum was five. The results of this experiment were recorded 30 days after infesting the soil and planting the seeds and are given in Table 26.

Table 26. Effect of seed treatments at different inoculum concentrations

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Percentage emergence at concentration</th>
<th>Percentage post-emergence kill at concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td>Ceresan</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Semesan</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>thiram</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>chloranil</td>
<td>44</td>
<td>60</td>
</tr>
<tr>
<td>captan</td>
<td>60</td>
<td>76</td>
</tr>
<tr>
<td>infested soil</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>no seed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-infested</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>soil, no seed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Based on five pots of five seeds each.

b Inoculum concentration 1 consisted of one 50 ml, 8-day old V-8 juice culture blended 15 seconds.

The plants were stunted and bushy (only 5 inches tall as compared to an average height of 12 inches of other plants) in the case of Ceresan-treated seeds. Apparently, Ceresan caused injury to the seeds and to the plants. This injurious effect of this fungicide might have been the cause of the 100% non-emergence of the seedlings in the 1/4 inoculum concentration treatment. The pre-emergence
damping-off was much less in the treatments where the seeds were
treated with the fungicides. Post-emergence kill was high in all
seed treatments except captan.

**Soil fungicides in vitro.** Four different concentrations—
50, 100, 150, and 200 ppm, of SD 345 and SD 4741 were incorporated
in V-8 juice medium for testing the effectiveness of these chemicals
against the pathogen. SD 345 was found to be very effective, since
no growth occurred in any of the four concentrations. Dry weight of
the mycelium on cultures containing the fungicides was determined
at the end of 14 days.

Table 27. Dry weight of mycelium of *P. cryptogea* (isolate 1322)
after growing on V-8 medium containing concentrations of SD 345
and SD 4741

<table>
<thead>
<tr>
<th>Concentration in ppm</th>
<th>Dry weight of mycelium in mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD 4741</td>
</tr>
<tr>
<td>50</td>
<td>28.5^a</td>
</tr>
<tr>
<td>100</td>
<td>26.5</td>
</tr>
<tr>
<td>150</td>
<td>23.7</td>
</tr>
<tr>
<td>200</td>
<td>20.2</td>
</tr>
<tr>
<td>Check</td>
<td>43.0</td>
</tr>
</tbody>
</table>

^aEach value is an average of three replicates incubated
14 days at room temperature.

**Soil fungicides in greenhouse experiments.** Five chick pea
seeds per pot were planted in soil infested with the pathogen. Water
solutions at 50, 100, 150, and 200 ppm of SD 345 and SD 4741 were
poured in the pots at the rate of 100 ml per pot. For each
concentration of the chemicals, there were two treatments with five pots each. Treatment one consisted of infesting the soil, planting the seeds and applying 100 ml of the water solution of the chemicals. Treatment two was like treatment one but without the infestation of the soil. In addition, there were two sets of checks; one consisted of the infested soil and no chemical treatment and the other with no infestation and no chemical treatment. The final results were recorded after 27 days and are given in Table 28.

Table 28. Number of plants surviving 27 days after planting seeds in soil infested with *P. oryptogea* and treated with soil fungicides

<table>
<thead>
<tr>
<th>Concentration in ppm</th>
<th>SD 345</th>
<th></th>
<th>SD 4741</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emergence</td>
<td>Post-emergence</td>
<td>Height (in.)</td>
<td>Emergence</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>5</td>
<td>8.8</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>17</td>
<td>12</td>
<td>8.5</td>
<td>1</td>
</tr>
<tr>
<td>150</td>
<td>14</td>
<td>6</td>
<td>5.2</td>
<td>1</td>
</tr>
<tr>
<td>200</td>
<td>21</td>
<td>3</td>
<td>4.3</td>
<td>3</td>
</tr>
<tr>
<td>Check</td>
<td>25</td>
<td>0</td>
<td>8.9</td>
<td>25</td>
</tr>
</tbody>
</table>

*a* Number emerged out of a total of 25 seeds.

*b* Eighteen days after planting.

SD 4741 was not effective against the pathogen whereas SD 345 gave fairly good control of seed decay at 100, 150, and 200 ppm. It was phytotoxic at 150 and 200 ppm and caused a severe stunting of the plants.

Since 100 ppm of SD 345 was found to be noninjurious to the host and also effective against the pathogen, the possibility of best
controlling the disease was investigated using this concentration of
the chemical. Two-week old cultures of 1, 1/2, 1/4, and 1/8 con-
centrations of inoculum of the pathogen were used for each concen-
tration of the inoculum. Treatment one consisted of infesting the
soil with the inoculum and watering this with 100 ml of the soil
fungicide while treatment two was without chemical treatment. A
week later, 100 ml of the water solution of the chemical were poured
again in pots belonging to the treatment one and the seeds planted.
In addition, there were two sets of checks: one treated with the
chemical and the other without. One hundred ml of 100 ppm water
solution of the fungicide were added to each pot once a week. Re-
sults were recorded 28 days after planting the seeds (Table 29).

Table 29. Number of plants surviving 28 days after planting seeds in
treated and non-treated soil infested with \(P. \) cryptogae. One hundred
ppm of SD 345 was used for treating the soil

<table>
<thead>
<tr>
<th>Inoculum concentration</th>
<th>Soil treated with SD 345</th>
<th>Soil not treated with SD 345</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emergence</td>
<td>Post-emergence kill</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>1/2</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>1/4</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>1/8</td>
<td>22</td>
<td>1</td>
</tr>
<tr>
<td>Check</td>
<td>23</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\)Number emerged out of a total of 25 seeds.
SD 345 at 100 ppm, when used twice for drenching the infested soil before planting the chick pea seeds, resulted in excellent control of the disease. No difference of any kind was noticed between the chemically treated check plants and those that were not treated with the chemical.

In another experiment, roots of 8-day old seedlings of chick pea were allowed to remain in water solutions of 0, 50, 100, 150, and 200 ppm of SD 345 and SD 4741 for 18 hours, after which they were planted in infested and non-infested soil at the rate of five plants per pot. Results of this experiment were recorded 30 days after planting and are given in Table 30.

Table 30. Number of plants killed 30 days after soaking the roots in SD 345 and SD 4741 water solutions and planting the seedlings in soil infested with *P. cryptogea*

<table>
<thead>
<tr>
<th>Fungicides in ppm</th>
<th>Infested soil</th>
<th>Non-infested soil</th>
<th>Infested soil</th>
<th>Non-infested soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD 345</td>
<td>SD 4741</td>
<td>SD 345</td>
<td>SD 4741</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>3</td>
<td>4</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>100</td>
<td>3</td>
<td>3</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>150</td>
<td>2</td>
<td>3</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
<td>3</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
</table>

*a*Total of 15 plants used for 0 treatment, 20 plants used for other treatments.  

*b*Total of 10 plants used.
SD 4741 was not effective against the pathogen as compared to SD 345. However, SD 345 was phytotoxic because plants treated with SD 345 and planted in non-infested soil were also killed. This might be because the time (18 hours) was too long for the seedlings to be in water solutions, and it could very well be that the number of plants injured would have been reduced if the plants had remained in the solutions for less than 18 hours.
DISCUSSION

This investigation deals with a disease of *Cicer arietinum* L. caused by *Phytophthora cryptogea* Pethybridge and Lafferty. According to Frezzi (16), two *Phytophthora* species, namely *P. citrophthora* and *P. megasperma* have been isolated from rotted roots of *Cicer arietinum*. Whether or not these organisms were causal agents was not established. Erwin and Kennedy (11), while testing the host range of *P. cryptogea* isolated from rotted roots of alfalfa, discovered that the fungus was pathogenic on chick pea.

*Phytophthora cryptogea* has a wide host range, is widely distributed and has been reported from many areas where chick pea is cultivated. In this investigation *P. cryptogea* was found to be pathogenic and destructive to chick pea. So far, it has not been reported to cause a disease of this legume in the fields, but the very fact that the potential of this pathogen is great and that it occurs in many parts where chick pea is cultivated, the danger of this fungus causing a disease of chick pea is great. It is also probable that a disease occurs but has not been discovered. This was true of alfalfa root rot caused by *P. cryptogea* in California (10). It is also possible that strains of *P. cryptogea* pathogenic to alfalfa and *Cicer* are not widely distributed. Both in this study and that of Erwin (10) there is evidence that alfalfa isolates have different host range from the *P. cryptogea* described by Tucker (34) and isolated from many parts of the world.
The development of a disease depends upon the establishment of host-pathogen relationship and the factors affecting this relationship. To have some understanding of this disease of chick pea caused by *P. cryptogea*, it was desirable to obtain as much information as possible on the factors which could influence the prevalence and severity of the disease and the physiology of the pathogen.

Three isolates of *P. cryptogea*, one from California and two from Ohio, were obtained and the pathogenicity of 85 single zoospore isolates of these was investigated on chick pea. All single zoospore isolates except two (#1301 and #1321) were highly virulent. Since isolate 1301 differed markedly in pathogenicity from most of the isolates, the former and one (isolate 1322) of the latter were selected for further pathological and physiological studies, from which it was ascertained that these isolates were different physiologically and pathologically.

The variability of a fungus can be brought about by two ways. One, by a genetic change caused by hybridization, mutation, heterokaryosis or parasexuality resulting in a new biotype; and the other, by a biochemical modification not involving a change in biotype.

In the study of carbon sources for the two isolates, isolate 1322 grew best with starch and second best with sucrose. Good mycelial growth also occurred on dextrose, mannose, d-mannitol, d (+) galactose, and d (+) lactose. Isolate 1301 grew best with starch, mannose, sucrose, and d (+) galactose (no significant difference in dry weights of mycelia) and did not grow at all with d (-) levulose. Erwin and Katznelson (13) reported that the growth of *P.*
cryptogea on sucrose was slightly better than glucose and about 14% greater than on starch. Taking utilization of carbon sources as a criterion for physiological differentiation, it was discovered that isolate 1322 utilised the carbon sources more efficiently than isolate 1301 except one test where environment may have affected results (Table 6).

Light and different concentrations of sucrose affected the mycelial growth of isolate 1322. The dry weights of the fungus increased as the amount of sucrose increased. According to Erwin and Katznelson (13), the optimum concentration of sucrose for the growth of P. cryptogea was between 15 and 25 g/liter.

Light and darkness produced different effects, the growth of the isolate being more in darkness than in light. In this investigation no attempt was made to determine the basis of this difference. Light of the visible range (400-800 m) exerts influences on the mycelial growth. According to Cochrane (6), exposure to "white light" may, depending upon the source, involve exposure to some ultraviolet; heating effects too cannot be disregarded. The growth of Sclerotinia fructigena, Karlingia rosea, and Botrytis squamosus is retarded by light (8).

In the study of the utilization of different nitrogen compounds, the two isolates differed not only in the ability to utilize different nitrogen compounds, but also in the amounts utilized by them. Isolate 1301 utilized yeast extract the best and L (+) asparagine the second best whereas isolate 1322 utilized almost equal amounts of L (+) asparagine and yeast extract and ammonium nitrate.
the least. Dry weights of isolate 1301 were greater than those of isolate 1322 on all nitrogen sources except one (tryptophan). Erwin and Katznelson reported that the mycelial weights of *P. cryptogea* were always the best on either L-asparagine or Casein hydrolysate and none on ammonium sulfate. In general it appears that isolate 1322 utilizes more organic nitrogen than inorganic nitrogen.

The end effect of different temperatures on mycelial growth was similar for both isolates, though the dry weights of isolate 1322 were more between 10°-20° and 25°-30°C. The mycelial growth of the isolates occurred between 10° and 30°C with an optimum at 20° and 25°C for isolates 1301 and 1322 respectively. There was no significant difference in dry weights of the two isolates at 20° and 25°C, therefore it is inferred that the optimum temperature for both might be 20° or 25°C. The isolates did not grow at 5°, 35°, and 40°C. According to Erwin (10), the cardinal temperatures of *P. cryptogea* from alfalfa were 8°, 25°, and 30°-33°C with the optimum at 25°C. These findings and those obtained in this investigation seem to be similar.

The end effect of hydrogen ion concentration on mycelial growth was similar for both isolates, though isolate 1322 grew more than isolate 1301 at pH 6 and 7. The pH range of the isolates was from 5 to 8 with an optimum at pH 7. Erwin and Katznelson reported that the maximum growth of *P. cryptogea* occurred at pH 5.6 to 7.2, but much less at 5.1 and virtually none at pH 4.9 and 7.8. The effect of different pH values on the isolates used in this investigation and on the isolate used by Erwin and Katznelson seems quite similar. The
pH values of the filtrates at the end of the experiments were lower than the initial values in practically all cases. This decrease in alkalinity may be explained on the basis of the production of CO₂ or organic substances as a result of metabolic activities of the fungus.

The host range of isolates 1301 and 1322 was investigated on 47 varieties and species of plants taking percentages of germination of seeds as a criterion. The percentages of germination of seeds of soybean (Blackhawk), hairy vetch, ladino clover, Kenland red clover, yellow sweet clover, Empire bird's foot, and onion (Evergreen White Bunching) were low in soils infested with the isolates. In the soil infested with isolate 1322, the percentages of germination of seeds of yellow sweet clover, crimson clover, sweet pea (Mixed Colors), alfalfa (Atlantic and Dupuits), and pea (Laxtonian and Wando) were low. *Phytophthora cryptogea* causes a root rot disease of alfalfa (10) but has not been reported to be pathogenic on pea. In this investigation, the fungus (isolate 1322) caused considerable seed decay and pre-emergence damping-off of pea, variety Laxtonian. In fact, this isolate could be of possible significance as a causal agent of pea rot on the basis of these investigations. More work is desirable to find out the pathogenicity of *P. cryptogea* on different varieties of pea.

Isolate 1322 was used in the major portion of this investigation for studying the factors influencing disease development. There are three phases of the disease. They are seed decay and pre-emergence damping-off, seedling blight, and root and crown rot. All three phases of the disease were found to occur in three different
soils (loam, sand, and a mixture 1 part of sand, 1 part of peat, and 2 parts of loam). The killing of the plants by the pathogen is thought to be not only through cell wall decomposition but also by toxic effects. Portions of the plants which when rotted disrupt the movement of water to the top of the plants. The evidence of this is that the killed plants are always extensively rotted at the crown region, disease incidence was much higher when the inoculum was located on or near the crown area than when it was located below the crown area.

The severity of the disease was dependent upon the inoculum level. It seems that a certain minimum amount of inoculum must be present for the development of disease. The inoculum level in the soil, if reduced by some means, will greatly reduce the incidence of the disease. This can be achieved by soil fumigation, soil sterilization, rotation of crops, and other sanitary measures.

The age of plants affected susceptibility, the incidence of disease was least in the oldest plants (4 weeks old at the time of infestation). The kill decreased with progressively older age groups of plants. Young plants were more susceptible than older plants shortly after soil infestation, but as the time of infestation of the soil increased, the differences in the number of plants killed among age groups became smaller. From these findings it is postulated that if the disease could be controlled for the first 4 or 5 weeks of the growing season, the losses in yield could be greatly reduced. Protection of the crown area of the plants from invasion probably could be accomplished for this period of time by treating the soil with a suitable fungicide.
The reduction of kill with increasingly older groups of plants can be considered a type of resistance, which could be due to increased size of the tap root system and change in morphology and physiology of the host, which necessitate a more extensive rotting before the plants are killed.

Almost all through this investigation in the greenhouse, secondary organisms, particularly species of *Fusarium*, were found associated with the pathogen in the rotted tissues of the host. The pathogen is capable of rotting and killing plants in steam-sterilized soil, but it is also very likely that secondary organisms may grow rapidly in this soil because the rate of re-colonization of autoclaved soil by fungi is very rapid. A sterile agar culture technique was used to demonstrate that the pathogen can rot and kill chick peas in the absence of other organisms. The rotting of the host tissue might be due to the death of the cells or to the dissolution of the middle lamella. The young and growing tissues of seedlings contain large amounts of pectic substances, and the main enzymatic process operating during initial invasion is the degradation of pectic substances by the invading fungi (19).

A species of *Fusarium*, which was isolated from the rotted tissues of the diseased plants caused by *P. cryptogea*, was found pathogenic on the host. One species of *Fusarium*, *F. lateritium* f. *ciceri*, has been reported to cause a wilt disease of *Cicer arietinum* in the United States (12). The species of *Fusarium* isolated by the investigator was not identified. When this *Fusarium* species was mixed with *P. cryptogea* at different concentrations of inoculum
level and the soil infested with this mixture, the severity of the disease was enhanced, which might be due to the fact that both are pathogenic on the host.

The susceptibility of chick pea was investigated using six different species of Phytophthora. Three isolates of P. parasitica from tomato, peperomia, and gloxinia were found pathogenic. The isolate from tomato caused 82% seed decay and pre-emergence damping-off. This suggests that Cicer arietinum is not only susceptible to P. cryptogea but also to some other species of Phytophthora. Frezzi (16) has already reported the isolation of P. citrophthora and P. megasperma from the rotted roots of the host.

The effect of different hydrogen ion concentrations of soil on the incidence of disease could not be satisfactorily demonstrated because of the variability of the adjusted pH values of the soil in spite of the fact that the soil was not treated for four weeks after adjusting the pH and the plants were watered with distilled water all through this experiment. However, taking into consideration the effect of different pH values on the dry weights of the pathogen in vitro, it appears that the pathogen is capable of growing and causing the disease at a sufficiently wide pH range of soil.

The difference in the severity of disease in relation to nitrogen was not significant. The ammonium compounds, though have been reported to be toxic to several Phytophthora species (15, 33) failed to suppress the incidence of the disease.

The control of soil-borne pathogens is extremely difficult. Resistant crop varieties are used extensively, especially in the
control of vascular diseases. Crop rotation has proved beneficial in the control of many plant diseases, but is not practiced always by the agriculturists for economic reasons. Improved cultural practices, the use of proper cover crops and soil tillering have helped. Soil treatments with heat and chemicals have been useful. Heating the soil to destroy the pathogens is very effective but is quite impractical for field cultivations. Seed treatments to date have been the most practical method of chemical control and are quite effective against only the earliest phases of seedling attack but not at a later stage of the development of the plants.

The soil treatment affects organisms other than those which are pathogenic. A chemical may kill indiscriminately both the detrimental and favorable organisms of the soil population, and thus bring about an imbalance in the microbial population between beneficial and detrimental organisms. Therefore, one of the measures to control the soil-borne pathogens involves a change in the soil microflora with a resultant biological control of the pathogens by non-pathogenic soil organisms.

In this investigation, two types of chemical treatments were evaluated in the control of the disease. First, chick pea seeds were treated with Ceresan, Samean, thiram, chloranil, and captan, and second, soil was treated with soil fungicides. Seed decay and pre-emergence damping-off were considerably reduced in the experiment where the seeds were treated with the fungicides. However, post-emergence damping-off could not be effectively controlled with the seed treatment method. Ceresan appeared to be injurious to the plants.
Captan and thiram gave the best protection against the early stages of seedling attack.

Two experimental soil fungicides (SD 345 and SD 4741), which were obtained from the Shell Development Company, Agricultural Research Division, Modesto, California, were tested for their effectiveness against *P. cryptogea*. The chemical name of SD 345 is 2-propene-1, 1-diol diacetate (allylidene diacetate) and that of SD 4741 is O,O,O-trimethyl phosphorothioate. SD 345 was effective against the pathogen at 50, 100, 150, and 200 ppm in vitro as well as in vivo. One hundred fifty and 200 ppm of this chemical were phytotoxic. One hundred ppm gave the best protection when the soil infested with the pathogen was treated twice with 100 ml of the water solution of the chemical, first before planting the seeds and second a week later at the time of planting the seeds.
SUMMARY

Seed decay, seedling blight, and root rot of *Cicer arietinum* L. caused by *Phytophthora cryptogea* Pethybridge and Lafferty as yet have not been investigated. Frezzi (15) reported that *P. citrophthora* and *P. megasperma* were isolated from rotted roots of *Cicer arietinum*. Whether or not these organisms were causal agents of the root rot was not established.

In this investigation *P. cryptogea* was found to be highly destructive and pathogenic to the chick pea. There are three phases of the disease caused by the pathogen. They are seed decay and pre-emergence damping-off, seedling blight, and root and crown rot.

Three isolates of *P. cryptogea*, one from California and two from Ohio, were obtained and the pathogenicity of the single zoospore isolates of these was tested on the chick pea. Some single zoospore isolates were found to be different in their pathogenicity. Isolates 1301 and 1322 were selected for further pathological and physiological studies because they differed markedly in their pathogenicity on chick pea.

For physiological studies, Lopatecki's and Newton's solution, which was modified after carbon utilization test by incorporating sucrose in place of dextrose, was used.

In the carbon utilization studies for the two isolates, soluble starch was found to be the best for both the isolates. Good
mycelial growth of both occurred in sucrose, dextrose, and mannose also. Differences were noticed only in the amounts utilized.

The effect of different amounts of carbon and light on the growth of isolate 1322 was investigated by using sucrose as the variable carbon source. The growth of the fungus increased as the amount of sucrose increased. Light and darkness produced different effects on the isolate. The growth of the fungus was more in darkness than in light.

In nitrogen utilization study, the two isolates differed not only in the ability to utilize different nitrogen compounds but also in the amounts utilized by them. Isolate 1301 utilized yeast extract best and L (+) asparagine the second best whereas isolate 1322 utilized almost equal amounts of L (+) asparagine and yeast extract and ammonium nitrate the least.

The pH range of the isolates was from 5 to 8 with an optimum at pH 7, the only difference between the two isolates being the different mycelial growths.

Both the isolates grew at 10°, 15°, 20°, 25°, and 30°C. The optimum temperature for the mycelial growth of isolates 1301 and 1322 was between 20° and 25°C. No growth of both occurred at 5° and 35°C.

Six different species of Phytophthora were used for testing the susceptibility of the chick pea. Three isolates of P. parasitica (tomato, peperomia, and gloxinia) were found pathogenic besides P. cryptogea.
Host range of the pathogen was tested on 47 different varieties and species of plants and germination percentages of seeds in infested (isolates 1301 and 1322) and non-infested soils recorded. The germination percentages of seeds of soybean (Blackhawk), hairy vetch, ladino clover, Kenland red clover, yellow sweet clover, Empire birdsfoot, onion (Evergreen White Bunching), alfalfa (Atlantic and Dupuits), and pea (Laxtonian and Wando) were low in the infested soil.

The relation of age of plants to susceptibility was investigated. Young plants were more susceptible than older plants shortly after the soil infestation, but as the time after infestation of the soil increased, the differences in the number of plants killed among age groups became smaller. The killing decreased with progressively older age group of plants and the incidence of the disease was least in the oldest plants (4 weeks old at the time of infestation).

The relationship of inoculum concentration and disease incidence was investigated. The severity of the disease was dependent upon the inoculum level. Disease incidence was much more severe when inoculum was placed at the crown level of the chick pea plants than when it was placed on or near the roots below the crown area.

The effect of different hydrogen ion concentrations of soil on the incidence of disease could not be satisfactorily investigated due to the variability of the adjusted pH values of the soil. However, it appears that the pathogen is capable of growing and causing the disease at a sufficiently wide pH range of soil.
The severity of disease in relation to nitrogen was investigated. The differences in the severity of disease in the infested soils treated with different nitrogenous compounds were not significant.

The possibility of controlling the disease was investigated. Chick pea seeds treated with Ceresan, Semesan, thiram, chloranil, and captan were planted in the soil infested with the pathogen. Captan and thiram gave the best protection against the early stages of seedling attack. Two experimental soil fungicides—SD 345 and SD 4741—were tested for their effectiveness against the pathogen. SD 345 was effective at 50, 100, 150, and 200 ppm in vitro as well as in vivo. One hundred ppm gave the best control when the infested soil was treated twice (at one week's interval) with 100 ml of the water solution of the chemical before planting the seeds.


I, Peter Wales Bhelwa, was born in Ghatula, Tahsil Dhamtari (M. P.), India, September 25, 1926. I received my high school education from the Mennonite Mission High School, Dhamtari (M. P.), India, and my Intermediate (Junior College) education from the Ewing Christian College, Allahabad (U. P.), India. I joined the College of Science, Nagpur (M. S.), affiliated with the Nagpur University, Nagpur (M. S.), India, which granted me the Bachelor of Science degree in 1950 and the Master of Science degree in Botany in 1952. In July, 1952, I was appointed as a lecturer in botany at the Hislop College, Nagpur (M. S.), India, and have been a permanent member of the faculty of the college since then. In 1959, I was awarded the Hazen Foundation Fellowship, Edward W. Hazen Foundation, New Haven, Connecticut, which enabled me to come to the United States for higher studies. This fellowship I held for the maximum period of two years (1959-61). In June, 1961, I was awarded the 'Special Study Grant' by the Danforth Foundation, St. Louis, Missouri, the fellowship I hold to date. I have been on study leave from the Hislop College, Nagpur (M. S.), India, while completing the requirements for the Doctor of Philosophy degree.