A PHYTOPHTHORA-INDUCED DISEASE OF ENGLISH HOLLY,
ILEX AQUIFOLIUM L.

by

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A PHYTOPHTHORA-INDUCED DISEASE OF ENGLISH HOLLY, 
ILEX ACUTIFOLIUM L.

INTRODUCTION

English holly, Ilex aquifolium L., is grown commercially in Oregon for use as decorating greenery at Christmas time. The Pacific Northwest is the major source of the English holly sold throughout the United States. Oregon alone has over 1000 acres in holly orchards at the present time and acreage is increasing (4). Most of this acreage is in the Portland area, with the remainder along the northern coast and in the southern Willamette Valley.

With the increasing interest in holly has come increasing interest in its diseases. Although holly has been considered relatively free from diseases, disease occurs, and is even the limiting factor in holly production in the Tillamook area. Little previous work has been done on holly diseases, and the existing reports did not explain all the symptoms observed in the field. The present work was initiated to clarify and contribute to the knowledge of holly diseases. The work was amply rewarded with the discovery that a Phytophthora species causes the main holly disease in Oregon. Other organisms believed to cause this disease were eliminated as suspects. Studies were conducted to determine the epiphytology of the Phytophthora disease, the mechanism of defoliation, and the taxonomic standing of the Phytophthora pathogen. This work was especially challenging
to carry out, for the disease, previously unreported, and all its aspects were virtually unknown.
Many fungi have been reported as saprophytic on *Ilex aquifolium* L. A smaller number have been reported as pathogenic, but only one of these reports, that of *Thielavia basicola* (B. & Br.) Zopf, was verified by positive inoculations (8). This pathogenicity was somewhat artificial in that it caused the failure of holly grafts.

*Boydia insculpta* (Oud.) Grove, one of the organisms considered pathogenic, was reported from England in 1921 by Grove (6) who combined the name from two previous reports and reviewed all previous work on the organism. Milbrath (14) first reported *B. insculpta* from the Pacific Northwest in 1939. Young and Deep (26), in Oregon in 1952, mainly on the basis of these works, again described *Boydia* as pathogenic on holly. The recovery of *Boydia* from isolations and the apparent association between black stem cankers and fructifications of *Boydia* led these writers to assume its pathogenic nature. Later inoculations made by Young, which were negative, led to the initiation of work on the problem by the author.

*Phomopsis crustosa* (Sacc.) Bomm. & Rouss. was reported by McWhorter (13) as the pathogen of a serious holly disease in Oregon in 1935. This report states that inoculation tests were in progress; however no inoculation results were ever reported. That *Phomopsis* was the cause
of this disease was inferred from its fruiting in typical cankers— even though it was later stated that such fruiting was rare. A *Diaporthe*, *Diaporthe eres* Nits., was associated with the *Phomopsis*. A brown canker and defoliation were the symptoms described. The defoliation which occurred in the winter agrees well with defoliation proven in this thesis to be caused by *Phytophthora*. An examination of material upon which McWhorter's report was based showed two types of cankers to be present; one is the canker described in this work as reddish canker-cork formation, and the other resembles the *Phytophthora* canker.

The *Phytophthora* disease described in this thesis has never been reported before, although personal conversation with Milbrath has revealed that a *Phytophthora* was isolated from small holly plants by Milbrath and Steenland in 1947.

Table No. 1 includes all the reports from the United States of microorganisms associated with *Ilex aquifolium*. No attempt was made to unsramble the synonymy. In

1 Conversation took place in 1954. John A. Milbrath is Professor of Plant Pathology at Oregon State College.

2 References checked were: Review of Applied Mycology (1922-February 1954); Botanical Abstracts (1918-1922); Biological Abstracts (1953-March 1954); Journal of Agricultural Research (1913-1949); Agricultural Index (1916-1951); Experiment Station Record (1910-1946); Plant Disease Reporter (1936-1950); and Plant Disease Reporter Supplements (Nos. 83-189).
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<td>Boydina insculpta (Gud.) Grove</td>
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<td>Not proven as cause of condition reported</td>
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<tr>
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<td>stem canker &amp; defoliation; pathogenic (13)</td>
<td>Not proven as cause of condition described</td>
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<tr>
<td><em>Phyllosticta sp.</em></td>
<td>leaf-spot (24)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Based on state report</td>
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<tr>
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<td>dead twigs; saprophytic (3); leaf spot; twig blight (24)&lt;sup&gt;3&lt;/sup&gt;</td>
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<td>diseased or lead leaves; saprophytic (3)</td>
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<sup>3</sup> Many of the entries compiled by Weiss (24) are based on herbarium specimens or personal correspondence, so the citation given is all that was found or exists, unless stated otherwise under remarks.
addition to the organisms tabled, Grove (5) reported twenty-six fungi on *I. aquifolium* in Europe. There is some duplication with the organisms tabled, and much confusion exists as to specific rank and synonymy of the twenty-six.

Most of the fungi listed by Grove were obtained from long-dead tissue and were considered saprophytes. However, *Coniothyrium Ilicis* Sm. & Ramsb. was stated to cause an epidemic. No proof for this was presented.

Reports concerning other species of *Ilex* are not included, although some of the organisms tabled have also been found on other *Ilex* species. The most interesting of these reports is by Bender (1); it describes a severe dieback of American holly, *I. opaca* Ait., caused by *Fusarium solani* var. *martii* (App. & Wr.) Wr.
METHODS AND MATERIALS

General

Intensive work was begun in July 1953, although some work was done the preceding spring. Twenty different orchards were surveyed, but only a few of these were subsequently checked every month or two. These latter, more seriously diseased orchards were Sander's near Tillamook; the Old Huckleberry orchard near Wheeler; Timmerman's near Astoria. Other diseased orchards which yielded valuable information were Wieman's near Portland; Lehman's near Junction City.

Isolations

Isolations from stems and leaves were made by washing the plant material, surface-sterilizing in 20 per cent Clorox for one minute, and washing in sterile distilled water; then sections were taken from the edge of the diseased part and planted on potato dextrose agar in Petri plates. The epidermis of stems was removed before taking sections from the cortex. The Phytophthora was not recovered when isolation plates were kept at room temperatures above 20° C. The Phytophthora pathogen was isolated easily from fresh material that was dying rapidly, but was difficult to isolate from leaf-spots or stems that had been diseased for some time.
Since agar-plate isolations from large stem cankers yielded numerous nonpathogenic organisms, the following technique was used to recover the pathogen. The canker was washed and surface-sterilized, and sections from the canker edges were transferred to slits made in stems of healthy holly plants in the greenhouse. If cankers then developed on the inoculated stems, isolations were made from them in the usual manner. This method effectively screened the other organisms and occasionally yielded the pathogen.

**Media**

1. Stock cultures were maintained on freshly made 2 per cent potato dextrose agar slants. The *Phytophthora* pathogen grew very slowly on Difco *p.d.a.*, so this was not used.

2. Pea broth was prepared by boiling 150 grams of dried whole peas for thirty minutes in a liter of distilled water. The peas were then discarded, the water strained through cheesecloth and measured into 250 c.c. Erlenmeyer flasks. These were sterilized for fifteen minutes at fifteen pounds pressure. This method produced clear pea broth. The pea broth was used in tests to obtain fruiting of *Phytophthora* as described by Leonian (10). The pea broth was inoculated and the culture allowed to grow for seven days. Mycelia were then removed, washed in two changes of sterile
distilled water in Petri plates and incubated in a third plate of sterile distilled water for three to five days, at which time examination was made for fruiting bodies.

3. Oatmeal agar.

Oat meal................60 grams
Agar...................17 grams
Distilled water........1 liter

4. Proteose peptone nutrient solution.

Proteose peptone......2 grams
Dihydrogen potassium phosphate........0.5 gram
Magnesium sulfate......0.2 gram
Succinic acid...........0.2 gram
Dextrose................5 grams
Distilled water........1 liter

5. Leonian's malt extract agar No. 1 (10).

Malt extract..........3 grams
Yeast extract..........2 grams
Dihydrogen potassium phosphate........0.5 gram
Magnesium sulfate......0.2 gram
Bacto-agar.............20 grams
Distilled water........1 liter

6. Leonian's malt extract agar No. 2 (11).

Malt extract..........5 grams
Dihydrogen potassium phosphate........0.6 gram
Magnesium sulfate......0.3 gram
Bacto-agar.............20 grams
Distilled water........1 liter

7. Malt extract agar No. 3.

Difco malt extract...20 grams
Agar...................20 grams
Distilled water........1 liter

8. Difco malt extract agar.

Difco malt extract agar................45 grams
Distilled water........1 liter
Inoculations

Stem inoculations were made on potted plants and in a few instances on orchard trees by slitting the cortex longitudinally for approximately one inch and inserting a small portion of mycelium and substrate under the flap made by the slit. The slit was then closed, and absorbent cotton or cheesecloth was put around the inoculated stem and wet with sterile distilled water. Leaves were inoculated by scratching the lower surface and placing a small amount of mycelium and substrate on the scratched surface. Modifications were tried at many temperatures and humidities, but the usual method was to place the plants in a large enclosed case where the humidity was high and the temperature varied from 18° to 22° C.

Flask inoculations were made with several organisms by taking five-inch holly stem tips, surface-sterilizing them in 20 per cent Clorox, and denuding them of all but the apical two leaves. Fungus and substrate were then inoculated at the base of the stems and the inoculated portion stuck into water-agar in 500 c.c. Erlenmeyer flasks. These flasks were plugged with cotton and left at room temperatures. Uninoculated holly in these flasks remained green and succulent for several months.

Leaf inoculations in Petri plates were used to study Phytophthora development. Leaves were clipped from holly
twigs, washed, surface-sterilized, and rinsed with sterile distilled water. The leaves were then wounded with a needle (five scrapes approximately one-fourth inch long, side by side) and inoculated with a small portion of mycelium and agar. Control leaves were wounded and inoculated with sterile agar. The leaves were then placed lower surface down in Petri plates and enough sterile distilled water to cover the bottom was poured into each plate. Three to five replications were used for each treatment. This method enabled many inoculations to be carried out easily; temperature relations, sporangial production and germination, and pathogenicity of other species of *Phytophthora* were studied in this manner.

**Propagation**

Eight hundred cuttings were made from several varieties of holly in order to obtain plants for inoculation trials. Cuttings were handled according to recommendations of Roberts and Boller (20). The cuttings were taken in September and consisted of approximately six inches of that year's stem growth. All but the top three leaves were removed and the ends were immersed in fifty parts per million of 3-indolebutyric acid for twenty-four hours. The lower four inches of the cuttings were placed in wet sand maintained at 18°C. by a soil heating cable. Low greenhouse temperatures prevailed at that time. The cuttings were
kept only moderately moist, for excessive moisture caused their leaves to fall. Approximately eighty per cent of the cuttings rooted within three months.
MINOR

1. Midge and leaf spots.

In November, leaves that contained a circular black spot one-fourth to one-half inch in diameter were collected from several areas. The spots were visible only on the upper surface of the leaves. Invariably a midge was present on the lower surface at the center of the spot (Figure 1). The midge was dead and in some cases a fungus was fruiting on its body. The proboscis was penetrating the leaf in one case. There appears to be a connection between the insect and the spot but the nature of the connection is unknown. It is possible that the midge provided entry for an organism that spotted the leaf, but one was not recovered from isolations. Leaves affected were few enough to be of no economic importance. This material was turned over to members of the Entomology Department.

2. Reddish swellings on leaves.

Tan to reddish raised areas may occur on the lower leaf surface (Figure 2). These resemble somewhat the transparent swellings described below, and may be related to them, but are up to one centimeter in diameter and of irregular outline. The lower leaf surface appears splotchy due to deposition of colored substances within the cells. Usually no organism could be isolated from these spots.
Fig. 1. Midge and associated leaf spots. Spots on upper surface (left and right) and midges at center of spots on lower surface (center).

Fig. 2. Red swellings on holly leaves. Upper surface (center) and lower surface (left and right).

Fig. 3. Transparent swellings on holly leaves. Upper surface (center) and lower surface (left and right).
3. Transparent swellings on leaves.

Small raised transparent swellings frequently occur on the lower surface of leaves (Figure 3). These swellings are circular and are usually one to two millimeters in diameter and one to two millimeters thick; they consist of proliferated cells and deposited waxes. By transmitted light these swellings show up as transparent spots in the leaf. These swellings often appear to surround leaf-spine punctures and it is probable that they are initiated by leaf puncturing. Usually no organism could be isolated from these spots. Affected leaves do not fall and appear normal in every way except for the minute swellings.

4. Purple leaf spots.

Purple spots are commonly present on the upper surface of leaves in many orchards. The spot appearance is due to deposition of colored products in and around the palisade cells. Often such spots are associated with spine injury which is common on holly leaves, or with spray injury. It is assumed that this condition is sometimes associated with frost injury or nutritional disturbances.

5. Reddish canker-cork formation.

Reddish areas occur on twigs and stems which grade in appearance from corky patches to small cankers (Figure 4). *Phomopsis* is sometimes, though uncommonly, found fruiting in these cankers. The affected areas are tan to reddish-brown
in color and small in size. They seldom extend more than an inch or two in length and, even on small twigs, rarely girdle the twig. Gradations exist from small circular spots of cork, and corky areas around lenticels, to the larger canker-cork formation. Cracks develop along the margins and within the canker-cork area. Stubs from pruning may die back a few inches and have a ring of reddish cork formed at the margin of healthy and dead tissue. Several canker-cork areas may develop on stems of small potted holly plants in greenhouses. The canker-cork formation is prevalent on bare branches exposed to sunlight and in orchards on windy sites along the coast. The holly hedge on the experimental farm at Astoria, which was severely wounded by moving a house along it, contains many canker-cork formations. Microscopic examination of incipient canker-cork patches has shown typical cork development such as occurs as a response to wounding.

This type of canker-cork is commonly called *Phomopsis* canker after McWhorter (13), although he probably was compounding this condition with the *Phytophthora* disease when he described it. Some herbarium material collected in 1934 and labeled "Phomopsis canker" by McWhorter is definitely the canker here described as reddish canker-cork formation. Many isolations made during this study have yielded no organism consistently. Specimens showing gradations from active small *Phytophthora* cankers to this red-tan corking
further indicate that much of the canker-cork formation is a response to injury, either by Phytophthora or by mechanical means. Phomopsis and other weak parasites may later invade these cankers. Until further work is done, the true cause of this condition remains unknown.

Major

1. Algae.

Holly leaves, limbs, and trunks often become covered with a superficial growth of algae. The algae, usually a species of Protococcus, do not visibly damage the holly, but the leaves covered with algae are dull and unsalable. Many thousand pounds of holly are not sold each year because of algal infestation. The algal growth is more prevalent on the shady side of the trees and in dense trees. Moist shady situations in general promote algal growth.

2. Sooty molds and insects.

Several insects are pests of holly (21). Growth of sooty molds is associated with the presence of scale insects. These molds grow on the "honey dew" secreted by the scale insects. The fungi are superficial upon the leaf surface; however, they produce black mycelia which coat the holly leaf. Control of the scale controls the growth of the sooty molds.

3. Phytophthora disease.

This disease is the most serious disease of holly
in Oregon at the present time. Although it is most severe along the coast, the disease occurs throughout the holly growing region of Oregon and was also found in Washington. The disease affects both old trees and young plants in propagating beds. In old trees infection begins in the lower part of the tree and develops upward. The disease is characterized by a symptom complex which begins with black leaf spotting and defoliation, and is followed by twig die-back and formation of black cankers on the stems. Large-limb cankers and trunk cankers also associated with the other symptoms are believed to be caused by the Phytophthora but the causal fungus has not been isolated from such cankers. Photographs of the symptom complex are included in the Phytophthora epiphytology section of the thesis.

The disease develops in the fall or winter with the advent of cool, wet weather, and subsides with the coming of warm and dry summer conditions. The symptom appearance when the disease is not active is much different from active symptom expression. It is then that the black color, so characteristic of the active phase, turns to grey and other organisms fruit in the dead tissue.

Of the three conditions classified as being of major importance, the experimental work which follows deals only with the Phytophthora disease.
ISOLATIONS AND INOCULATIONS

Isolations

Six hundred isolation plates were made from diseased holly tissue from April 1953 to April 1954. A great variety of organisms was obtained; however, no one organism predominated in frequency. Before the Phytophthora was isolated in November it was not possible to connect any type of symptom with a particular organism obtainable by isolation, even though much time was spent attempting to do so.

Bovidia, Phomopsis, and Fusarium were the most commonly isolated fungi, but even these were obtained only 20 to 30 times. Species of Diplodia, Phoma, Cladosporium, Chaetomium, Pestalotia, Alternaria and many unidentified fungi were isolated. The imperfect stage of Nectria gallica Bres. was also isolated several times. Various bacteria were commonly isolated, often in conjunction with one or more fungi.

After the Phytophthora was first isolated from leaf-spots in November, it was readily isolated from dying stems and leaf-spots during the winter and spring, if fresh black material was used. If the tissue had been dead for even a short time, contaminating fungi and bacteria were recovered instead.

Phytophthora recovery from cankers on stems one to two inches in diameter was occasionally accomplished by
inoculating the canker tissue into healthy holly stems and recovering from them. This method was tried for trunk and large-limb cankers, but no cankers were produced on healthy holly stems and Phytophthora was not recovered. Petri plate isolations from the limb and trunk cankers yielded the typical diverse microflora, but never Phytophthora.

Inoculations

Inoculations were made with the more commonly isolated fungi and bacteria. More than one hundred inoculations were made with Boydia under a wide range of conditions and at different times of the year. Many modifications of the usual inoculation technique were made with Boydia, and of all these, the flask method was the only successful one. In this instance, inoculated stem tips in flasks died after one month and Boydia then produced fruiting bodies on the stems. No significance can be attached to this result, for uninoculated stems also died at about the same time. The fruiting of Boydia on the dead tips probably resulted from its invasion of the already dying tips.

Thirty-five inoculations of Phomopsis were made during the year. Thirty-four of these were negative; however, one was not only positive, but the canker produced killed the whole plant. In this instance the inoculation was made with a hypodermic needle containing spores and macerated mycelia. One month after inoculation the plant
appeared normal, but after two months a canker was developing and *Phomopsis* alone was reisolated from the canker. A second series of reisolations again recovered *Phomopsis* and shortly thereafter the plant died. The symptoms produced did not resemble the reddish canker-cork formation but consisted of a black progressing canker which was similar in appearance to the *Phytophthora* canker on small plants. All attempts to duplicate this positive inoculation failed. The one positive *Phomopsis* inoculation demonstrates that *Phomopsis* can be pathogenic to holly under certain conditions.

A smaller number of inoculations, made with *Fusarium*, *Diplodia*, *Phoma*, and bacterial isolates, were all negative. Inoculations with single spore cultures of *Nectria galligena* (*Fusarium wilkommii* Lindau) were also negative.

Positive *Phytophthora* inoculations were obtained on leaves, young or old stems, leaf scars, and berries. Active leaf spots and stem cankers produced by these inoculations were identical to those occurring naturally, and *Phytophthora* was readily reisolated from them. Inoculations made on stems when the humidity was low were not always successful. Inoculations without previous wounding were never successful even on succulent new growth, with the exception of inoculations on newly-abscisced leaf scars.

In attempting to obtain fruiting of *Bovdia* in tissue
killed by Phytophthora, Boydia and Phytophthora were inoculated concurrently, and Boydia was inoculated also after Phytophthora had killed the stems. These attempts failed. However, Boydia fruiting bodies appeared in cankers which developed from Phytophthora inoculations made on orchard trees.

Correlation of negative inoculation results with field observations

The extensive work done on Boydia lost its significance when it became evident that Boydia was not a cause of disease, and only a few remarks were included here. Boydia was collected and isolated from ten different orchards in the state and appears ubiquitous on dead holly stems (Figures 5, 6, and 7). The fungus often was associated with black cankers and was especially common where there were many cankers and recently killed twigs. It was noted to fruit on dying twig tissue that had been healthy a month previously. Boydia usually was isolated from cankers which appeared walled off, but it was also recovered from small surface spots beyond the edge of twig-tip dieback caused by Phytophthora. The development of hundreds of small surface spots (Figure 5c) beyond the necrotic edge of Phytophthora-killed twigs, occurred in spring and summer, and may have some connection with Boydia. Boydia spores were found in raindrops collected from holly in the summer time.
Fig. 4. (a and b) Reddish canker-cork formation. "Mature" cork-canker (a), and incipient cork spots (b, arrow). (c) *Phomopsis* pycnidia in stem of tree killed by gophers.

Fig. 5. *Boydia insculpta*, (a) perithecium on dead holly stem, (b) small walled-off canker containing a few *Boydia* perithecia, and (c) small sunken spots, from which *Boydia* was isolated, beyond the edge of *Phytophthora*-killed dieback.
Fig. 6. Ascospore of *Boydia insculpta*, 450x. Immature spores in ascus on the right.

Fig. 7. *Boydia insculpta*, 13 day old culture on potato dextrose agar, showing growth and fruiting characteristics.
Phomopsis was commonly found fruiting on dead holly twigs. Small trees girdled by rodents may be uniformly covered with pycnidia of Phomopsis (Figure 4c), indicating its ability to enter a dying tree.

Nectria galligena may fruit in black cankers and sometimes over large areas of dying trunks.

Fruiting bodies and spore masses of many other organisms were common on dead and dying holly tissue.

Trochilia ilicis (Chev. ex Fr.) Crouan and other Discomycetes were often found on dead holly leaves.
Inoculum development

Disease development in orchards begins in the fall with spotting of leaves on the lower branches. The symptom complex is shown in Figures 8 through 13. If conditions are favorable, the leaf spotting progresses steadily upward, while the first spotted leaves drop off. In investigating the means by which the pathogen is spread, it was found that sporangia are produced on sporangiophores that emerge from the stomata which are only on the lower leaf surface. This was observed on naturally infected leaves and was duplicated in the laboratory by inoculating leaves and incubating them in Petri plates. A thorough microscopic examination of infected leaves was made and sporangiophores were observed emerging only through stomata. The thick cuticle on the upper leaf surface and the thinner cuticle on the lower leaf surface apparently are not penetrated by the sporangiophores.

The build-up of inoculum does not end with sporangial production, for zoospores may subsequently be released from the sporangia. The effect of temperature upon type of sporangial germination was studied to determine whether the germination of sporangia usually is direct by means of germ tubes, or indirect by zoospore formation. This was done by inoculating leaves in Petri plates, permitting
spots one centimeter in diameter to develop at 20°C, washing the leaves and then incubating them at 5, 10, 15, 20, 25, and 30°C. The leaves were examined after forty-eight and sixty hours' exposure to the different temperatures. Sporangia were produced only at 10, 15, and 20°C. After sixty hours sporangiophores were developing at 5°C. At 20°C about three-fourths of the sporangia were germinating directly and the rest were empty, indicating zoospore release. Zoospores only were formed at 10 and 15°C. Considering the low winter temperatures prevailing at the time of leaf-spot development, these data indicate that inoculum for infection of the leaves consists mainly of zoospores.

Mechanism of infection and spread

Interest developed in how the pathogen spreads from leaf to leaf and how the zoospores or germ tubes enter the leaves. Inoculations were made on the lower surface of unwounded leaves using mycelia in small agar blocks. Even when this was tried on young succulent tissue, results were negative. Inoculations were then made by applying a small drop of water containing mycelia, oospores, and a few sporangia to the lower surface of leaves. No infection occurred. Although the ideal way to test entry into leaves would be to use a concentrated suspension of zoospores and sporangia, no way to obtain these in quantity was devised.

The five key points concerning the mechanisms of
infection and spread are numbered in the following paragraph.

(1) It appears that mycelial penetration through the epidermis or even entry through stomata is difficult to obtain; however entry of a zoospore germ tube is a different matter and is not ruled out at all by these observations. Observations in the field offer additional suggestions for means of entry. Holly leaves have very sharp spines and the leaves are easily whipped by the wind so that constant pricking of one leaf with another's spines occurs. During rainy weather, water runs off of holly leaves and hangs on the spines. (2) On infected leaves these water droplets hanging from the spines have been observed to contain zoospores and they make an ideal means for transferring the inoculum into a nearby leaf. Neighboring leaves have been found with spots which were situated at points where spine contact between them occurred. (3) Since most spots appear at the leaf edge by a spine, and it is mainly there that hanging water droplets wet the lower leaf surface, it is postulated that entry occurs through stomata underneath these water droplets. Peripheral leaf-spots which are centered around leaf-spines are illustrated in Figure 9. (4) Upward spread of infection probably occurs by the back-splashing of drops carrying the inoculum. Movement from tree to tree over a distance of at least thirty feet occurs. No experimental work was done to establish how
Figure 8. A Phytophthora-diseased holly orchard near Tillamook in March 1954, showing defoliated branches.
Fig. 9. Typical *Phytophthora* leaf-spots centered at spines on leaf edges, January 1954. Unusual case of leaves not falling from the stem.

Fig. 10. *Phytophthora* stem cankers developing around wounds.

Fig. 11. Springtime *Phytophthora* infection. Diseased berries and active cankering of stem under berry clusters.
Fig. 12. Old trunk-cankers. Natural appearance (left), with sap-sucker holes in canker. Cankers cleaned (right) showing depth and extent.

Fig. 13. Large-limb cankers. Note craters (arrow) from which slime oozes.
this movement occurred. However, sporangia produced in the laboratory are readily detachable from the sporangiophores. (5) It is postulated that the sporangia can be blown about in the wind.

**Twig infection**

Twig infection usually occurs after leaf infection is initiated and may continue all winter and into the spring. Infection of twigs may occur by the movement of the fungus through the leaf and into the twig. This is not the primary means of twig infection for most of the infected leaves fall off before the spots cover the leaf.

To test the possibility that infection can occur at the leaf scar, leaves were pulled from the stems and mycelium and agar were placed on the exposed leaf scars. These inoculations were positive. By this method even large limbs may become infected, for holly leaves are borne on stems as large as three inches in diameter. Small twigs with many leaf scars are commonly infected and the dieback may progress down the twig and into the parent branch. Such movement into parent stems was demonstrated by inoculation.

Since holly is harvested in November and December and often pruned after harvesting, the possibility exists that pruning wounds may offer entry points for infection. To test this possibility, wounds were made in December in
the cortex of branches in a heavily infected orchard. Typical Phytophthora cankers developed around these wounds (Figure 10).

In April, twigs were observed with purple leaves that eventually turned grey and produced a flagging effect. Closer observation revealed that the twig was girdled usually at the point of attachment of a berry cluster some distance from the tip of the twig. The berries would also be blackened (Figure 11). Inoculations of ripe berries in Petri plates proved that infection of the berries can occur, and the association of berry clusters with these stem infections indicates that stem infection can occur through the berries. Large-limb and trunk cankers (Figures 12 and 13) are associated with the other symptoms and are believed to be caused by the Phytophthora; however the pathogen was not recovered from them.

Oospore production

Possibilities of an inoculum source for the first leaf spotting in the fall are: (1) by oversummering of mycelium in twigs, cankers, fallen leaves or soil; (2) by oversummering of chlamydospores; or (3) by production and oversummering of oospores. Time did not permit the determination of whether one or more of these methods serve as a means of oversummering. However, work was carried out which favors the third possibility. Mycelia in agar culture
are killed after 6 days' exposure to 27°C; and chlamydomospores have never been found. Microscopic examination of dead twig tips showed that oospores were produced in the cortical tissues. Some twigs contained an abundance of oospores, others contained few, or none at all.

In addition to the oospores found in twigs, oospores were produced in leaves which were inoculated in the laboratory. Oospores were found both in the palisade tissue and among the cells of the spongy layer. Oospores and sporangia are illustrated in Figure 14. To test the effect of temperature upon oospore development in vivo, other leaves were inoculated, incubated at 20°C until spots were one centimeter in diameter, and then transferred to temperatures of 5, 10, 15, 20, 25, 27, and 30°C. Examination forty-eight hours later showed abundant oospore production in leaves held at 10, 15 and 20°C, and no oospore production at the other temperatures. After sixty hours the results were the same.

To test the effect of temperature upon oospore formation in the absence of holly tissue, the Phytophthora was grown in pea broth, mycelia were removed and washed in sterile distilled water, then incubated at the different temperatures in sterile distilled water in Petri plates. After three days, oogonia had formed abundantly at 20°C, and none had formed at any other temperature. After five
(a) Oogonium, amphigynous antheridium, and oospore; in water.

(b) Sporangia, smaller ones germinating by germ tubes; in water.

(c) Oospore in cortical tissue of a holly twig.

(d) Oospore in palisade tissue of a holly leaf.

Figure 14. Oospores and sporangia of the holly Phytophthora, 450x.
days oogonia had also formed at 10 and 15° C. In water culture oospores were liberated one to two weeks after formation.

**Temperature and disease development**

The effect of temperature upon disease development was tested by inoculating leaves by the Petri plate method and incubating them at 5, 10, 15, 20, 25, 27, and 30° C. Results after ten days are shown in Figure 15. Spots developed only at 10, 15, and 20° C. with an increase in diameter up to 20° C., although the spots at 15° C. were almost as large. Control leaves were not spotted. Mycelial growth on culture media varied similarly with temperature, except that slight growth also occurred at 5° C.
HOST RANGE OF HOLLY PHYTOPHTHORA AND PATHOGENICITY
OF OTHER PHYTOPHTHORA SPECIES

Host range

The pathogenicity of the holly Phytophthora to some other plants was tested. Results are presented in Table 2 and in Figure 16. The results indicate that the holly Phytophthora is pathogenic to four horticultural varieties of Ilex aquifolium. Petri plate leaf inoculations were positive on four other species of Ilex. Of especial interest is the pathogenicity to Ilex opaca, our native Eastern species. The results on plants other than Ilex indicate that the host range is very limited.

<table>
<thead>
<tr>
<th>Species inoculated</th>
<th>plant part</th>
<th>result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ilex aquifolium L.</td>
<td>leaf &amp; stem</td>
<td>+</td>
</tr>
<tr>
<td>Silver variegated</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Variegated hedgehog</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Green hedgehog</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Pinto</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Ilex pernyi Franch.</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Ilex pernyi Franch. forma veitchii</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Ilex crenata Thunb.</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Ilex cornuta Lindl.</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Ilex opaca Ait.</td>
<td>leaf</td>
<td>+</td>
</tr>
<tr>
<td>Species inoculated</td>
<td>plant part</td>
<td>result</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------</td>
<td>--------</td>
</tr>
<tr>
<td>Apple, flowering crab,</td>
<td>stem</td>
<td>0</td>
</tr>
<tr>
<td>Malus species</td>
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</tr>
<tr>
<td>Apple, red Delicious,</td>
<td>stem</td>
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<tr>
<td>Malus pumila Miller</td>
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<td>trace</td>
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<tr>
<td>Apricot, Wenatchee,</td>
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<tr>
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<td>Cherry, Bing,</td>
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<tr>
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<tr>
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<td>Peach, Veteran,</td>
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<tr>
<td>Prunus persica (L.) Batsch</td>
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<tr>
<td>Rose, Rosa L.</td>
<td>stem</td>
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Pathogenicity of other species of Phytophthora to Ilex aquifolium

Pathogenicity of eight other species of Phytophthora to leaves of *I. aquifolium* was tested by the Petri plate method of inoculation. Species used were *P. cryptogea* Pethybr., *P. cactorum* (Lib. et Colem.) Schrot., *P. parasitica* Dast., *P. boehmeriae* Sawada, *P. hibernalis* Carne, *P. cinnamomi* Rands, *P. lateralis* Milbrath & Tucker, *P. fragariae* Hickman. Results for the first three are shown in Figure 17. Only two others, *P. boehmeriae* and *P. hibernalis* produced leaf spotting. The results with these two organisms compared with *P. parasitica* and *P. cactorum* respectively.
Fig. 15. Effect of temperature upon Phytophthora leaf-spot development, 10 days after inoculation.

Fig. 16. Phytophthora leaf-spots on various hollies. (L. to r.) I. aquifolium "Pinto", I. opaca, I. cornuta, I. aquifolium "Variegated".

Fig. 17. Pathogenicity of four Phytophthora species to holly leaves. (Left to right) P. cryptogea, P. cactorum, P. parasitica, and holly Phytophthora. Leaf-spot size 15 days after inoculation.
Attempts to increase pathogenicity by isolating and reinoculating were made but rate of leaf-spot development did not increase after two successive inoculations. The above pathogenicity trial indicates that five of the eight *Phytophthora* species tested can colonize wounded holly leaf tissue and slightly invade unwounded tissue. They do not approach the pathogenicity of the holly *Phytophthora*, and, although pathogenicity and host range cannot be used in species determination, these results show that the holly *Phytophthora* is a different entity from the species with which it was compared.
MECHANISM OF DEFOLIATION

The observation of the falling of apparently healthy leaves which were near infected ones led to a series of tests to determine the mechanism of defoliation. The dropping of noninfected leaves suggested that a toxic substance was produced which diffuses for short distances in the tissue. Since it has been shown (15) that minute concentrations of ethylene can defoliate holly, a test was made to determine whether the toxic substance produced was ethylene or at least a gas. The results of this test are shown in Figure 18. The experiment was set up in two-quart jars, with healthy holly stems placed in small water-filled vials within the jars. Wound-inoculated holly leaves were placed in the bottoms of the jars along with a small amount of water to keep them moist. Three series of jars were used. In one series ten inoculated holly leaves were placed in the jars, in another five, and in the third series, one inoculated leaf was used. Four replications were used for each treatment and control jars were set up which differed only in having wounded but noninoculated leaves placed in the bottoms of the jars. The jars were sealed with standard jar lids. A ripe apple was placed in one jar for comparison of defoliation time. The stem in this jar defoliated on the fourth day. Spots on inoculated leaves had developed to a diameter of about one centimeter by the sixth
Fig. 18. Defoliation of a healthy twig after 10 days by gas produced by five Phytophthora-inoculated leaves placed in the bottom of the jar (left). Control (right) contains five wounded but non-inoculated leaves in the bottom of the jar.
day. On the seventh day leaves began to drop in the jars containing five and ten inoculated leaves, and the stems were completely defoliated by the eighth day. On the tenth day the stem was defoliated in the jar containing one inoculated leaf. At the end of three weeks, when the experiment was terminated, no stems in the control jars had become defoliated. A repetition of the experiment was modified by first letting the spot develop and then putting the spotted leaf in the jar with a healthy twig. One leaf was used for each jar. The time before defoliation was then shortened to five days.

This experiment demonstrates that a gas is produced by diseased holly tissue and/or the fungus, which can defoliate holly.

The similarity to defoliation known to be induced by ethylene led to further tests to determine if ethylene specifically is the defoliating agent of stems containing diseased leaves. Ten inoculated leaves were placed in a 500 c.c. Erlenmeyer flask which was provided with two outlets. The outlets were closed with screw clamps which were opened only for a few seconds each day. A control flask contained ten uninoculated leaves. After leaf-spots had developed, Hansen\(^4\) using a new method (27), tested the air

\(^4\) Dr. Elmer Hansen is Professor of Horticulture at Oregon State College.
in the flasks for ethylene. The general method was to pass air through the flasks and then through mercuric oxide in perchloric acid, an ethylene absorbent. The ethylene was then released from the solution by the addition of hydrochloric acid, and any ethylene evolved was measured manometrically. This method can detect ethylene in concentrations as low as 0.5 p.p.m. The results from this test were negative. It is concluded that ethylene was either not produced at all, or was produced in quantities below 0.5 p.p.m. Hansen believes that holly will be defoliated at concentrations of ethylene as low as 0.1 p.p.m. Biological tests which are sensitive to this low concentration were begun but are not yet completed.
TAXONOMY

Publications dealing with the taxonomy of the genus Phytophthora de Bary are not numerous although the literature on individual species is voluminous. The usefulness of much of the literature dealing with Phytophthora diseases is lessened because the species of Phytophthora involved were not identified. This has been due to the extreme difficulty of species determination within the genus. Although Tucker monographed the genus in 1931 (23), this did not provide the average worker with the means to readily distinguish species. In fact, Leonian (10) considered several of Tucker's "species" indistinguishable and invalid.

Leonian (10) in 1934 made a thorough study of the then-existing species and set up a key to the genus based mainly upon physiological characters. Leonian did not consider such characters as morphology and pathogenicity to be valid bases for species separation within the genus. He based his work on the principle that an organism should be retained as a species only if it could be easily recognized and sharply defined. Leonian's work (10) is used as a basis for the classification of the Phytophthora discussed in this thesis. Leonian studied all Phytophthora species known prior to 1934, and retained twenty-two as distinct species. Unfortunately not all workers subsequently have applied Leonian's criteria to new Phytophthora isolates.
Since 1934, eighteen new species and two new varieties have been described; some rest upon no more firm foundation than morphological characters. In addition, many species merged by Leonian on the basis of his experiments are still referred to in the literature by their old designations. A total of at least sixty-two Phytophthora species can be found in the literature. Tucker listed thirty-seven other names as synonyms, so more than one hundred names refer to members of the genus Phytophthora.

**TABLE 3. PHYTOPHTHORA SPECIES NAMED FROM 1934 TO FEBRUARY 1954 (16, 17, 18)**

<table>
<thead>
<tr>
<th>Species</th>
<th>Year</th>
<th>Synonyms</th>
</tr>
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<tbody>
<tr>
<td>canavaliae Hara</td>
<td>1930</td>
<td>imperfecta Sarej. ? 1949*</td>
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<tr>
<td>(nom. seminud.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chinchonae Sawada</td>
<td>1937*</td>
<td>lateralis Tucker &amp; Milbrath</td>
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<tr>
<td>cyperi (Ideta) Ito &amp; Tokunaga (Peronospora cyperi Ideta)</td>
<td>1935</td>
<td>macrospora (Sacc.)Ito1940</td>
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<tr>
<td>cyperi-bulbosi Seet &amp; Hamak. ?</td>
<td>1954*</td>
<td>(Sclerospora macrospora Sacc.)</td>
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<tr>
<td>devastatrix (Lib.)</td>
<td>1936</td>
<td>quininea Crandall 1947</td>
</tr>
<tr>
<td>Futt. (P. infestans (Mont.) de By.)</td>
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<td>primulae Tomlinson 1952</td>
</tr>
<tr>
<td>fagopyri Takimoto</td>
<td>1935</td>
<td>speciosa Mehlisch 1935</td>
</tr>
<tr>
<td>fragarlicae Hickman</td>
<td>1940</td>
<td>stellata Shanor 1938</td>
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<tr>
<td>himalayensis Dastur</td>
<td>1948</td>
<td>verrucosa Alcock &amp; Foister</td>
</tr>
<tr>
<td>inflata Caroselli &amp; Tucker</td>
<td>1949</td>
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</tbody>
</table>

The one exception, canavaliae, was named in 1930 but was not included by Leonian. Species with asterisk* are not in Index of Fungi but are in Review of Applied Mycology volumes of the dates indicated.
Keying characters

The distinguishing characters employed by Leonian and used on the holly *Phytophthora* were:

1. Presence or absence of growth on malt extract agar after six days at 20° C.
2. Presence or absence of growth at 27° C.
3. Formation or absence of formation of sexual bodies by sterile hyphae after being transferred from pea broth to distilled water.
4. Formation of predominantly paragynous antheridia or formation of predominantly amphigynous antheridia.
5. Presence or absence of growth after two weeks when exposed to malachite green concentrations of 1:2,000,000; 1:3,000,000; 1:4,000,000; 1:8,000,000; or 1:12,000,000.
6. Presence or absence of growth at 8° C.

Using Leonian's criteria (10), the holly *Phytophthora* keyed to *Phytophthora hibernalis* Carne. However, this depended only upon reactions to criteria 1-3 in the above list. Specifically there occurred (1) growth after six days on malt extract agar at 20° C., (2) no growth at 27° C. and (3) formation of sexual bodies from sterile mycelia in distilled water after transferring from pea broth6. Since

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6 Although Leonian obtained fruiting bodies from *P. hibernalis* under these conditions, the writer, working with a *hibernalis* culture obtained from Zentmøller at Riverside, California, obtained no fruiting bodies from it under these conditions.
P. hibernalis produces paragynous antheridia (23) and the holly Phytophthora was found to produce amphigynous antheridia and the appearance of the two organisms in culture is not the same, further tests were employed. The appearance of the holly Phytophthora in culture is compared with that of P. hibernalis and two other Phytophthora species in Figure 19.

The effect of temperature upon mycelial growth rate was studied and results are presented in Table 4.

**TABLE 4. THE EFFECT OF TEMPERATURE UPON GROWTH OF HOLLY PHYTOPHTHORA**

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>27</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative growth</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The above results were the same for six different isolates obtained from southwestern Washington and northwestern Oregon, whether they were grown on malt extract agar, or potato dextrose agar. Average diameter of growth after seven days on p.d.a. at 20° C. was 2.8 centimeters. Spots developed on inoculated leaves at the same temperatures that growth occurred on media. The relative diameter of leaf spots at the different temperatures was the same as the relative diameter of mycelial growth on media, except that spots at 5° C. were very slow in developing. The character of the hyphae changed markedly with
Fig. 19. Cultures of four Phytophthora species on potato dextrose agar after 12 days at 15° C.
temperature, became increasingly vesiculose at the lower temperatures and at 5° C. even resembled the hyphae of P. cinnamomi.

The effect of dilute concentrations of malachite green upon growth of the holly Phytophthora and P. hibernalis was studied and results are presented in Table 5.

<table>
<thead>
<tr>
<th>Organism</th>
<th>1/2ppm</th>
<th>1/3ppm</th>
<th>1/4ppm</th>
<th>1/8ppm</th>
<th>1/12ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>holly Phytophthora</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P. hibernalis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The above results were obtained after two weeks exposure to the malachite green concentrations incorporated into proteose peptone nutrient solution in test tubes and held at 20° C. Discs of agar and mycelium cut with a cork borer were used for inoculum. The results for the two organisms were not the same, for the holly Phytophthora showed no growth even at concentrations of malachite green of 1:12,000,000 and the P. hibernalis showed growth at this concentration. However Leonian (10) found no growth of P. hibernalis even at the low malachite green concentration of 1:12,000,000. A discrepancy exists here, for in a previous work (9) Leonian found that P. hibernalis grew at concentrations of malachite green of 1:4,000,000. He does not
explain this discrepancy and makes no mention of it in the later work. It appears that reaction to malachite green concentrations is of only limited usefulness in *Phytophthora* classification.

Another discrepancy which has caused much confusion is the term "malt extract agar". In Leonian's work of 1934, he refers to malt extract agar (called here No. 1) but the listing in the paper of ingredients of the only medium which contains malt extract bears no heading. In a previous work (11) he writes of malt extract agar (called here No. 2) and lists ingredients which differ from the ingredients listed in the later work. The type of malt extract agar workers such as Hickman (7) have used when employing the growth reaction on malt extract agar in taxonomy is not known. That this is important can be seen from the reaction of holly *Phytophthora*, which produced no growth on Difco malt extract agar, and yet grew on the other malt extract media. To clarify the growth reaction on malt extract agar, the holly *Phytophthora* and *P. hibernalis* were grown on four different malt extract agar media and results are shown in Table 6.

The failure of holly *Phytophthora* to grow on Difco m.e.a. differed from the reactions of six other *Phytophthora* species, including *P. lateralis*.

The rate and aspect of growth of six *Phytophthora*
species differed on agar containing two per cent dextrose, two per cent sucrose, or two per cent maltose. The holly Phytophthora was the only species which failed to grow on two per cent maltose. These reactions suggest that growth on sugars alone may be an aid in distinguishing Phytophthora species.

TABLE 6. A COMPARISON OF GROWTH OF HOLLY PHYTOPHTHORA AND P. HIBERNALIS ON VARIOUS MALT EXTRACT AGAR MEDIA

<table>
<thead>
<tr>
<th>Media</th>
<th>Extent of growth after 6 days</th>
<th>Holly Phytophthora</th>
<th>P. hibernalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leonian's m.e.a.</td>
<td></td>
<td>✓ ✓</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>No. 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leonian's m.e.a.</td>
<td></td>
<td>✓</td>
<td>✓ ✓</td>
</tr>
<tr>
<td>No. 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malt extract agar</td>
<td>trace</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>No. 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco m.e.a.</td>
<td>0</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

The holly Phytophthora grew well and produced a few oogonia and sporangia on oatmeal agar. One-month-old cultures at 15° C. on potato dextrose agar developed a narrow ring of aerial mycelia at the culture edge. This ring was similar in appearance to the ring around the culture edge of P. hibernalis shown in Figure 19. It was found that the ring of aerial mycelia of the holly Phytophthora contained many sexual fruiting bodies. The ring was easily removed from the subsurface mycelia with a transfer needle. Observation of the rest of the culture plate showed the
usual nonsporulating mycelia. No oospores had previously been observed in p.d.a. cultures. A few sporangia were found in a p.d.a. culture plate in which both Boydia and holly Phytophthora were growing. The cases mentioned above are the only ones in which spores of any kind were found to be produced in culture media.

Microscopic characters and description

The holly Phytophthora shows a slightly scalloped growth in culture. The scalloping consists of layers of mycelia which are successively deeper in the agar toward the outer edge of the culture (Figure 19). Aerial mycelia occur on p.d.a., but the culture never appears fluffy.

Abundant oogonia are produced when the pea broth-sterile water method is employed, and they average 21 microns. The antheridia are amphigynous (Figure 14a); only one or two doubtful cases of perigyny have been observed. Oospores are spherical and they average 18 microns.

Sporangia are produced sympodially and those from holly leaves are 30-50 by 18-30 microns and average 39 by 24 microns (Figure 14b). The sporangial shape may vary considerably, those formed in distilled water are shorter and more spherical than those formed on the leaves; and the sporangia which germinate directly often become more elongate than the sporangia which release zoospores. The sporangia appear papillate with shallow papillae.
The papillate character varies with the age of the sporangia and is especially pronounced when the sporangia begin to germinate by germ tubes. Direct germination of sporangia by more than one germ tube may occur. Zoospores have sometimes been observed to germinate and directly produce small sporangia. The sporangia are truly pedicellate, a type described by Blackwell (2).

**Summary of taxonomy**

Taxonomic studies of the holly *Phytophthora* placed it in the *syringae* group, although it differed from *P. syringae*, *P. hibernalis*, and *P. porri*, the three species in that group. Members of this group are distinguished from all others mainly by their temperature limitations (10). The holly *Phytophthora* differed from *P. hibernalis* by forming amphigynous antheridia. Of doubtful value taxonomically, but of interest, were the pathogenicity differences, the differences in growth characteristics, and the differences in growth reaction when grown in dilute solutions of malachite green. The holly *Phytophthora* differed from *P. syringae* and *P. porri* (10) by the production of sexual bodies in distilled water after transferring from pea broth. The holly *Phytophthora* differed further from *P. porri* by being killed after an exposure of seven days to 27°C.

It is unknown how many of the species named since
Leonian fit into the syringae group, for physiological studies on many of them have never been made. However a check on the information available showed that one or more characteristics of seventeen of the eighteen new species differed from the characteristics of the holly Phytophthora. Information available on the other new species, P. speciosa, was insufficient to determine anything concerning it. Many of the other new species were described on almost no grounds and some were not even described, so comparisons with them were almost impossible.

Excluding these limitations, the holly Phytophthora appears distinct from any species described. It is possible that the holly Phytophthora would fall within another species if sufficient isolates of the other species were studied and its boundaries were extended to cover a greater range of characteristics. It is felt that more physiological studies should be carried out before final decision is made and the holly Phytophthora is either placed in an existing species or described as a new species.
The holly disease known in Oregon for some time as "Boydia canker" is now known to be caused by a Phytophthora species. The disease has been in the Astoria region for at least twenty years, for an herbarium specimen (stem) collected in 1934 was examined and found to contain many oo-spores of the Phytophthora.

The disease begins in the fall with black leaf-spotting and may increase to epiphytotic proportions with the advent of cool, rainy weather. Under these conditions sporangia and zoospores may develop abundantly on affected leaves and be washed or blown about, and subsequently produce twig and stem or other leaf infections during the winter. The winter nature of the disease agrees with the low temperature requirements (5-20°C) of the pathogen in culture. Periods of dry weather visibly slow leaf infection in the field. It is probable that the oospores produced both in leaves and twigs are the means of oversummering and the completion of the organism's life cycle.

The mistaken interpretation of Boydia and Phomopsis as the pathogen (6, 14 and 26) is readily understandable for three reasons: (1) the Phytophthora pathogen is difficult to isolate except when it is very active; (2) the other organisms, especially Boydia, are connected in some way with the chain of reactions initiated by the pathogen,
and (3) assumptions were made on the basis of the earliest report and were not confirmed by positive inoculations. That \textit{Boydia} is sometimes connected with the disease pattern is confirmed by its isolation from sunken spots beyond definite dieback margins and its frequent fruiting in recently killed holly stems, and in black cankers. The elucidation of the relationship between \textit{Boydia} and springtime disease development has yet to be made.

A determination of the source and type of inoculum which infects stems through the ripe berries also remains to be made, for when this occurs diseased leaves are no longer on the trees.

The cause of severe defoliation, which is the striking phase of the disease, was investigated. Leaves with small spots, or even spotless leaves near diseased ones, were observed to drop from the twigs. This suggested a volatile toxic product, and ethylene with its known defoliating effect on holly, was suspected. Experiments conducted to prove ethylene production by diseased leaves were not entirely successful for they demonstrated only the production of a gas. It is probable that ethylene will be proven to be the defoliating agent and it would be of interest to determine if the ethylene is a metabolite of the fungus. It would also be of interest to know if the disease is a factor in holly shipped at Christmas time, for
the presence of one or a few diseased leaves in a box of cut holly would probably defoliate all of it.

Limited studies of host range indicate that the Phytophthora can infect some other Ilex species and that the host range must be very narrow. The Phytophthora disease occurs throughout the holly-growing area in Oregon and is also found in Washington; it is likely that holly throughout the Pacific Northwest, including western British Columbia, can become infected. Considerations of the origin of the disease and its present extension over a large area involve several factors. Apparently the fungus is intimately connected with *I. aquifolium* and it is possible that a Phytophthora has evolved in this area and adapted itself to *I. aquifolium* in the short time holly has been here. It is more probable that the disease originated in Europe where holly is indigenous and was introduced with the host. The result of an attempt to find the disease in Europe would be of interest. It is probable that many of the old orchards, where the disease is now well established, were propagated from holly obtained from one or two sources. This would effectively spread the disease over a large area if it were present in the original planting. If it were not spread then, the interchange of holly which occurs continually, has offered means for distributing the disease. Possibly of no importance, but of interest is the eating
of the ripe berries by robins which migrate in the springtime. It is then that infected berries are on the trees. Cankers are often associated with sapsucker holes in the trunks of diseased trees. The holes made may offer entry for the pathogen.

Experiments on control were not made; however, observations indicate that the disease is more severe in moist and humid situations. Orchards which are closely planted and in which trees have grown together, restricting wind movement, are particularly susceptible to infection. For heavily infected orchards, a rigid pruning and sanitation program may be necessary to remove the oversummering stage. Protective sprays may be effective, but to be so, will have to protect in the rainy months of November and December. It is then that protective sprays are difficult to hold on a surface due to the rain and it is then that holly is cut. Cut holly is not marketable if a visible residue is on the leaves, so the residue factor will have to be taken into consideration.

An effort to identify the Phytophthora was discouraging for it not only revealed the confusion in the genus but it was found impossible to assign the Phytophthora to any present species. Leonian's work was taken as a foundation and by using it, the holly Phytophthora was assigned to the syringae group. However, it did not conform to any
of the three species in that group, differing from *P. hibernalis*, the most similar species, by the production of amphigynous antheridia. It was felt that more physiological tests should be conducted before final decision is made and the holly *Phytophthora* is either placed in an existing species or described as a new species.
SUMMARY

1. A severe disease of holly in Oregon is caused by a species of Phytophthora. The disease is new in the sense that it has never been reported in the literature.

2. Results from inoculation trials showed that Boydia and Phomopsis, organisms previously reported to cause phases of the disease, do not cause the phases reported. Boydia and Phomopsis may be connected with the disease as secondary invaders or saprophytes. These organisms are often found fruiting on recently killed holly tissue and Boydia can be isolated soon after Phytophthora killing occurs.

3. The disease is characterized by fall and winter black leaf-spotting, defoliation, twig-dieback and the production of black cankers. Trunk and large-limb cankers associated with the other symptoms are believed to be caused by the Phytophthora but the pathogen was not isolated from them.

4. Build-up of inoculum in the form of sporangia and zoospores, occurs on the diseased leaves. It is probable that spread occurs mainly by water movement, but also by wind movement. Infection occurs readily on spine-punctured leaves and may also occur by penetration through unwounded leaf tissue. Oospores are produced in leaf and twig tissue and it is postulated that the oospores are the over-summering stage of the fungus.

5. The fungus does not grow at a temperature of 25°C.
has an optimum between 20° C. and 15° C. and shows some growth at 5° C. Sporangia germinate by zoospore formation at lower temperatures and mainly by means of germ tubes at 20° C.

6. Defoliation is caused by a gas produced by the pathogen or diseased leaf tissue. It is postulated that this gas is ethylene.

7. Limited pathogenic studies indicate a narrow host range, although some species of Ilex other than I. aquifolium can be infected by the pathogen.

8. Taxonomic standing of the holly Phytophthora was not adequately established. Although tests using Leonian's physiological criteria showed that it conforms most nearly to the P. syringae group, the holly Phytophthora differs from the three members of that group. A study of the literature showed that it differs from all previously adequately-described species.

9. Seven other holly disease conditions are discussed in this thesis.
BIBLIOGRAPHY


