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TECHNICAL REPORT #12

OHIA DECLINE:

THE ROLE OF *PHYTOPHTHORA CINNAMOMI*

NATIONAL PARK SERVICE

CONTRACT NO. CX 8000 6 0006

Clifford W. Smith, Unit Director

The National Park Service and the University of Hawaii signed
the memorandum of agreement establishing this Cooperative
National Park Resources Studies Unit (CPSU UH) on March 16, 1973.
The CPSU UH provides a multidisciplinary approach to studies on
the biological resources in the National Parks in Hawaii, that is,
Hawaii Volcanoes National Park, Haleakala National Park, City of
Refuge National Historical Park, and Puukohola Heiau National
Historic Site. Through the Unit Director, projects are undertaken
in areas identified by park management. These studies provide
information for resource management programs. The involvement
of University faculty and students in the resource management of
the National Parks in Hawaii leads to a greater awareness of the
problems and needs of the National Park Service. At the same time,
research not directly or immediately applicable to management is
also encouraged through the CPSU UH.

PHYTOPHTHORA CINNAMOMI:

ITS SURVIVAL IN SOIL AND RELATION TO OHIA DECLINE

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PREFACE

The investigation presented in this report is a first-result increment of the OHIA RAIN FOREST STUDY under National Park Service Contract CX 8000 6 0006, from which it was partially funded.

The study was restricted to well-drained soils in the ohia dieback terrain on Mauna Kea and Mauna Loa and establishes that the distribution of the soil fungus Phytophthora cinnamomi is not correlated with the dead standing ohia trees on these sites. Therefore, the fungus cannot be considered the killer of ohia in the dieback terrain as was formerly suspected. The second part of the study focuses on the survival behavior of this fungus in ohia forest soils and indicates that it can exist as a saprophyte. Therefore, the fungus is not an obligatory parasite and it may be considered one of the fungal decomposers occurring normally in well-drained ohia rain forest soils.

The report contains the main part of a Ph.D. dissertation done under the direction of Dr. Wen-hsing Ko. Other members of the dissertation committee were Minoru Aragaki, Oliver V. Holtzmann, Dieter Mueller-Dombois and Goro Uehara, all of the University of Hawaii.

Dieter Mueller-Dombois
Principal Investigator
OHIA RAIN FOREST STUDY

ABSTRACT

The population of Phytophthora cinnamomi in soils and the amount of ohia rootlets infested with this fungus were determined at four locations, each with healthy and decline forest located close together. The fungus was detected in 34% of soil samples collected from healthy areas and 29% of those from decline areas. Average population of P. cinnamomi in healthy areas (0.1-1.7 propagules/g soil) was not significantly different from that in decline areas (0.1-2.2 propagules/g soil). About 37% of declining trees and also 37% of healthy trees had rootlets infested with P. cinnamomi. Declining trees had an average of 5.2% rootlet segments infested with P. cinnamomi, and healthy trees had 9.4% with this fungus. For a total of 199 ohia trees surveyed, there was no correlation between percentage of rootlets infested with P. cinnamomi and severity of tree decline. There was no indication that P. cinnamomi in decline areas was more pathogenic than that in healthy areas. These results suggest that P. cinnamomi is not a major cause of ohia decline.

By contrast, in avocado tree decline caused by P. cinnamomi, the population of P. cinnamomi in soil collected from the root zone of declining trees was significantly higher than that collected from healthy trees. The fungus was isolated from 97% of the declining trees. Moreover, the severity of tree decline was directly correlated with the percentage of roots infested with P. cinnamomi. Declining trees had an average of 29.2% of root segments infested with P. cinnamomi while healthy trees had only 8.7% with this fungus.

iv

Colonies of P. cinnamomi recovered from natural soil originated mainly from chlamydospores, and occasionally from zoospores. Chlamydospores occurred as free spores or imbedded in organic matter. Results of this study indicated that sporangia of P. cinnamomi also existed in natural soil, and zoospores found on the isolation plates were released from sporangia during incubation.

Among the three spore types of P. cinnamomi tested, chlamydospores were the most persistent in soil, sporangia were intermediate, while zoospores were the least persistent. Survival of P. cinnamomi in soil was better under moist than submerged conditions. The population of chlamydospores remained detectable for one year in moist soil, while only for 3 months in submerged soil. Similarly, over a 12-month period the population of P. cinnamomi in a naturally infested avocado soil declined faster under submerged than moist conditions. Results of survival studies also showed that P. cinnamomi in the root tissue was more persistent than as free chlamydospores in soil. Under moist conditions, the percentage of root tissues from which the fungus was recovered declined only slightly after one year of incubation, while the population of chlamydospores in soil decreased to undetectable level in the same period.

Phytophthora cinnamomi is a good saprophyte. It was able to colonize about 52% of ohia stem segments at a population as low as 10 chlamydospores/g of soil. Among the three spore types of P. cinnamomi tested, chlamydospores were also the most effective in colonizing dead ohia stems, while colonization potential of motile and encysted zoospores was about the same.

Motility of zoospores is important in disease development. At inoculum level of 2.5 x 10^3 propagules/g of soil or above, encysted zoospores were the least infective to ohia seedlings, while infection potential of chlamydospores and motile zoospores was about the same. At inoculum levels below 2.5 x 10^3 propagules/g of soil, however no significant difference in infection potential was found among these three spore types tested.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS (Continued)

INTRODUCTION

Phytophthora cinnamomi Rands is an important root-infecting fungus causing diseases on a wide range of host plants in many parts of the world. It causes root rot of avocado (71), and has been reported associated with littleleaf disease of Pinus radiata in New Zealand (54), and Australia (28), and P. echinata in southeastern United States (10). Phytophthora cinnamomi also has been implicated in the severe decline of jarrah (Eucalyptus marginata) and other native forest tree species in Australia (47, 48, 58, 75, 77).

In Hawaii, P. cinnamomi is of special interest because of its wide occurrence in the ohia rain forests (30, 34). It is widespread on the island of Hawaii (34), and has been isolated from roots of various plant species (35). However, the role of P. cinnamomi in ohia decline is still not clear. If P. cinnamomi is the major cause of ohia decline, the first rule of Koch's postulates, viz. that of intimate association of pathogen with diseased specimens should be sustained. Also, as an important cause of ohia decline, the number of rootlets infected with P. cinnamomi should be correlated with the severity of tree decline. In this study, intensive isolations of P. cinnamomi from soils and roots of ohia trees were conducted at four locations, each with decline and healthy areas located close together in order to establish the association. The association was also closely examined in declining avocado trees in which the role of P. cinnamomi has been well established.

Phytophthora cinnamomi has been shown to be readily transported from place to place by water movement and by any activity involving

soil transportation (35, 55). However, these studies do not adequately explain the widespread occurrence of this fungus. Very little is known how the fungus establishes itself in the new area after being dispersed. Phytophthora cinnamomi produces chlamydospores, oospores, sporangia, and zoospores in or on host roots and in soil (32, 51, 56). Although chlamydospores and zoospores of P. cinnamomi have been considered as the main survival and infection structures, respectively (24, 26), no comparisons had been made among these spore types of P. cinnamomi as to their relative potential for infection of hosts and survival in soil. The type of structure which colonizes organic matter in soil is also unknown. Therefore, the survival potential of zoospores, sporangia, and chlamydospores of P. cinnamomi in moist and saturated soils and the ability of zoospores and chlamydospores to induce disease and to colonize substrates in soil were also studied. Oospores were not included in these studies because they were not found in roots or soils collected from ohia forests.

CHAPTER I

ASSOCIATION OF PHYTOPHTHORA CINNAMOMI WITH OHIA DECLINE

LITERATURE REVIEW

Phytophthora cinnamomi has been implicated in many large scale

tree declines, but its contribution to different tree declines varies.

The following section is to review the relation of P. cinnamomi to ohia

decline and other three major tree declines.

Ohia Decline

Large scale dieback of forest trees including ohia in Hawaii was

noticed as early as 1875 (13). The problem was reported again early in

this century (40, 42, 43, 44). Recent concern about the degeneration

or decline of ohia forests on the island of Hawaii (6, 39, 52) origi-

nated from the observations of this phenomenon by Mueller-Dombois and

Krajina (53) during their ecological studies under the Island Ecosystems

Integrated Research Program of the U. S. International Biological

Program. The decline has affected thousands of acres of native ohia

forests and the affected area has increased considerably (57). Various

fungi, insects and mammals had been considered as possible causes of

ohia decline (3, 6, 30, 33, 39). Phytophthora cinnamomi has received

the most attention among them because of its reported association with

other tree declines. The fungus, isolated from roots of ohia trees,

was shown to be able to cause ohia rootlet necrosis by Kliejunas and

Ko in 1973 (30). Phytophthora cinnamomi was found to be of widespread

occurrence on the island of Hawaii and was isolated from roots of both

endemic and introduced plant species in 22 different plant families (3, 34, 35). Although the frequency of isolation of P. cinnamomi from decline areas was higher than that from healthy areas, Kliejunas and Ko (34) did not consider this fungus to be the only cause of ohia decline. Only 5 to 30% of rootlets of declining trees was infested with P. cinnamomi and the fungus could not be detected in roots of declining trees in three decline areas. Moreover, the population of P. cinnamomi in soils of decline and healthy forests was about the same.

Kliejunas and Ko (32, 34) showed that declining trees produced numerous new leaves and shoots and appeared healthy after treatment with complete fertilizer alone or in combination with fungicides, but not with fungicides alone. Their results have demonstrated that ohia decline symptoms arise from a nutrient deficiency in the tree. However, the exact cause of nutrient deficiency is still unknown. They suggested that it could be a result of root infection by pathogens, low soil fertility, or a combination of both. Recently, Mueller-Dombois (52) has proposed the succession hypothesis to explain ohia decline. He viewed the death of ohia trees in the forests of the island of Hawaii as a normal phenomenon in the primary succession of the rain forest ecosystem on an isolated island. According to the succession hypothesis the ohia decline is the result of site changes in combination with replacement of the pioneer variety of Metrosideros by a seral variety, which in turn is replaced by a climax variety.

Pine Decline

Decline of pine trees which is commonly called littleleaf disease occurs in southeastern United States on P. echinata (10), and in New

Zealand on P. radiata (25, 54). Littleleaf disease of P. radiata has also been reported recently in Australia (28). Reports generally agree that the disease is associated mainly with poor internal drainage and low fertility of soil (1, 10, 15, 25, 28, 56, 81). The disease was controlled by application of nitrogen fertilizer in the United States (66, 67) and superphosphate in New Zealand (1, 14, 81). This has been considered by Campbell and Copeland (10) evidence that littleleaf symptoms arise from a nutrient deficiency in trees.

Phytophthora cinnamomi was isolated from rootlets of both P. echinata and P. radiata and was shown to be pathogenic to both species of pine (7, 9, 54). However, results of many reports suggest that P. cinnamomi may not be the major cause of littleleaf disease of pine. The fungus was detected in soil of both decline and healthy areas (8, 10, 28, 54). Although P. cinnamomi was detected more frequently in soil under diseased trees than healthy trees, there was no correlation between the abundance of this fungus in soil and the severity of the disease (9, 54, 56). In the United States, P. cinnamomi was isolated only from 2% of the roots of diseased trees (9) and in Australia, it was isolated from only 1 out of 258 trees showing symptoms of little-leaf (20).

Eucalypt Decline

According to Australian plant pathologists, decline of Eucalyptus in Australia was first noticed in 1921 and recorded about 1928 (58, 59). However, it actually had been reported much earlier than that. When Clarke (13) reported the large scale degeneration of various tree species in Hawaii in 1875, he also mentioned that dying out of

Eucalyptus in many localities of Australia had been reported in an Australian paper.

In Australia P. cinnamomi occurs in a wide range of forest, woodland, and heath communities. The fungus causes a severe dieback of jarrah (E. marginata) in western Australia (58, 59), and has been reported to be associated with many other native plant species including various species of Eucalyptus in other parts of Australia (29, 48, 55, 75, 78, 80). There is a general acceptance of P. cinnamomi as the major cause of eucalypt dieback in Australia. The fungus was intimately associated with the disease, and had never been isolated from healthy forests despite repeated attempts (47, 75, 76, 77, 80). The spread of P. cinnamomi was associated with logging, road making, and water and vehicle movements, and the extension of the disease also followed the same pattern (77, 78, 80). Koch's postulates had been satisfied under field conditions and typical dieback symptoms were produced on previously healthy mature trees after pure-culture inoculation (58). In addition, dieback symptoms on eucalypt trees disappeared and the spread of disease was prevented when the disease areas were treated with fungicides (76, 77).

Avocado Decline

Avocado root rot caused by P. cinnamomi was first reported by Tucker in 1929 (71), and the fungus was subsequently shown to be the main cause of avocado decline (18, 86). Phytophthora cinnamomi had been shown to be intimately associated with declining avocado trees (18, 23). Crandall (18) was able to isolate P. cinnamomi from 97% of

the rootlets of diseased trees during the wet season. A survey made in California by Harvey (23) showed that roots of 164 out of 307 declining trees were infected with P. cinnamomi. Many of the 143 declining trees which were not infected by the fungus showed evidence of improper care. Of the 268 healthy trees surveyed, 222 were not infected by P. cinnamomi. All 46 healthy trees that yielded P. cinnamomi were in close proximity to declining trees. Phytophthora root rot of avocado had been controlled by treatment of soil with steam, chloropicrin and alfalfa meal in the greenhouse (83, 86) and by repeated application of Dexon in the field (84).

Necessity for abundant soil moisture in the development of Phytophthora root rot of avocado had been reported frequently (18, 71, 73, 86, 87). Abundant soil moisture was shown to favor spore production and infection by the pathogen (85). Root injury by lack of oxygen or accumulation of materials such as nitrite under high soil moisture conditions was shown to be insignificant in disease development (19, 85).

MATERIALS AND METHODS

Description of Ohia Forests under Investigation

The ohia forest stands selected for this study (hereafter referred to as location Nos. 1, 2, 3, 4, 5, 6, 7, and 8) were located on the island of Hawaii (Fig. 1). Soil and root isolations of P. cinnamomi were conducted in the first four locations, each with decline and healthy ohia forests located close together (Fig. 2). Soil
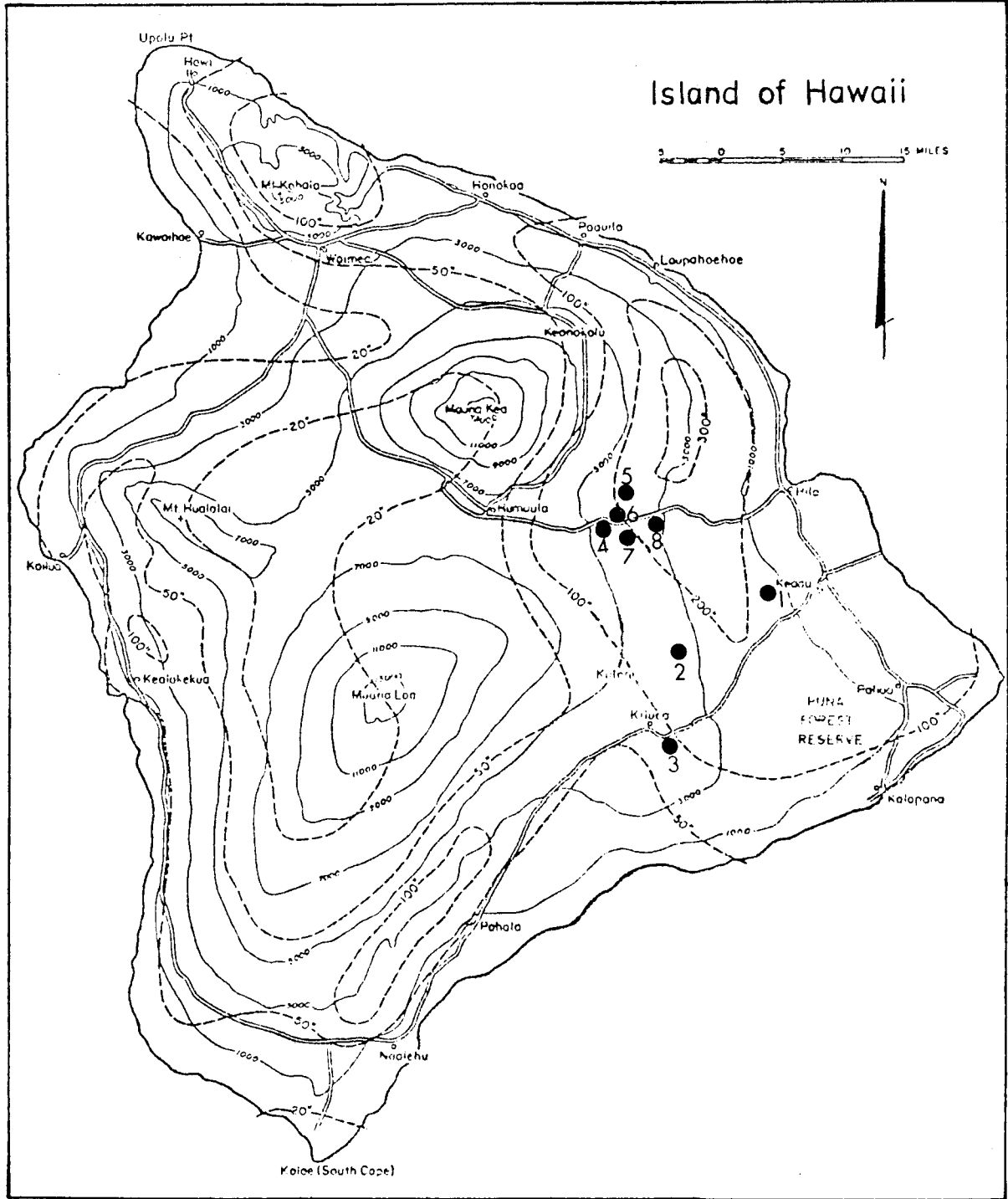
Fig. 1. Locations of ohia forest stands selected for the present study. The elevational contours and isohyet lines are illustrated.

Fig. 2. Decline (A) and healthy (B) ohia forests at location No. 2. They are located close together.

characteristics, elevation, annual rainfall, and mean soil temperature
of these eight locations are summarized in Table 1 (69).

Population Determination of Phytophthora cinnamomi in Soil

Twenty soil samples, combined from five subsamples each, were
collected from each healthy and decline areas at location Nos. 1, 2, 3,
and 4. Samples were taken from a depth of 0-10 cm after the surface
litters were cleared. Twenty soil samples were similarly collected
beneath declining avocado trees and another 20 samples beneath healthy
avocado trees in Puna and Kona areas on the island of Hawaii. The
population of P. cinnamomi in each soil sample was determined by using
a combination of wet sieving and the selective medium (45). Fifty g of
soil was passed through a 38 μ sieve with 300 ml of tap water. The
filtrate was collected, and the materials retained by the sieve was
washed into a 400 ml beaker with tap water to a total volume of 300 ml.
For the first 40 soil samples collected from location No. 1, the
portion retained by the sieve and the filtrate were plated separately
on the medium. Phytophthora cinnamoni was recovered only from the
portions retained by the sieve but not from the filtrates. Therefore,
only the materials retained by the sieve were plated on the medium in
subsequent samples. After sieving, 3 ml of soil suspension was plated
on each plate, and 10 plates were used per soil sample. After 36 hr
of incubation at 24 C, plates were gently washed free from soil par-
ticles with the running tap water, and the number of P. cinnamomi
colonies on each plate was counted under a dissecting microscope at
30 X. Since mycelia of P. cinnamomi were highly branched and consisted

Table 1. Soil characteristics, elevation, annual rainfall, and mean soil temperature
of eight locations of ohia forests selected for study (69)

Location	Soil type	Elevation (m)	Annual rainfall (cm)	Mean soil temperature (C)
1	Keaukaha series, well-drained, thin organic soil over pahoehoe lava bedrock, strongly acid	415	230-380	22-23
2	Kiloa series, well-drained, thin extremely stony organic soil over fragmental aa lava, strongly acid	966	230-380	18-19
3	Kekake series, well-drained, thin organic soil over pahoehoe lava bedrock, strongly acid	1128	130-200	11-13
4, 5, 6 7 and 8	Keei series, well-drained, thin organic soil overlying pahoehoe lava bedrock, strongly acid	1000-1506	230-380	17-18

of abundant swollen vesicles, colonies of this fungus were distinguishable from those of other Pythiaceous fungi which were also capable of growing on the selective medium. Colonies considered to be P. cinnamomi were marked on the bottom of petri plate, and they were examined again after another 3 days of incubation under the same conditions. The fungus produced chlamydospores usually in clumps on the medium within 4 days of incubation.

By using this method about 83% of P. cinnamomi was recovered from soil artificially inoculated with chlamydospores of this fungus. For those soil samples from which P. cinnamomi was not recovered by the use of this method, isolation was repeated using the lupine baiting technique of Chee and Newhook (11). However, results were all negative.

Root Isolation

Root samples were collected from 20 healthy trees from each healthy area and 20 declining trees from each decline area at location Nos. 1, 2, 3, and 4, and from 39 declining trees at the other four locations. Each root sample consisted of three subsamples which were dug out from within 1 m around trunk base of tree. The percentage of crown dieback for each tree was estimated visually. Because most rootlets were dark brown, the necrotic lesions were not distinguishable from the healthy tissue even after washing. As a result, 100 segments (1.5 cm long with diameter less than 2 mm) were cut at random from necrotic and healthy rootlets of each root sample. They were plated on the selective medium after washing under running tap water for 1 hr (at the rate of about 5 liters/min), or sometimes overnight (at the

rate of about 1 liter/min), followed by surface sterilization with 0.5%
NaOCl for 30 sec and single rinse in sterilized distilled water. The
number of rootlet segments from which P. cinnamomi was recovered was
determined under dissecting microscope after 48 hr of incubation at
24 C.

Inoculation Tests

Ohia seedlings of 8-17 cm in height were transplanted to soils
collected from healthy and decline areas at location Nos. 1, 2, and 3.
Three 1.5-liter containers with five seedlings in each were used for
each soil sample, and the experiment was repeated once. Soil samples
collected from the same areas were also used to inoculate ohia trees
of 1.5-4.8 m in height grown in an area where P. cinnamomi had not been
isolated. Three lateral roots per tree about 1 m around the trunk
perimeter were uncovered from aa rock. A plastic bag was placed
underneath each lateral root and about 1,400 g of soil was then placed
around it. Inoculated roots were covered with aa rock again. Three
trees were used for each soil sample, and the experiment was repeated
once. For controls autoclaved soil, composite of soil samples from
three locations, was used in both greenhouse and field tests. Per-
centages of rootlet segments infected with P. cinnamomi were determined
one month after inoculation by the same method as described previously.

RESULTS

Population of Phytophthora cinnamomi in Soil

Ohia forests -- The population of P. cinnamomi in soils was de-
termined at four locations, each with healthy and decline areas located

close together. The fungus was detected in 34% of soil samples collected from healthy areas and 29% of those from decline areas (Fig. 3). It was most frequently isolated from soils of location No. 1 where it was detected in 55% of soil samples of healthy area, and 65% of those of decline area. At the other three locations, the fungus was detected in less than 30% of soil samples with the exception of healthy area at location No. 2 which had 42% of soil samples with P. cinnamomi. The percentage of soil samples under each population class was about the same between healthy and decline areas at each location or in total combination. The average population of P. cinnamomi was 1.7 and 2.2 propagules/g of soil, respectively, in healthy and decline areas at location No. 1, while that of the other three locations was lower than 1.0 propagule/g of soil (Table 2). The population of P. cinnamomi in soils collected from healthy areas was not significantly different from those collected from decline areas.

Avocado soil -- Phytophthora cinnamomi was detected in about 65% of soil samples collected from the root zone of declining avocado trees, but was detected in only 30% of those taken from healthy avocado trees. The population of this fungus in soils under healthy trees averaged 0.2 propagule/g of soil. On the contrary, the population in soils under declining trees averaged 11.0 propagules/g of soil which was about 50 times that of healthy trees. The highest level of P. cinnamomi in soils collected under declining avocado trees was 52 propagules/g of soil.
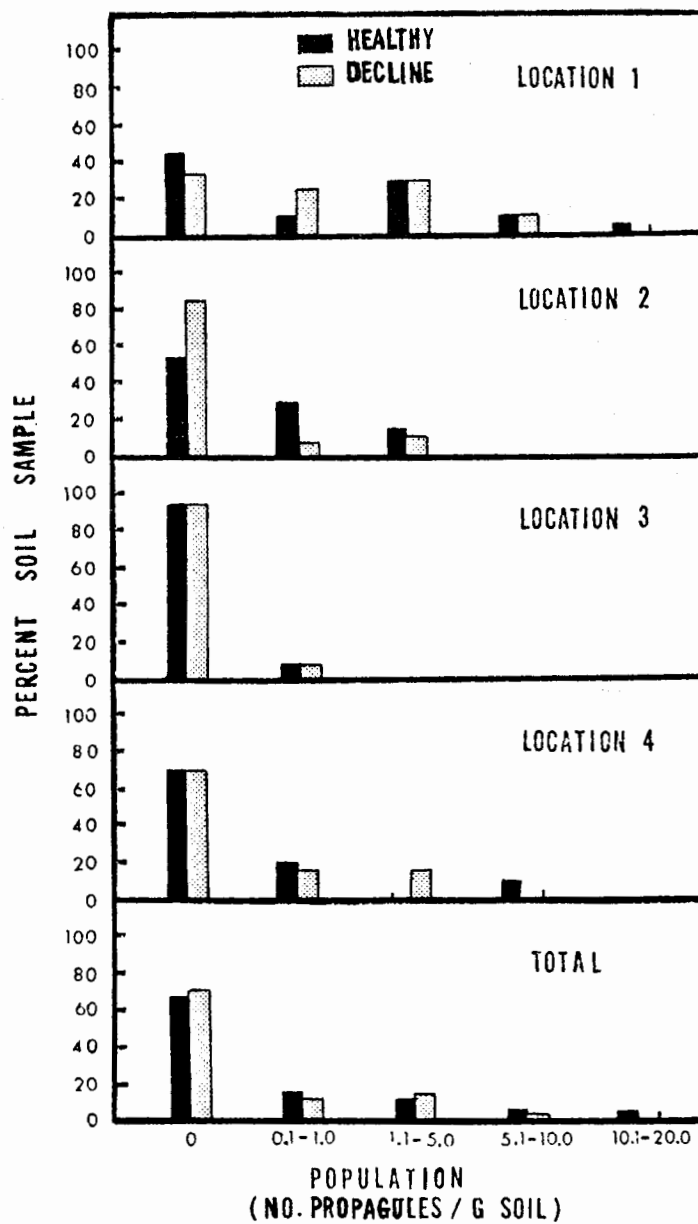
Fig. 3. Comparison of population of <u>Phytophthora</u>
<u>cinnamomi</u> in soils taken from healthy and
decline ohia forests at four locations.

Table 2. Comparison of average population of <u>Phytophthora</u>
<u>cinnamomi</u> in healthy and declining ohia forests
at four locations

Location	Population[a] (No. propagules/g of soil)	
	Healthy area	Decline area
1	1.7 AB[b]	2.2 A
2	0.6 BC	0.5 C
3	0.1 C	0.1 C
4	1.0 BC	0.5 C
Average	0.9	0.8

[a]Average of 20 soil samples, each consisting of five subsamples.

[b]Duncan's multiple range test: means followed by the same
letter are not significantly different at P = 0.05. Analysis
of data of total samples was done separately.

Isolation of Phytophthora cinnamomi from Rootlets of Ohia and Avocado
Trees

Ohia -- When ohia rootlets and root sections with diameters greater
than 2 mm taken from 10 trees were separately plated on the selective
medium, P. cinnamomi was recovered only from rootlets, but not from
larger root sections. Therefore, for ohia only rootlets (less than
2 mm in diameter) were plated out in all the subsequent studies.
About 37% of declining trees and also 37% of healthy trees had rootlets
infested with P. cinnamomi (Fig. 4). The average number of rootlets
from which P. cinnamomi was isolated was about the same for declining
and healthy trees at location Nos. 2, 3, and 4 (Table 3). At location
No. 1, healthy trees had higher percentages of rootlets infested with
the fungus than that of declining trees. However, when the data of
the four locations were combined, the number of rootlets with P.
cinnamomi from healthy and declining trees was about the same. De-
clining trees had an average of 5.2% rootlet segments infested with
P. cinnamomi, and healthy trees had 9.4% with this fungus.

Avocado -- Root samples of 20 healthy and 60 declining avocado
trees were collected from the districts of Puna and Kona on the island
of Hawaii. The healthy trees were growing in close proximity to the
declining trees. Phytophthora cinnamomi was isolated from roots of
97% of declining avocado trees, and also from 75% of healthy avocado
trees. However, percentage of roots infested with this fungus of
declining trees was significantly higher than that of healthy trees.
Declining trees had an average of 29.2% root segments infested with P.
cinnamomi, while healthy trees had only 8.7% with this fungus.
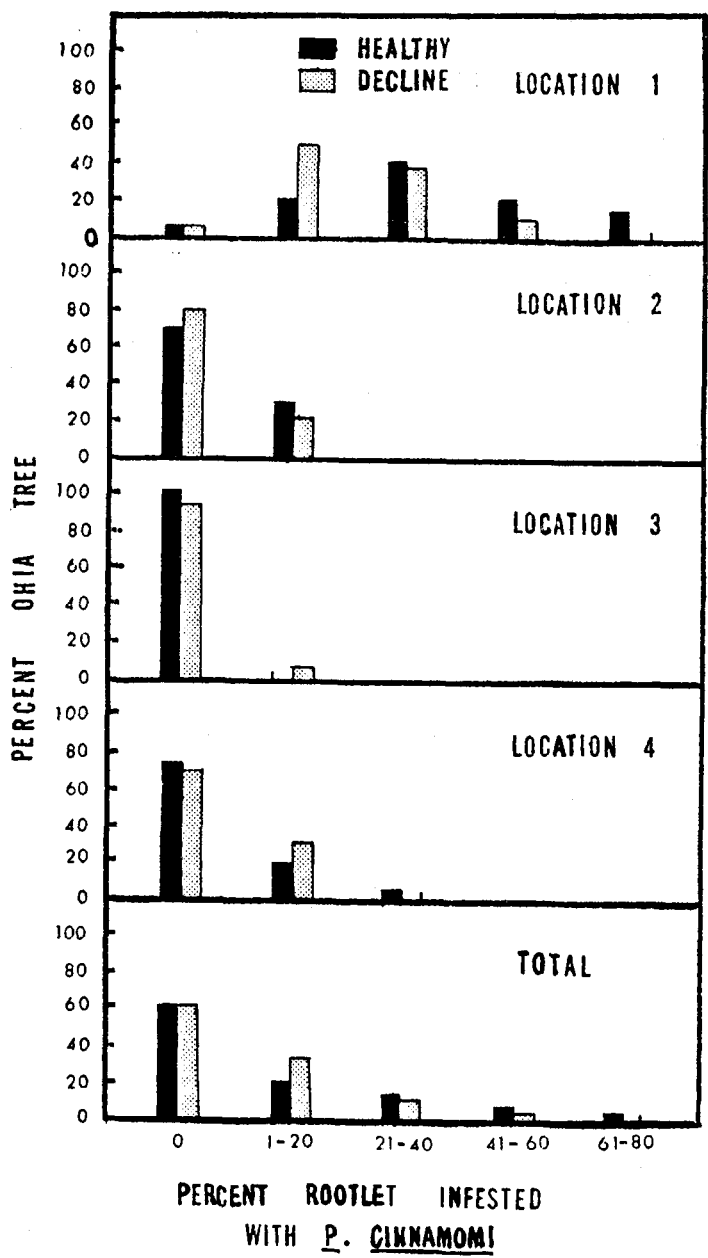
Fig. 4. Comparison of percentage of rootlets infested
with Phytophthora cinnamomi between healthy
and declining ohia trees at four locations.

Table 3. Comparison of average of rootlets infested with Phytophthora
cinnamomi of healthy and declining ohia trees at four loca-
tions, in individual or in total combination

Location	Rootlet segments infested with P. cinnamomi (%)[a]	
	Healthy trees	Declining trees
1	33.6 A[b]	19.2 B
2	0.6 CD	0.3 D
3	0.0 D	0.8 CD
4	3.5 C	0.6 CD
Average	9.4	5.2

[a]Average of 20 ohia trees, 100 rootlet segments from each root
sample being plated on the selective medium and assayed for the
presence of P. cinnamomi.

[b]Duncan's multiple range test: means followed by the same letter
are not significantly different at P = 0.05. Analysis of data
of total samples was done separately.

Moreover, the difference in the root appearance between declining and healthy avocado trees was also striking. Most of the feeder roots of declining avocado trees were completely rotted. Consequently, root isolations could be made only from larger roots, 3-10 mm in diameter. On the contrary, root samples taken from healthy trees contained abundant feeder roots, and isolations were made from these feeder roots for healthy trees.

Relationship between Abundance of Phytophthora cinnamomi in Rootlets and Severity of Tree Decline

Ohia -- In the total of 199 ohia trees, there was no correlation between percentage of rootlets infested with P. cinnamomi and severity of tree decline (Fig. 5). The calculated regression equation, $Y = -0.02 X + 34.13$ had a correlation coefficient of -0.01 which is not significantly different from zero at $P = 0.05$.

Avocado -- With a total of 80 avocado trees, severity of tree decline was directly correlated with percentage of roots infested with P. cinnamomi (Fig. 6). The calculated regression equation, $Y = 0.91 X + 25.34$ had a correlation coefficient of 0.54 which is significantly different from zero at $P = 0.01$.

Infection Potential of Soils Collected from Healthy and Decline Ohia Forests

When ohia seedlings were grown for one month in soils collected from healthy or decline areas of location Nos. 2 and 3, the percentage of rootlets infested with P. cinnamomi was about the same (Table 4). However, more rootlets were infested with the fungus in healthy than
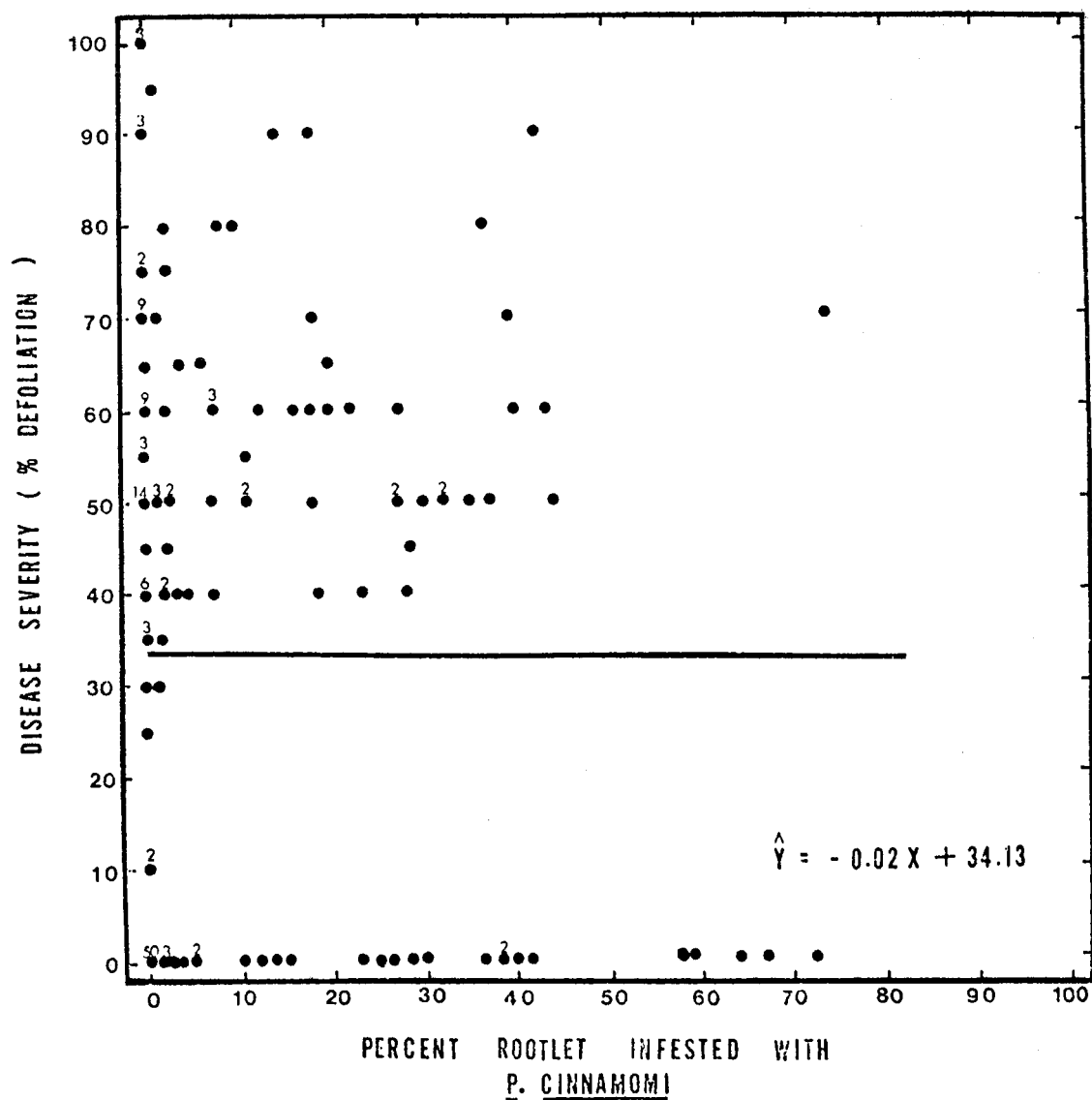
Fig. 5. Relationship between amount of rootlets infested with
Phytophthora cinnamomi and severity of decline for the
combined data of 199 ohia trees collected from eight
locations. The correlation coefficient is -0.01 which
is not significantly different from zero at P = 0.05.
The figure immediately above each point indicates the
number of trees.
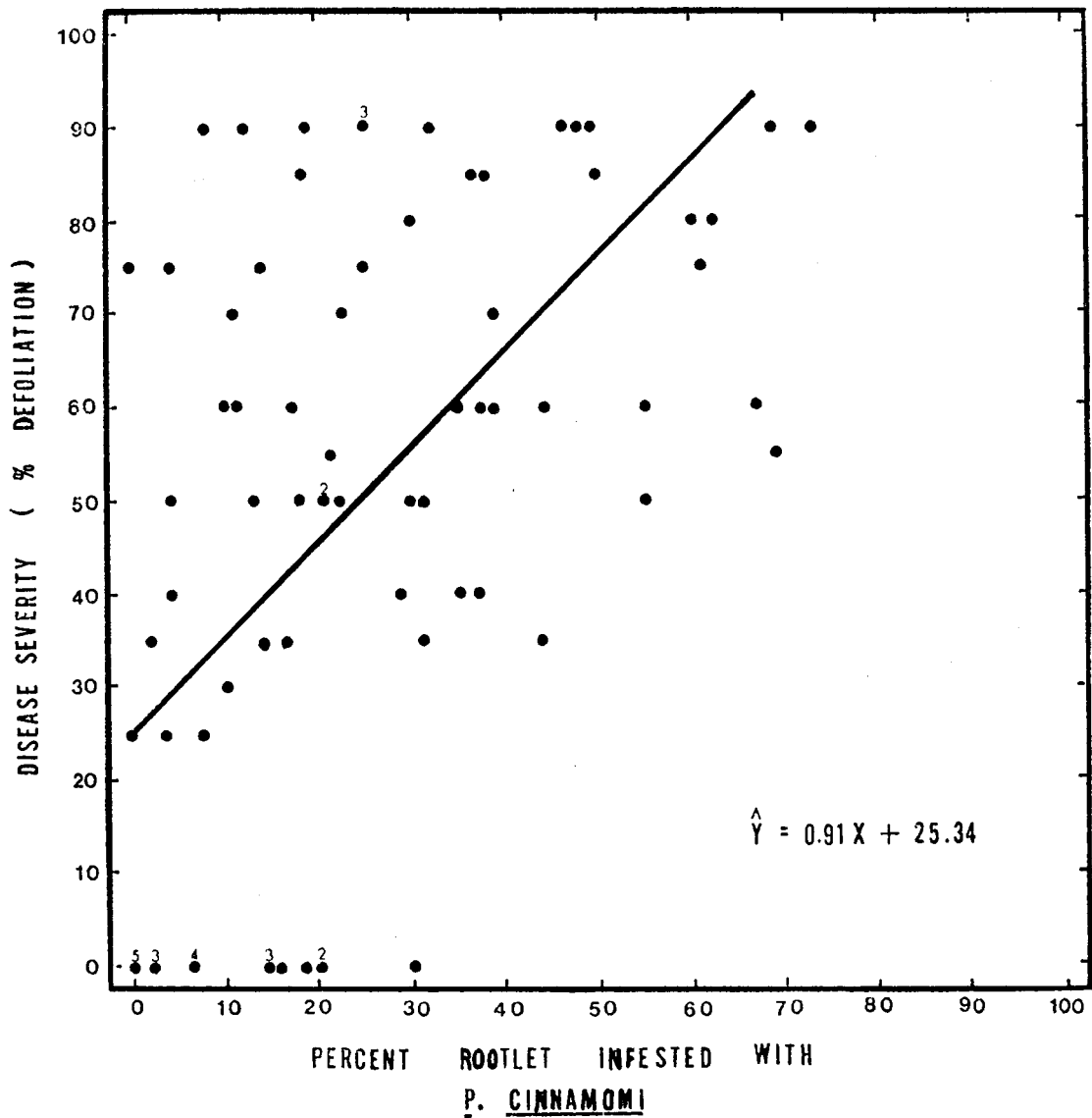
Fig. 6. Relationship between amount of roots infested with
Phytophthora cinnamomi and severity of decline for the
combined data of 80 avocado trees taken from four fields.
The correlation coefficient is 0.54 which is significantly
different from zero at P = 0.01. The figure immediately
above each point indicates the number of trees.

Table 4. Greenhouse comparison of infection potential
of soils collected from healthy and decline
areas at three locations

Location	Infection potential[a] (% rootlets with $\underline{P}$. $\underline{cinnamomi}$)	
	Healthy soil	Decline soil
1	40.3 A[b]	23.3 B
2	12.3 BC	4.0 C
3	0.0 C	0.0 C
Control	0.0	0.0

[a]Average of six pots, five ohia seedlings per pot.

[b]Means followed by the same letter are not significantly dif-
ferent at P = 0.05.

in decline soils from location No. 1. Similar results were obtained in
the field tests with larger ohia trees (Table 5). Therefore, there was
no indication that P. cinnamomi in decline areas was more pathogenic
than in healthy areas.

DISCUSSION

Phytophthora cinnamomi has been suspected to be the possible
primary cause of ohia decline for the following reasons: (i) the
fungus was isolated from rootlets of ohia trees and was able to cause
rootlet necrosis and death of ohia seedlings and small trees in
greenhouse tests (30); (ii) it was found to be of widespread occurrence
in ohia forests and was detected more frequently in declining forests
than in healthy forests (34, 35); and (iii) it has been associated
with other tree declines. As the major cause of ohia decline, one
would expect a constant association of P. cinnamomi with declining
ohia trees (first rule of proof of Koch's Postulates) (64). Results
of this study showed that P. cinnamomi was not consistently associated
with declining ohia trees. The fungus was isolated from 37% of de-
clining trees, but approximately the same percentage of healthy trees
were also with rootlets infested with this fungus. Kliejunas and Ko
(34) also failed to isolate P. cinnamomi from roots of declining trees
in three decline areas. Also, as an important cause of ohia decline,
the number of rootlets infected with P. cinnamomi should be correlated
with the severity of tree decline. Among 199 trees surveyed in this
study, there was no correlation between severity of tree decline and
percentage of rootlets infested with P. cinnamomi. In fact P.

Table 5. Field comparison of infection potential of
soils collected from healthy and decline
areas at three locations

| Location | Infection potential[a] (% rootlets with P. cinnamomi) | |
	Healthy soil	Decline soil
1	33.3 A[b]	14.3 B
2	11.2 B	14.0 B
3	0.3 C	0.0 C
Control	0.0	0.0

[a]Average of two replicates, three ohia trees per replicate.

[b]Means followed by the same letter are not significantly dif-
ferent at $P = 0.05$.

cinnamomi could not be detected in roots of three recently dead trees and three seriously declining trees (Fig. 5). These results suggest that P. cinnamomi is not a major cause of ohia decline.

Effect of rootlet necrosis caused by P. cinnamomi on the growth of ohia trees appears to be minimal. Results of isolation study showed that 37% of healthy ohia trees were also with rootlets infested with this fungus. Moreover, 5 out of 80 ohia trees surveyed had more than 50% of their rootlets infested with P. cinnamomi and yet showed no declining symptoms (Fig. 5). It is not known whether infection of P. cinnamomi occurs after the ohia rootlets are dead or when they are still alive. Phytophthora cinnamomi is a good saprophyte, as indicated in the second part of present study; it readily invades dead ohia tissues in the soil. Therefore, the association of P. cinnamomi with ohia rootlets may be of saprophytic nature.

The death of ohia seedlings and small ohia trees resulting from inoculation with P. cinnamomi in the greenhouse (30) was probably due to the use of excessively large amounts of inoculum (40,000 zoospores/ seedling or 1 plate of culture/small tree). In decline areas the average population of P. cinnamomi ranged only from 0.1-2.2 propagules/g of soil (Table 2). Moreover, according to the decline survey, small ohia trees were generally healthy in appearance and no dead small trees were observed in declining forests (34).

Phytophthora cinnamomi was isolated from soils of all the locations chosen for the present study. The population of the fungus in soils collected from decline areas was about the same as that collected from healthy areas. These results are in accord with those of

Kliejunas and Ko (34) who also showed no significant difference between P. cinnamomi population in soil of decline and healthy forests. In an island wide survey, Kliejunas and Ko (34) reported that the frequency of isolating P. cinnamomi from decline areas was higher than that from healthy areas. In the present study the difference in environmental conditions in decline and healthy areas at location Nos. 1, 2, 3, and 4 was minimal because they were close together. Under such conditions P. cinnamomi population varied among the locations, but did not differ significantly between decline and healthy areas at each location (Fig. 3; Table 2). This suggests that the difference noted in their study may be a result of variations of environmental conditions which influence the distribution of P. cinnamomi in soil. Effects of environmental conditions on distribution of P. cinnamomi in soil have been documented (46, 48, 77, 79).

Results of soil and root isolation also showed that the amount of rootlets infested with P. cinnamomi was correlated with the population of this fungus in soil (Figs. 3, 4). For instance, the fungus was detected in rootlets of 95% of both healthy and declining ohia trees at location No. 1 where the population of this fungus in soil was the highest among these four locations tested. On the contrary, P. cinnamomi was detected in rootlets of 1 out of 40 trees surveyed at location No. 3, and the fungus was detected in 2 out of 40 soil samples collected from there. This was further supported by the results of inoculation trials. Soils collected from location No. 1 caused much more rootlet necrosis than those from location No. 3 in both greenhouse and field tests.

Phytophthora cinnamomi has also been implicated in many other tree declines, but its contribution to decline of different tree species varies. Several lines of evidence suggested that P. cinnamomi is the primary cause of avocado decline in many parts of the world, and of eucalypt dieback in Australia. (i) The fungus was intimately associated with the diseased trees (18, 23, 47, 75, 76, 77, 80). Crandall (18) was able to isolate the fungus from 97% of the rootlets of diseased avocado trees during the wet season, while in Australia the fungus was consistently isolated from diseased forests and had never been isolated from healthy forests (47, 75, 76, 77, 80). (ii) Koch's postulates had been satisfied under field conditions and typical dieback symptoms were produced on previously healthy trees after pure culture inoculation (58, 89). (iii) Spread of disease was associated with irrigation water movement in the case of avocado decline (4), and with road making activities and presence of drainage channel in the case of eucalypt dieback (77, 78, 80). (iv) Diseased symptoms disappeared when the disease areas were treated with fungicides (76, 77, 84).

Results of this study with avocado decline also showed that P. cinnamomi was isolated from 97% of the declining avocado trees, and that there was a good correlation between the percentage of roots from which the fungus was isolated and the severity of tree decline. In addition, the population of the fungus in soils collected from the root zone of declining trees was significantly higher than that collected from the healthy trees.

The association of P. cinnamomi to littleleaf disease of pine has been extensively studied. The fungus was pathogenic to rootlets of both P. radiata and P. echinata and was detected in soil under both decline and healthy areas (7, 8, 9, 28, 54). Although P. cinnamomi was isolated more frequently from soil under diseased trees than healthy trees, there was no correlation between the population of this fungus in soil and the severity of the disease (9, 54, 56). Phytophthora cinnamomi was isolated from only 2% of the roots of diseased trees in the United States (9), and from 1 out of 258 pine trees showing symptoms of littleleaf in Australia (20). Jehne (28) showed that the frequency of isolation of P. cinnamomi from dead pine trees was not significantly different from that obtained from healthy trees in both healthy and decline areas. Therefore, the lack of association between P. cinnamomi and littleleaf disease of pine suggests a similarity to ohia decline. Another similarity between ohia decline and littleleaf of pine is that symptoms of both diseasea arise from a deficiency in the tree of inorganic nutrients. Littleleaf symptoms were relieved by application of nitrogen fertilizer in the United States (66, 67) and superphosphate in New Zealand (1, 14, 81), while dieback symptoms of ohia were alleviated by treatment with complete fertilizer (32).

SUMMARY

The population of P. cinnamomi in soils and the percentage of ohia rootlets infested with this fungus were determined at four lo-cations, each with healthy and decline ohia forests located close together. Twenty soil samples, each combined from five subsamples,

were collected from each area and the population was determined using the combination of wet sieving and selective medium. Phytophthora cinnamomi was detected in 29% of soil samples collected from decline areas and 34% from healthy areas. Average populations of the fungus in healthy areas (0.1-1.7 propagules/g of soil) were not significantly different from that in decline areas (0.1-2.2 propagules/g of soil). Results of inoculation trials showed that infection potential of P. cinnamomi in soils collected from decline and healthy areas was about the same, except at one location where the infection potential was higher in healthy than decline soils.

Root samples were collected from 20 trees from each area and 100 rootlet segments per sample tree were plated on selective medium after overnight washing in running tap water and surface sterilization. The percentage of declining trees infested with P. cinnamomi was not significantly different from that of healthy trees, except at one location where healthy trees had a higher percentage of rootlets infested with this fungus than had the declining trees. About 37% of declining trees and 37% of healthy trees had rootlets infested with P. cinnamomi. Also, there was no correlation between percentage of rootlet segments infested with P. cinnamomi and severity of tree decline. Declining trees had an average of 5.2% of rootlets with P. cinnamomi, while healthy trees had 9.4% with this fungus. Results of soil and root isolation indicate that P. cinnamomi is not the major cause of ohia decline.

By contrast, results concerned with avocado decline, caused by P. cinnamomi, showed that the population of P. cinnamomi in soil

collected from the root zone of declining trees was significantly higher than that collected from healthy trees. The fungus was isolated from 97% of the declining trees. Moreover, the severity of tree decline was directly correlated with the percentage of roots infested with P. cinnamomi. Declining trees had an average of 29.2% of root segments infested with P. cinnamomi while healthy trees had only 8.7% with this fungus.

CHAPTER II

BIOLOGY OF PHYTOPHTHORA CINNAMOMI IN SOIL

LITERATURE REVIEW

Biology of Phytophthora Species in Soil in General

Infection potential -- Zoospores generally have been considered to

be the most effective form of inoculum of Phytophthora for infection of

host plants (26). They are motile and capable of oriented movement

toward plant roots. Kliejunas and Ko (31) showed that motile zoospores

of P. palmivora were much more effective in killing papaya seedlings

than nonmotile zoospores, thus demonstrating the importance of motility

and tactic response of zoospores in disease development. However, when

infection potential of zoospores was compared with that of sporangia and

chlamydospores at the same inoculum density, zoospores were the least

infective among these three types of spores (36). Similar results were

obtained by Ramirez and Mitchell (62) who showed that a much higher con-

centration of zoospores than chlamydospores of P. palmivora was needed

to induce the same amount of disease on papaya seedlings. Comparison of

infection potential among different spore types of other Phytophthora

spp. have not been made.

Colonization potential -- Little information regarding colonization

of organic matter by Phytophthora spp. in soil have been published.

Zentmyer and Mircetich (88) showed that P. cinnamomi was able to

colonize organic matter in natural soil especially under conditions of

high soil moisture. Infection potential of P. palmivora was increased

when field soil was amended with papaya tissues (70) and population of

P. parasitica in soil was also increased when organic matter was added

(27) thus indicating their ability to colonize organic matter in soil.
Ko and Chan (36) reported that among the three spore types of P.
palmivora studied, sporangia were the most effective in colonizing dead
papaya stems, while the colonization potential of zoospores and
chlamydospores was about the same.

Survival in soil -- Phytophthora drechsleri formed chlamydospores
in the roots of several common weed species artificially inoculated
with the fungus (17), suggesting that the fungus may be able to survive
in the absence of host plants by parasitizing roots of nonhost plants in
the same area. However, presence of nonhosts did not have beneficial
effects on the survival of P. parasitica and P. megasperma (21, 61).
In the absence of hosts, most species of Phytophthora usually survive
in soil as dormant spores. Among the four spore types produced by
members of Phytophthora, chlamydospores and oospores have been con-
sidered as the main survival structures. Studies on soil isolation have
shown that colonies of P. parasitica and P. cinnamomi on the plates
frequently originated from chlamydospores (24, 27). Chlamydospores also
have been recognized in a number of other species including P. arecae,
P. boehmeriae, P. cactorum, P. citrophthroa, P. colocasiae, P.
drechsleri, P. palmivora, and P. syringae (16, 74). Long term survival
of oospores of P. cactorum and P. megasperma in soil has been demon-
strated by Sneh and McIntosh (68) and Legge (41), respectively. Zoo-
spores of P. cactorum, P. drechsleri and P. megasperma became non-
detectable within a short period of time in soil (49, 50). However,
zoospores of P. palmivora remained viable for at least 6 months in
soil (72). Although sporangia of P. cactorum were not able to survive

in soil (2, 68), sporangia of P. palmivora remained viable in soil for 2 years (72) and sporangia of P. parasitica were also found in natural soil (27).

Biology of Phytophthora cinnamomi in Soil

The behaviour of P. cinnamomi in soil is basically similar to that of other Phytophthora spp. described above. Zentmyer and Mircetich (88) found that P. cinnamomi was still detectable after 6 years in naturally infested soil at 20 C. However, Kuhlman (38) reported that 19 months was the maximum survival period in forest soil of Oregon at outdoor conditions. In both papers, low soil moisture was stated to be detri-mental to survival of this fungus. Survival of P. cinnamomi in soil under saturated conditions is not known.

Phytophthora cinnamomi was found to be of widespread occurrence in ohia forests on the island of Hawaii and was isolated from roots of both endemic and introduced plant species in 22 different families (3, 34, 35). It has been shown to be readily transported from place to place by water movement or any activity involving soil transportation (35, 55). However, it is still not known how the fungus establishes itself in the new areas after being dispersed. The fungus may establish itself in the new areas through host infection, substrate colonization, or exist in the form of dormant spores.

Of the four spore types produced by P. cinnamomi, chlamydospores were frequently found as the origins of P. cinnamomi colonies on soil isolation plates (24, 35, 45). Results of these studies suggest that chlamydospores are the primary survival structure in soil. Since high

soil moisture has always been associated with root rot caused by P. cinnamomi (18, 71, 73, 86, 87) and the fungus produces zoospores under wet soil conditions, zoospores are considered the primary infection unit. They are motile and capable of oriented movement toward plant roots. Oospores of P. cinnamomi have also been found in diseased root tissues (51), but the role of oospores in the life cycle of this fungus is not understood. Since oospores are produced only in the presence of both mating types or under special conditions (5, 60, 65), their population in soil is probably extremely low in comparison with that of chlamydospores. Reeves (63) showed that when buried in soil, mycelia of P. cinnamomi were able to produce sporangia, chlamydospores, or less commonly oospores. Therefore, mycelia may also contribute indirectly to the long term survival of this fungus in soil.

Reports regarding the saprophytic ability of P. cinnamomi are not consistent. By showing that P. cinnamomi was capable of invading wheat straw and dead avocado roots in natural soil, Zentmyer and Mircetich (88) considered P. cinnamomi to be a good saprophyte. However, Kuhlman (38) regarded P. cinnamomi as a poor saprophyte because he found that this fungus was able to colonize Douglas-fir twigs only at high inoculum density. After the death of forest trees, populations of P. connamomi in soils decreased considerably (46, 54, 79), thus indicating the requirement of host roots for survival of this fungus in the forest soil.

In this study, persistence of zoospores, sporangia, and chlamydospores of P. cinnamomi in moist and submerged soils was compared. Also,

the ability of motile zoospores, encysted zoospores, and chlamydospores
to induce disease and to colonize substrates in soil were studied.

MATERIALS AND METHODS

Preparation of Fungal Propagules

Phytophthora cinnamomi (58F) obtained from an ohia rootlet was
maintained on vegetable juice agar (per liter: V-8 juice, 200 ml;
$CaCO_3$, 2 g; agar, 20 g) at 24 C with continuous fluorescent light. Ten
to 15 agar discs (6 mm in diameter) from a 2- to 3-day-old culture of
P. cinnamomi growing on 20% V-8 agar were transferred to a sterilized
disc of washed, uncoated cellophane (90 mm in diameter) laid on the
same medium. After incubating for 24 hr at 24 C, the cellophane mem-
brane containing the mycelia was removed from the medium and placed in
a petri dish into which about 25 ml of 5% V-8 juice browth (per liter:
V-8 juice, 50 ml; $CaCO_3$, 2 g) were added. The V-8 juice broth was
centrifuged at 1,500 rpm for 5 min before use. Abundant young mycelia
were produced on the cellophane after 24 hr. The broth was drained
from the petri dish, and mycelia on the cellophane were rinsed with
20 ml of mineral solution of Chen and Zentmyer (12). The mycelia were
rinsed again with the same solution immediately and incubated in 20 ml
of mineral solution at 24 C with continuous fluorescent light. Mycelia
started to produce sporangia after 9 hr, at which time the mineral
solution was drained to prevent premature release of zoospores from
the sporangia. Abundant sporangia were produced after 12 hr more in
moist conditions, mostly at the margin of each mycelial mat. Sporangial
suspension was obtained by spraying the mycelial mats with distilled

water by means of an atomizer. For release of zoospores, plates were washed three times with sterile distilled water, chilled at 5 C for 20 min, and then returned to 24 C. Most zoospores were released within 1 hr after chilling. Encysted zoospore suspension was obtained by agitating motile zoospore suspension in a test tube for 1.0-1.5 min with Vortex Mixer.

After the zoospore suspension was drained, the plate was further incubated under the same conditions. Abundant mature chlamydospores were produced within one week. Agar pieces in the center of each colony were removed before mycelial mats were scraped off the cellophane with a spatula. Mycelial mats in distilled water were triturated in an Omni-Mixer chamber at 4,500 rpm for 3 min. The resultant suspension was passed through a 105 µ sieve which retained the larger mycelial fragments. Chlamydospores were further separated from small mycelial fragments by sedimentation in a 150-ml beaker two to three times. Most mycelial fragments were eliminated from the suspension after this treatment. Chlamydospore suspension contained about 4% of pyriform spores which did not release zoospores even with cold treatment. Concentration of spore suspensions was determined by the microsyringe method (37).

Survival of Phytophthora cinnamomi in Soil

Persistence of zoospores, sporangia, and chlamydspores added to soil -- Fifty ml of zoospore and sporangial suspension at concentrations of 2.6 x 10^6/ml and 1.2 x 10^3/ml, respectively, were separately mixed with 400 g of air-dry soil (a clay loam). After mixing

thoroughly, half of each soil placed in a 400-ml beaker was adjusted to moist conditions (60% soil moisture on an oven dry weight basis), and another half was brought to submerged conditions (200 g soil/50 ml water). Studies of survival of chlamydospores in moist and submerged soils were conducted separately. Twenty-five ml of chlamydospore suspension at concentration of 1.2×10^3-3.2×10^3/ml was added to 200 g of air-dry soil adjusted to either moist or submerged conditions as described above. All the soils were stored in a moist chamber following adjustment to the appropriate moisture level. At various time intervals, 5 g of soil was removed and the population of each propagule in soil was determined by plating the diluted soil suspension on selective medium. Two replicates with five plates per replicate were used.

Naturally infested soil -- The soil used in this study was collected from the root zone of a declining avocado tree. After larger rocks and undecomposed organic matters were removed, 500 g of soil placed in a beaker was added with water to submerged conditions, while another 500 g was adjusted to moist conditions. At various time intervals during a period of one year at 24 C, 30 g of soil was removed and the population of P. cinnamomi in each soil was determined by the method of wet sieving and selective medium.

Survival of Phytophthora cinnamomi in Root Tissue

Avocado -- Root sample was collected from a severely declining avocado tree. After washing in running tap water for 1 hr, root segments 1 cm long and 3-10 mm in diameter were cut and buried in soil

adjusted to 60% moisture level and held at 24 C. At various time intervals during a period of one year, 50 segments were removed and isolation of P. cinnamomi from root tissue was made by the same method as previously stated.

Ohia -- Ohia root segments, 1 cm long with diameters of 3-10 mm, were washed in running tap water for 1 hr, and surface sterilized with 75% alcohol for 10 min. To establish the fungus in the tissue, these root segments placed in petri dishes were inoculated with 20 agar discs per petri dish obtained from a 4-day-old culture of P. cinnamomi grown on 20% V-8 agar. After 2 weeks of incubation at 24 C when mycelia of the fungus had covered the surface of root tissues, root segments were removed and buried in soil and adjusted to moist conditions. The percentage of root segments with P. cinnamomi was determined at various time intervals in a one-year period.

Nature of Propagules Recovered from Soil

Two avocado field soils and two ohia forest soils known to have high population of P. cinnamomi were processed through the same procedure as that used in determining P. cinnamomi population in soil. Both filtrate and the materials retained on the sieve were plated on the selective medium, and 10 plates each were used for each soil sample. After 36 hr of incubation at 24 C, plates were washed gently free from soil particles with running tap water. Each colony of P. cinnamomi on the plate was marked on the bottom of the petri dish under a dissecting microscope. The origin of each colony was examined under a microscope at 100 X. Whenever pieces of organic matter were

recognized as the origin of colonies, they were removed with a needle to a glass slide, pressed under the cover glass, and examined for the presence of fungal propagules.

Infection Potential of Motile Zoospores, Encysted Zoospores, and Chlamydospores

Ohia seeds were sowed on a layer of mica peat (about 3 mm thick) laid on the surface of 200 g of soil in a plastic tray (12 x 12 x 2.5 cm). Seedlings were thinned to 70 per pot 3 weeks after sowing. Inoculations were made 3 months after sowing when ohia seedlings had 4-6 leaves and were about 1 cm in height. Thirty ml of propagule suspension was evenly distributed over the soil surface in each pot by using a disposable pipette. All seedlings were flooded with water for 24 hr following treatment and then watered once daily. Each treatment was replicated twice and the experiment was repeated once. During a period of one month the number of ohia seedlings killed in each pot was recorded.

Colonization Potential of Motile Zoospores, Encysted Zoospores, and Chlamydospores

Thirty ml of propagule suspension was mixed with 200 g of soil in a 500-ml beaker. The soil moisture was adjusted to near saturation (70% on an oven dry weight basis). One hundred stem segments (1.5 cm long and about 2 mm in diameter) obtained from young shoots of ohia trees were dried in an oven at 65 C overnight, and buried in the infested soil. After one week of incubation at 24 C, 50 segments were removed from soil, washed in running tap water for 2 hr, surface

sterilized in 0.5% NaOCl for 30 sec, rinsed once in sterilized distilled water, and placed on selective medium. The number of segments containing P. cinnamomi was recorded. The experiment was repeated once.

RESULTS

Survival of Phytophthora cinnamomi in Soil

Persistence of zoospores, sporangia, and chlamydospores added to soil -- Among the three spore types of P. cinnamomi tested, chlamydospores were the most persistent in soil, sporangia were intermediate, while zoospores were the least persistent (Figs. 7, 8). The population of chlamydospores decreased to an undetectable level after one year under moist conditions, and 3 months under submerged conditions. Sporangia and zoospores were not recovered from both moist and submerged soils after 2 months and 3 weeks, respectively.

To test if sporangia released zoospores in soil, 10 g of soil used in the survival study was removed, and the population of zoospores in soil was determined by the following method. Soil suspension was passed through a 38 μ sieve which retained sporangia while allowed zoospores to pass through. The filtrate was incubated on the selective medium, and the number of colonies of P. cinnamomi that originated from zoospores on the isolation plates was counted 36 hr after incubation.

Over a 2-week period after sporangia were added to the soil, zoospores were recovered from both moist (Fig. 9) and submerged soils (Fig. 10). After incubation for one day, the ratio of zoospores to sporangia was about 4 and 1 in submerged and moist soils, respectively. The population of zoospores reached the maximal level on the fourth day

Fig. 7. Persistence of chlamydospores, sporangia, and zoo-
spores of Phytophthora cinnamomi in moist soil at
24 C.
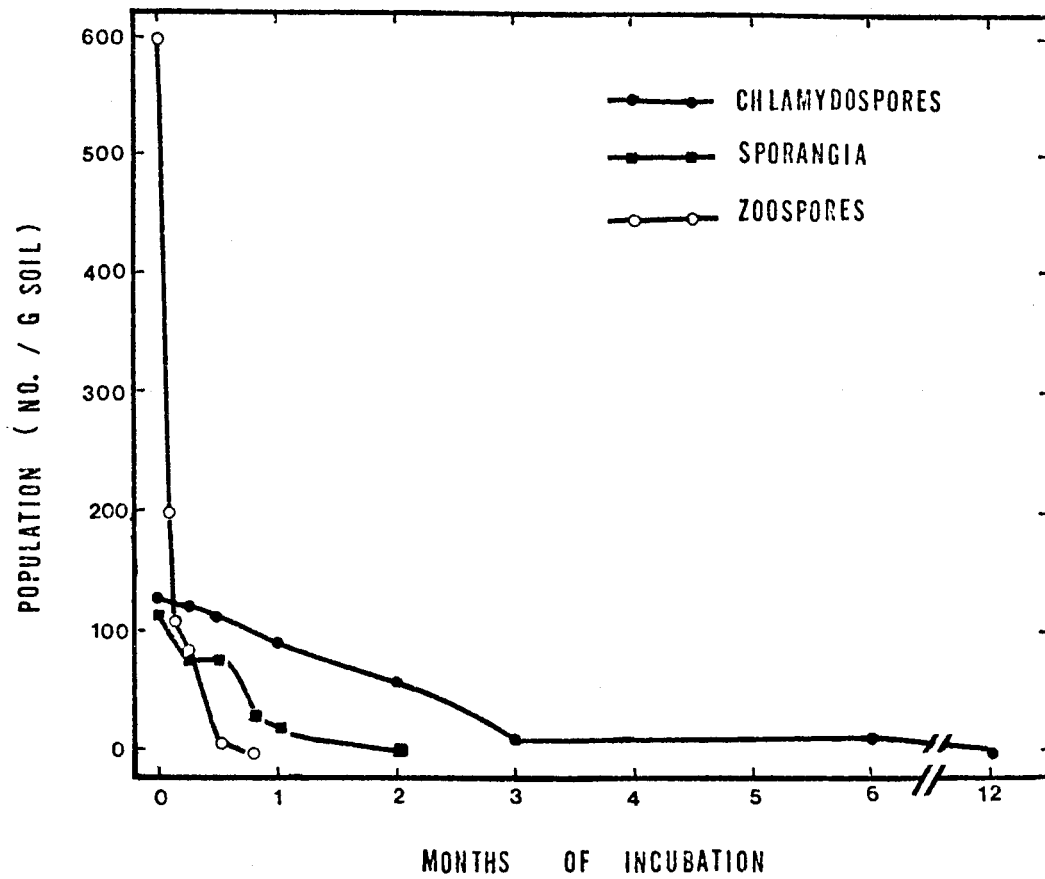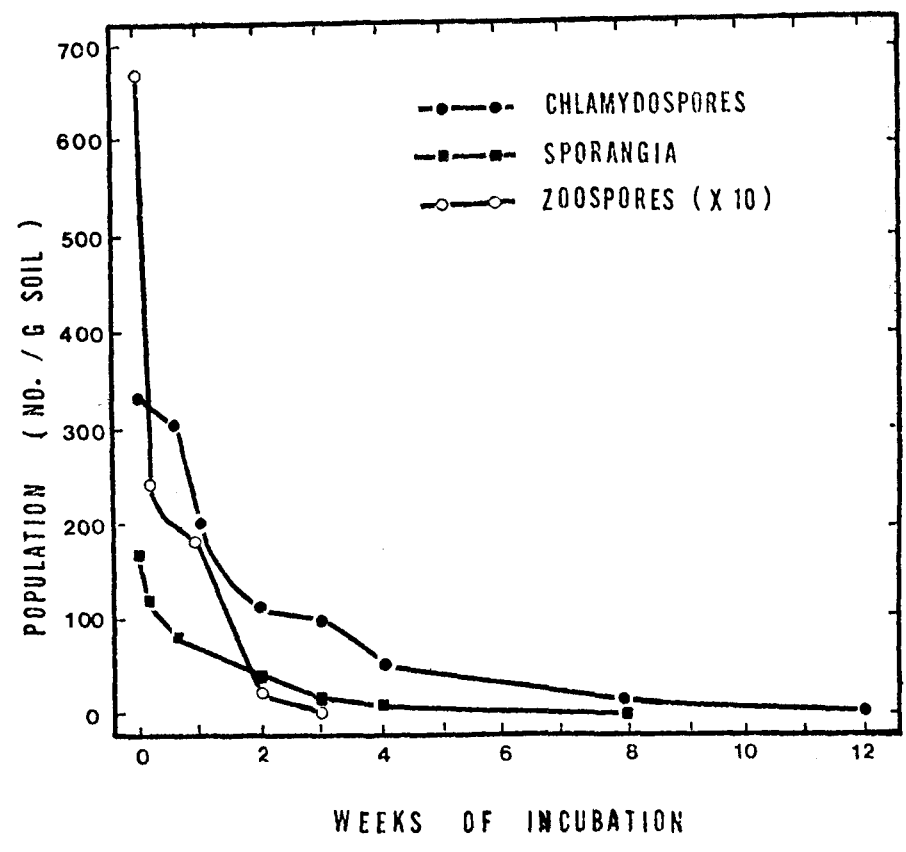
Fig. 8. Persistence of chlamydospores, sporangia, and zoospores of Phytophthora cinnamomi in submerged soil at 24 C.
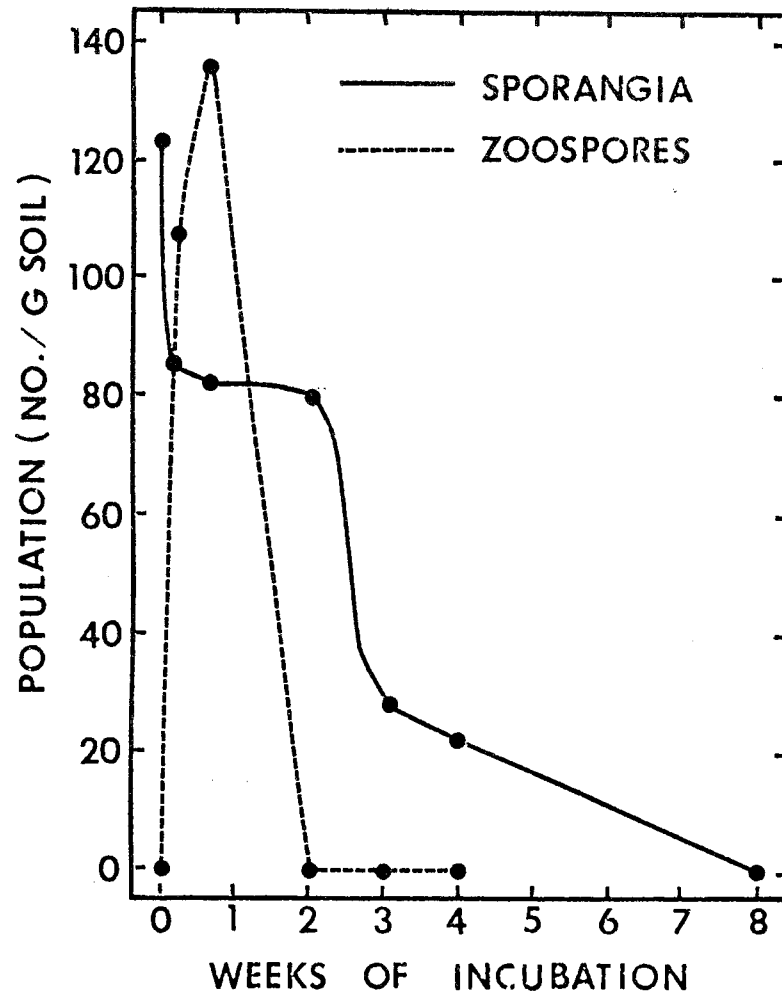
Fig. 9. Recovery of zoospores and sporangia
         from moist soil at various periods
         following addition of sporangia to
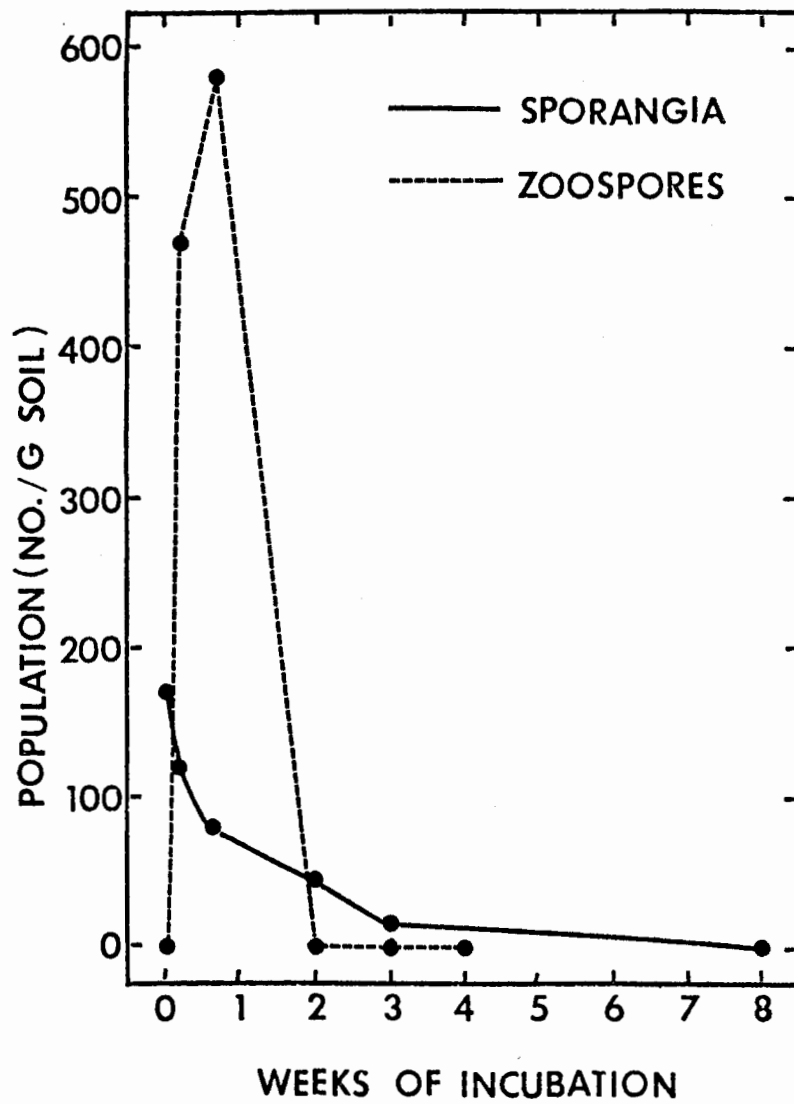   soil.

Fig. 10. Recovery of zoospores and sporangia from submerged soil at various periods following addition of sporangia to soil.

in both soils, and then decreased rapidly to an undetectable level after
2 weeks. Sporangia were still recoverable from both soils after 2 weeks.
In both soils the population of sporangia decreased to an undetectable
level after 8 weeks.

Naturally infested soil -- In naturally infested soil, P.
cinnamomi also survived better under moist than submerged conditions.
During the 12-month period the population of P. cinnamomi decreased only
from 14.3 to 8.0 propagules/g of soil under moist conditions, but from
14.3 to 0.6 propagules/g of soil under submerged conditions (Fig. 11).

Population in moist and submerged ohia forest soil -- In this
study, the population of P. cinnamomi in soils collected from moist and
submerged areas in the ohia forest was determined. During the investi-
gation on the association of this fungus with ohia decline, a large
area partially submerged in standing water was noted near location
No. 8 (Fig. 1). The ohia trees in this area were in severe decline.
Twenty submerged soil samples and 20 moist soil samples of the same
area were collected, and the population of P. cinnamomi in these soils
was determined by using the combination of wet sieving and the selective
medium as previously described. Phytophthora cinnamomi was detected in
70% of the soil samples collected from the moist area, while it was not
recovered from any of the soils collected from the submerged area. The
population of this fungus in the moist soils ranged from 1.2 to 4.4
propagules/g of soil.

Survival of Phytophthora cinnamomi in Root Tissue

When root segments taken from declining avocado trees were buried
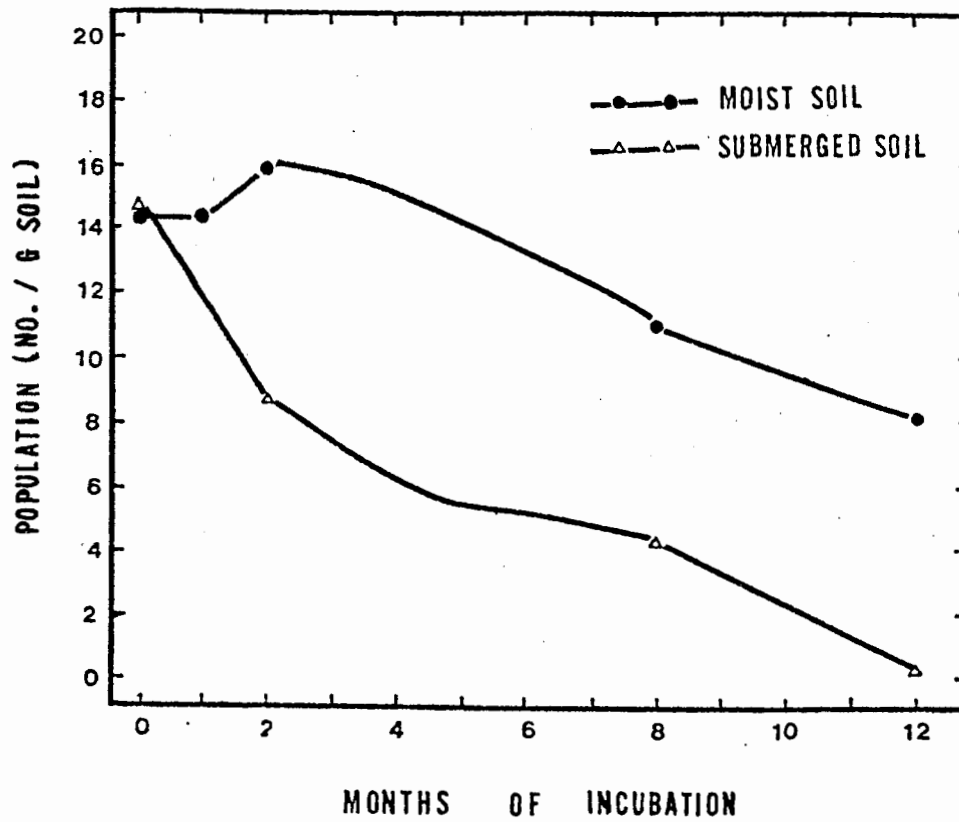in moist soil and held at 24 C, the percentage of root segments infested

Fig. 11. Survival of _Phytophthora cinnamomi_ in naturally
infested avocado field soil adjusted to moist
and submerged conditions.

with P. cinnamomi decreased only slightly over a 12-month period
(Fig. 12). The fungus was recovered from 39% of the root segments im-
mediately after burial in soil, and was recovered from 37% after incu-
bation for 12 months.

Similarly, when artificially inoculated ohia root segments were
used the percentage of root segments containing this fungus was 83% at
the beginning, and still remained at 50% after 12 months in soil.
Chlamydospores of P. cinnamomi were observed in both avocado and ohia
root tissues.

Factors Affecting the Survival of Zoospores in Soil

Zoospores of P. cinnamomi were very short-lived in soil as indi-
cated in the above study. However, when they were added to moist soil
mixed with ohia stems (2.5 x 10 mm) or leaves, they were able to
colonize these tissues and remained viable for a longer period of time
in soil. Phytophthora cinnamomi was consistently isolated from stem
segments and leaves for 1 year and 9 months, respectively. After
9 months of burial in soil leaf tissues were completely decomposed, but
the fungus in the form of free chlamydospores was still recovered from
soil when the last isolation was made after 1 year of incubation.

When zoospores were added to soils planted with ohia, papaya,
tomato or pepper seedlings, they were able to infect roots of these
plants and thus extended their survival time in soil. Within one month
after inoculation, all the ohia seedlings were killed, and roots of
papaya, tomato, and pepper seedlings were infested with P. cinnamomi
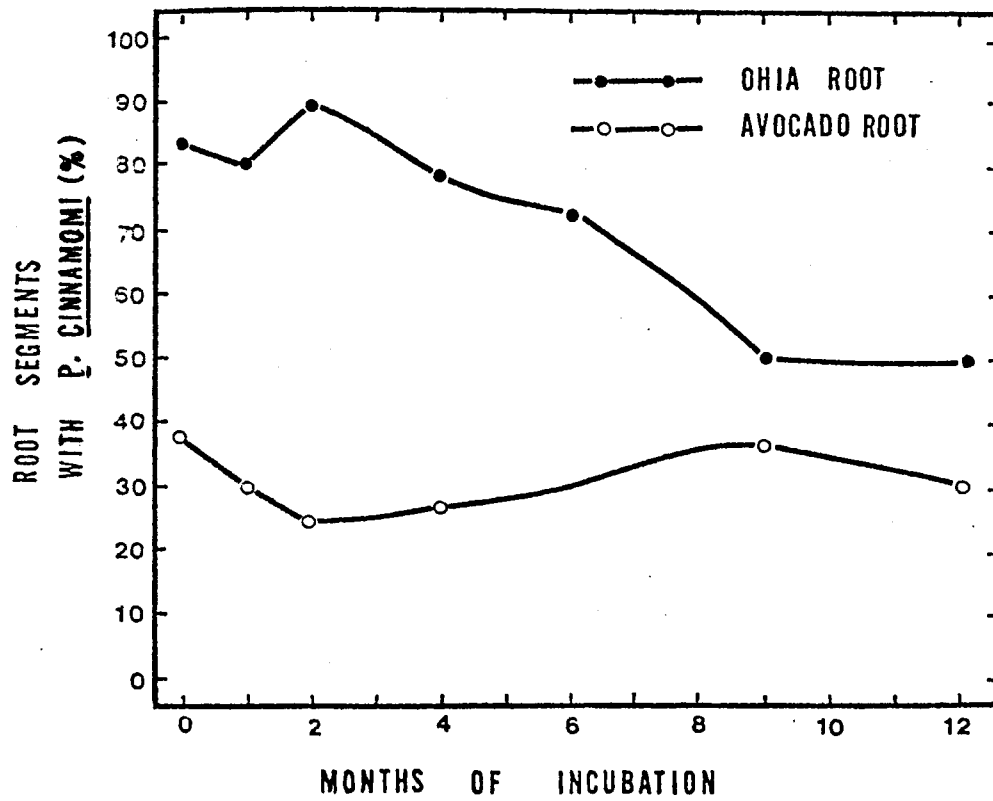without showing visible symptoms. The fungus was recovered from roots

Fig. 12. Persistence of Phytophthora cinnamomi in diseased
avocado roots and in artificially inoculated ohia
roots buried in moist soil, and held at 24 C.
Isolations were made over a period of 12 months.

of all the ohia seedlings, and was also from roots of 70% of tomato, 11%

of papaya, and 14% of pepper seedlings after 1 month. Phytophthora

cinnamomi produced sporangia on the root surface and chlamydospores in

root tissue of ohia seedlings. No spores were observed in root tissues

of other plant species.

Nature of Propagules Recovered from Soil

To determine if zoospores of P. cinnamomi were present in natural

soil, 50 g of soil suspended in 300 ml of water was passed through a

38 μ sieve which permitted the passage of zoospores but not chlamydo-

spores and sporangia. Materials retained on the sieve and filtrate

were plated separately on the selective medium. Phytophthora

cinnamomi was recovered only from materials retained on the sieve, but

not from filtrate, thus indicating that P. cinnamomi zoospores were not

present in natural soil. Plates containing materials retained on the

sieve were examined microscopically to determine the origin of colonies

of P. cinnamomi. Results showed that P. cinnamomi existed mainly as

free chlamydospores in avocado soils and as chlamydospores imbedded in

organic matter in ohia forest soils (Table 6). Chlamydospores were

thin-walled and appeared either as single spore (Fig. 13), or in clusters

of two or three spores (Fig. 14). Zoospores were also observed on the

plates. Very often several zoospores were found close together

(Fig. 15), and occasionally empty sporangia were observed in the

vicinity of these zoospores. This suggested that P. cinnamomi sporangia

may be present in natural soil. In preliminary tests, it was found

that when P. cinnamomi sporangia added to soil were suspended in water

(50 g/100 ml) chilled at 5 C for 30 min, incubated at 24 C for 1 hr

Table 6. Nature of propagules of _Phytophthora cinnamomi_ recovered from soil

| Source of soil | No. of colonies examined | Origin of colony (%) | | | |
| | | Free chlamydospore | Zoospore | Organic matter | |
				Chlamydospore	Unknown
Soil A (Ohia)	69	9	7	58	26
Soil B (Ohia)	30	30	3	50	17
Soil C (Avocado)	100	71	0	20	9
Soil D (Avocado)	53	60	28	12	0
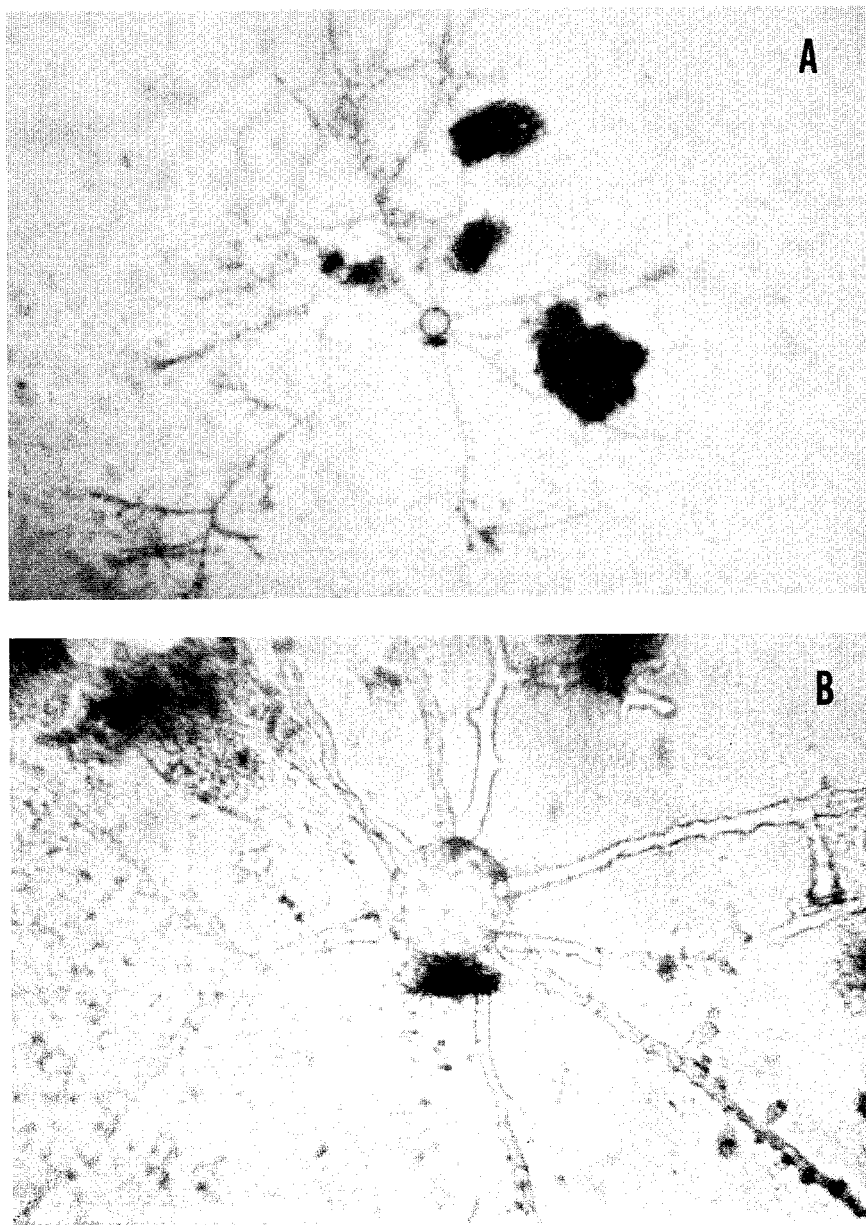
Fig. 13.   A free chlamydospore of Phytophthora
          cinnamomi recovered from ohia forest soil:
          A) After 36 hr of incubation on the selective
          medium (450 X); B) same as (A) at 1,800 X,
          note the chlamydospore is thin-walled and
          produces numerous germ tubes.
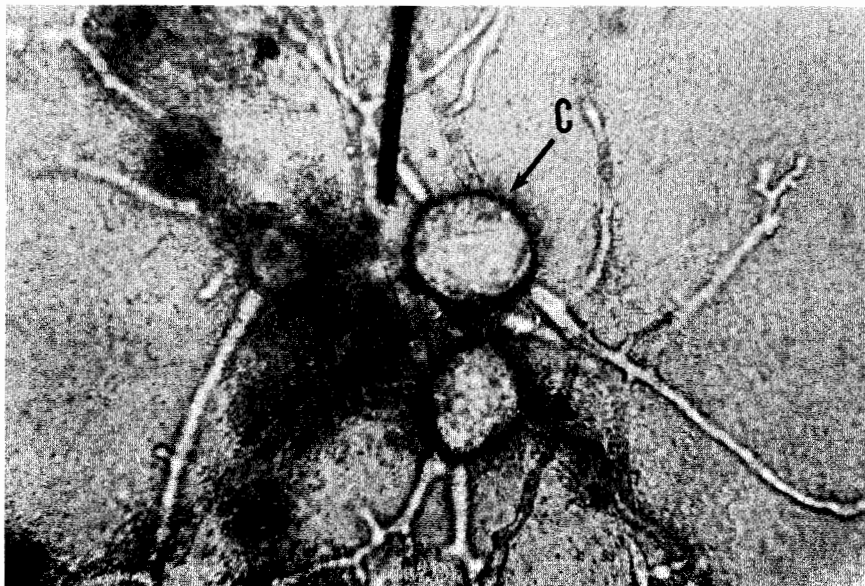
Fig. 14. A cluster of chlamydospores (C) of <u>Phytophthora</u> <u>cinnamomi</u> with three spores staying together re-covered from natural soil (1,800 X).

Fig. 15. Two encysted zoospores (Z) of <u>Phytophthora</u>
<u>cinnamomi</u> germinating on soil isolation
plate (1,800 X).

and then passed through a 38 μ sieve, zoospores were detected in the
filtrate. No zoospores were detected in the filtrate when P. cinnamomi
chlamydospores added to soil were similarly tested. By using this
technique, zoospores at the concentrations of 10 and 50/g of soil were
detected in the filtrate of ohia forest soil and avocado soil, res-
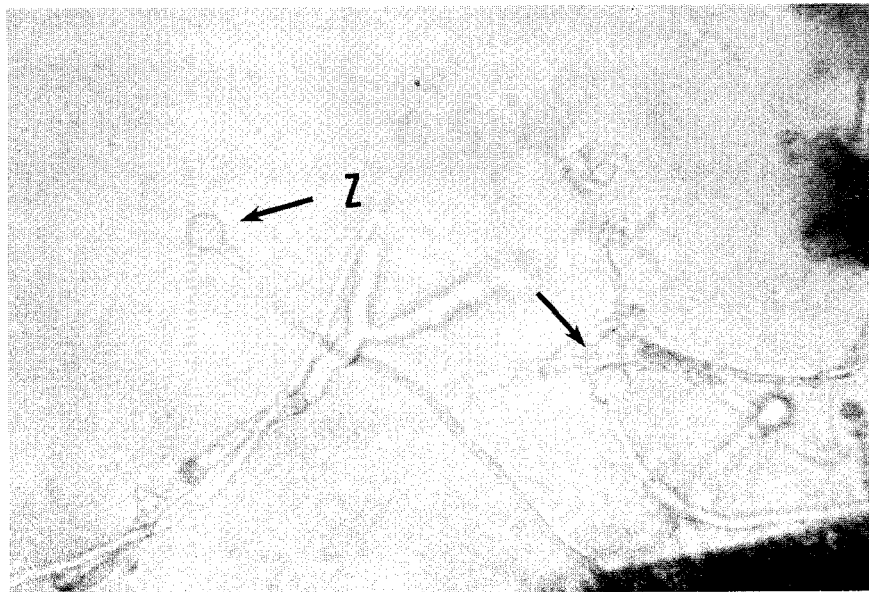pectively. Without chilling treatment no zoospores were detected in
the filtrate from either soil. These results further confirmed the
previous observations that sporangia of P. cinnamomi also existed in
natural soil and that zoospores retained on the sieve originated from
sporangia during incubation.

To determine whether chlamydospores germinated in soil, 3 ml of
chlamydospore suspension at the concentration of 5.0×10^5/ml were
added to 10 g of soil placed in a small petri dish (50 x 15 mm). The
soil was adjusted to either moist or submerged conditions and held at
24 C. Percentage of chlamydospore germination was determined under the
microscope by suspending 1 g of soil in 5 ml of distilled water. In
submerged soil, 23% of chlamydospores germinated by producing sporangia
on the tips of germ tubes after 1 month of incubation. Most sporangia
were empty due to release of zoospores under submerged conditions. In
moist soil, 18% of chlamydospores germinated after 1 month, but
sporangia formed under such conditions did not discharge zoospores
(Fig. 16), but many of them discharged zoospores after 24 hr incubation
in water. In one moist soil to which 1,900 chlamydospores/g of soil
were added, population of zoospores in soil determined by using the
combination of chilling treatment and wet sieving as described in the
above section is shown in Fig. 17. Zoospores, released from sporangia
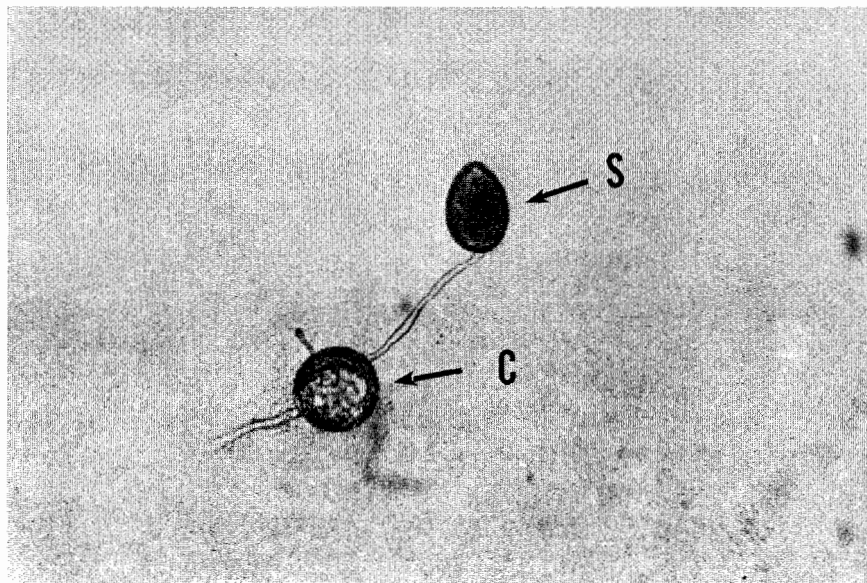
Fig. 16. A germinating chlamydospore (C) with a sporangium
          (S) on the tip of germ tube recovered from arti-
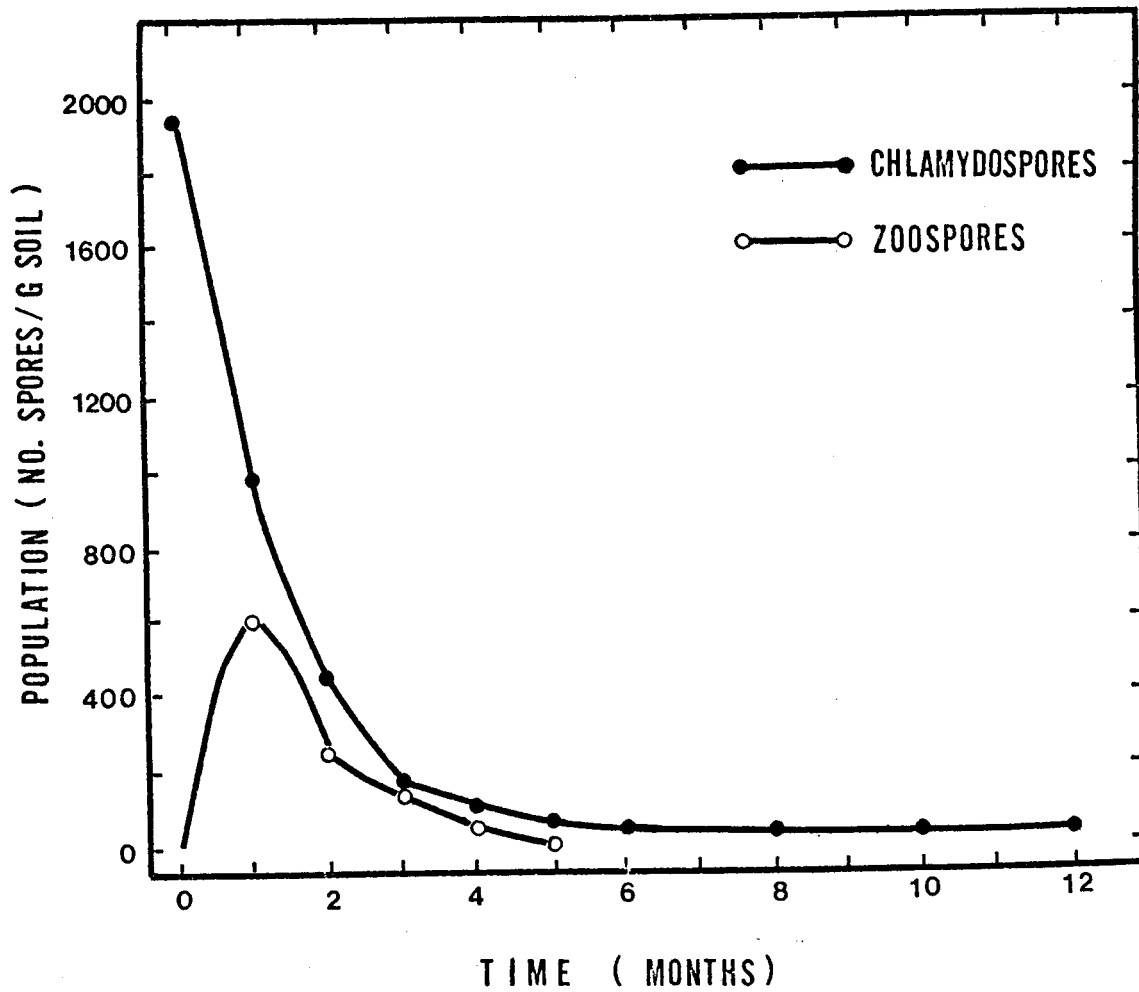   ficially inoculated soil (1,350 X).

Fig. 17. Recovery of zoospores from moist soil at various
periods after artificially infesting with chlamydo-
spores of Phytophthora cinnamomi.

in soil, were consistently recovered for the first 5 months of
incubation. The population of zoospores was at the maximum (600 zoo-
spores/g of soil) after 1 month and declined gradually thereafter. It
reached an undetectable level after four and a half months. The popu-
lation of chlamydospores decreased from 1,900 to 50 propagules/g of
soil during the same incubation period.

Infection Potential of Motile Zoospores, Encysted Zoospores, and Chlamydospores

Percentages of 3-month-old ohia seedlings killed by motile zoo-
spores, encysted zoospores, and chlamydospores of P. cinnamomi at
various inoculum levels were determined after 1 month. When the
inoculum level at or above 2.5×10^3 propagules/g of soil, encysted
zoospores were the least infective to the ohia seedlings, while in-
fection potential of motile zoospores and chlamydospores was about the
same (Table 7). For instance, at an inoculum level of 2.5×10^3
propagules/g of soil, percentages of ohia seedlings killed by encysted
zoospores, motile zoospores, and chlamydospores were 18, 65, and 45%,
respectively. At inoculum levels below 2.5×10^3 propagules/g of soil,
however no significant difference was found among these three spore
types tested.

Colonization Potential of Motile Zoospores, Encysted Zoospores, and Chlamydospores

Percentages of dead ohia stem segments colonized by motile zoo-
spores, encysted zoospores, and chlamydospores of P. cinnamomi at
various inoculum levels were determined after 1 week. Among these three

Table 7. Comparison of infection potential of chlamydospores, motile zoospores, and encysted zoospores of Phytophthora cinnamomi

No. propagules /g of soil	Infection potential (%)[a]		
	Chlamydospores	Motile zoospores	Encysted zoospores
5.0×10^3	--[b]	93 A[c]	42 B
2.5×10^3	45 A	65 A	18 B
5.0×10^2	13 A	25 A	5 A
2.5×10^2	11 A	3 A	--

[a]Average of 70 ohia seedlings per treatment.

[b]Not tested.

[c]Means followed by the same letter in each row are not significantly different at $P = 0.05$.

spore types tested, chlamydospore was the most effective in colonizing
ohia stems, while colonization potential of motile and encysted zoo-
spores was about the same (Table 8). For instance, at an inoculum
level of 1 x 10^2 propagules/g of soil, the percentages of ohia stem
segments colonized by chlamydospores, motile zoospores, and encysted
zoospores were 77, 8, and 19%, respectively.

DISCUSSION

Phytophthora cinnamomi has been shown to survive 6 years in
avocado field soil and 19 months in forest soil (38, 88). Results of
present study showed that in natural soil P. cinnamomi exists mainly as
free chlamydospores and chlamydospores imbedded in organic matter, and
less frequently as free sporangia. Free chlamydospores as the origins
of P. cinnamomi colonies on soil isolation plates had also been reported
previously (24, 35, 45, 56). Although chlamydospores of P. cinnamomi
were the most persistent among the three spore types tested, their
population also decreased to undetectable levels after 12 months.
When root tissues naturally or artificially infested with P. cinnamomi
were incubated in soil, the fungus remained viable in more than 50% of
the pieces after 12 months, and chlamydospores were observed within the
tissues. This suggests that chlamydospores imbedded in organic matter
are responsible for long term survival. After decomposition in soil of
leaf tissues colonized by the fungus, free chlamydospores were observed
on soil isolation plates, indicative that these were released from plant
tissue. Results of this study also showed that free sporangia in soil

Table 8. Comparison of colonization potential of chlamydospores, motile zoospores, and encysted zoospores of Phytophthora cinnamomi

No. propagules /g of soil	Substrate colonization (%)[a]		
	Chlamydospores	Motile zoospores	Encysted zoospores
1×10^3	94 A[b]	18 B	21 B
1×10^2	77 A	8 B	19 B
1×10	52 A	3 B	3 B

[a]Average of 50 dry ohia stem segments per treatment.

[b]Means followed by the same letter in each row are not significantly different at P = 0.05.

could originate from those produced on the surface of infected root tissues or from germinating chlamydospores.

Both sporangia and zoospores of P. cinnamomi were relatively short-lived in soil. This is in agreement with previous reports on survival of other Phytophthora species in soil (49, 50, 82). The only one exception to this is that of P. palmivora reported by Turner (72) who showed that zoospores and sporangia of this fungus, respectively, remained viable for 6 months and 2 years in soil. However, he did not observe the exact structure of this fungus which survived in soil.

Phytophthora cinnamomi zoospores were the primary structure which were dispersed in rain splash and runoff water in ohia forests (35). In the presence of dead tissue, P. cinnamomi zoospores were able to establish themselves in soil through colonization and production of chlamydospores in plant tissue. They were also able to prolong their survival by infecting roots of both susceptible and nonsusceptible plants. This may account in part for the widespread occurrence of this fungus in ohia forest (34).

Both zoospores and chlamydospores of P. cinnamomi were able to colonize dead tissues in soil. This result is in accord with that of Zentmyer and Mircetich (88), but disagrees with that of Kuhlman (38) who reported that dead tissue in soil was rarely invaded by P. cinnamomi. Since P. cinnamomi grown in a mixture of alfalfa meal and sand was used as the inoculum by Kuhlman, activity of this fungus in soil could have been suppressed by other microbial activities enhanced by alfalfa meal carried along with the inoculum. Zentmyer (83) and

Gilpatrick (22) had demonstrated the suppression of avocado root rot

caused by P. cinnamomi by amendment of soil with alfalfa meal.

Chlamydospores of P. cinnamomi are more efficient in colonizing

dead tissues than zoospores, but their infection potential is no better

than zoospores. On the other hand, the colonization potential of

chlamydospores and zoospores of P. palmivora was about the same, but

chlamydospores of this fungus had higher infection potential than zoo-

spores (36). Like P. palmivora (31), motile zoospores of P. cinnamomi

had higher infection potential than nonmotile zoospores. This further

indicates the importance of zoospore attraction toward plant roots in

disease development.

SUMMARY

Among the three spore types of P. cinnamomi tested, chlamydospores

were the most persistent in soil, sporangia were intermediate, while

zoospores were the least persistent. Survival of P. cinnamomi in soil

was better under moist than submerged conditions. The population of

chlamydospores remained detectable for one year in moist soil, while

only for 3 months in submerged soil. Similarly, over a 12-month period

the population of P. cinnamomi in a naturally infested avocado soil de-

clined faster under submerged than moist conditions. Results of

survival studies also showed that P. cinnamomi in the root tissue was

more persistent than as free chlamydospores in soil. Under moist

conditions, the percentage of root tissues from which the fungus was

recovered declined only slightly after one year of incubation, while the

population of chlamydospores in soil decreased to undetectable level in the same period.

Zoospores were able to colonize dead ohia stems and leaves, and to parasitize roots of ohia, tomato, papaya, and pepper seedlings. Therefore, survival time of zoospores in soil can be extended either through substrate colonization or root infection.

Colonies of P. cinnamomi recovered from natural soil originated mainly from chlamydospores, and occasionally from zoospores. Chlamydospores occurred as free spores or imbedded in organic matter. Results of this study indicated that sporangia of P. cinnamomi also existed in natural soil, and zoospores found on the isolation plates were released from sporangia during incubation. A method of detecting the presence of P. cinnamomi sporangia in soil was described.

Among the three spore types of P. cinnamomi tested, chlamydospores were also the most effective in colonizing dead ohia stems, while colonization potential of motile and encysted zoospores was about the same. Results of the colonization study suggest that P. cinnamomi is a good saprophyte. The fungus was able to colonize about 52% of ohia stem segments at a population as low as 10 chlamydospores/g of soil.

At inoculum level of 2.5×10^3 propagules/g of soil or above, encysted zoospores were the least infective to ohia seedlings, while infection potential of chlamydospores and motile zoospores was about the same. At inoculum levels below 2.5×10^3 propagules/g of soil, however no significant difference in infection potential was found among these three spore types tested.

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