

A ROOT ROT DISEASE OF FUCHSIA
CAUSED BY PHYTOPHTHORA PARASITICA

by

SURESH SIDDHESHWAR PATIL

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE

June 1959

APPROVED:

Redacted for privacy

Professor of Plant Pathology and
Chairman of Department of Botany

In Charge of Major

Redacted for privacy

Chairman of School Graduate Committee

Redacted for privacy

Dean of Graduate School

Date thesis is presented May 1, 1959

Typed by Rose Amos

ACKNOWLEDGMENT

The author sincerely expresses his indebtedness to Roy A. Young, Chairman of the Department of Botany and Plant Pathology for his guidance and encouragement throughout the course of this investigation and in the preparation of the manuscript.

Thanks are also due to Dr. R. O. Belkengren and Dr. L. F. Roth for their valuable criticism of the manuscript and to Mr. H. H. Millsap for making the photographs. Valuable suggestions and help from fellow students is also acknowledged.

TABLE OF CONTENTS

<u>Title</u>	<u>Page</u>
INTRODUCTION	1
LITERATURE REVIEW.	3
METHODS AND MATERIALS.	8
Media	8
Culture	9
Isolation	10
Inoculation	11
IDENTIFICATION OF THE FUNGUS	12
GROWTH ON PLANT MEDIA	15
EFFECT OF pH ON GROWTH OF <u>PHYTOPHTHORA PARASITICA</u>	17
SPORULATION IN CULTURE	19
Formation of Sporangia.	19
Formation of Oospores and Chlamydospores.	20
PATHOLOGY OF <u>P. PARASITICA</u>	22
Pathogenicity Studies	22
Nature of the Influence of Temperature.	27
DISCUSSION	30
SUMMARY.	35
BIBLIOGRAPHY	37

LIST OF TABLES AND FIGURES

<u>Table</u>		<u>Page</u>
1	The influence of temperature on rate of growth of <u>P. parasitica</u> on different plant media.	16
2	Influence of temperature on sporangial production.	20
3	Time required for infection and disease development.	28
4	Temperature data for June, July, and August 1955, 1956, 1957, and 1958.	33

<u>Figure</u>		
1	The influence of temperature on rate of growth of <u>P. parasitica</u> on different plant media.	18
2	General symptoms of Fuchsia root rot showing wilt, defoliation and reduced root system.	24
3	Effect of temperature on development of Fuchsia root rot.	25

A ROOT ROT DISEASE OF FUCHSIA
CAUSED BY PHYTOPHTHORA PARASITICA

INTRODUCTION

In the Pacific Northwest, several varieties of Fuchsia sp are grown in gardens, greenhouses and lath houses. Fuchsia species are native to New Zealand and tropical America but different hybrids and varieties of these plants are adapted to climatic conditions in this area. There are sun tolerant fuchsias but a great majority of the varieties require cool sites and partial shade. Garden fuchsias are long-day sensitive plants and are very sensitive to dry atmosphere. Some varieties are winter hardy and can be grown outdoors in regions like coastal California. Fuchsia plants which are grown in the greenhouse grow best at 50-60°F. Temperatures above 70°F. result in slender and weak plants that do not bloom adequately.

In July 1958, reports were received of a serious disease of fuchsias in greenhouses and lath houses in the Portland area. Apparently vigorous plants of all ages and sizes began wilting and dying. Affected plants wilted suddenly and subsequently died within a few days. Preliminary isolations consistently yielded a white phycomycetous fungus which was tentatively identified as Phytophthora parasitica Dastur. Inoculation trials showed that under

certain conditions the fungus was capable of infecting the roots of fuchsia plants and causing a wilt disease.

Prior to 1958, there had been no reports of such a disease on fuchsias. The wide spread loss of plants, even old stock plants in hanging baskets, in the summer months of 1958 indicated that the plants might have been infected for sometime but that conditions had not been favorable for disease development.

The studies reported in this thesis were conducted to determine the cause of root rot of fuchsias and the influence of environmental factors on disease development.

LITERATURE REVIEW

There has been no previous report of a root rot disease of Fuchsia although Frezzi (2, p. 129) isolated Phytophthora parasitica Dastur from Fuchsia plants. There have been, however, many reports of diseases on other plants caused by Phytophthora parasitica. The following is a summary of the description of morphological characters of Phytophthora parasitica given by Dastur (1, p. 191).

The comparatively young mycelium consists of thin, unseptate, not much branched hyphae. In older cultures branches and septa appear. The sporangia are borne on simple or irregularly branched stalks and are generally apical as in a typical Phytophthora. Intercalary and lateral sporangia are also seen. Sporangia are pear-shaped and vary a great deal in size. The variation in size, on an average, is 25-30 x 20-40 and the number of zoospores in the sporangia varies according to the size of sporangia. They fall off their stalks with or without any vestige of their stalks and discharge the zoospores within 5 minutes when suspended in water.

The zoospores swim for 20 minutes to 2 hours before rounding up. Varying temperature, moisture, and possibly light, are the factors that determine zoospore formation. Zoospores measure 8-12 x 5-8 in motile stage and 7-11

in resting stage. They are reniform and have 2 cilia. Their germination takes place 15 minutes after rounding up.

Dastur further stated that the germination of sporangia takes place in three ways:

1. When in water they form zoospores which give rise to germ tubes.
2. They give rise to one or more short germ tubes which may form zoospores or tertiary sporangia.
3. They produce conidia which in turn germinate giving rise to mycelium.

On media chlamyospores are produced which range from 20-60 in diameter. They are spherical and have smooth, thin walls with yellow color. Extremes of temperature which retard the formation of sporangia do not retard the formation of chlamyospores. Chlamyospores do not necessarily need a resting period before germination.

Phytophthora parasitica oospores have been observed by Dastur on French bean agar, and oat juice agar but were obtained only when these were inoculated from cultures growing on a medium different than the above mentioned ones. In all cases oogonial origin was within the antheridium. The antheridia were hypogenous.

Several workers have attempted in past years, to study the genus Phytophthora and find out some distinctive characteristics of its member species. Despite these

attempts there is not a generally usable key for the identification of Phytophthora species.

Leonian (5, p. 446) published his physiological studies on the genus Phytophthora in 1925 and gave a key based on these studies. He used M/100 solutions of a number of sugars, amino acids, nitrates, sulfates, phosphates, chlorides and carbonates to study the different species in the genus. Physiological reactions of the species plus morphological characters such as colony growth, nature of hyphae and oogonia were used. But when the saltation phenomenon occurred, the physiological characteristics were found to be as variable as the morphological characteristics, and therefore this key did not solve the problem of the classification of this complicated genus. However, his key includes some additional characteristics which in conjunction with the morphological and pathological characteristics, help to a certain extent in identification of species.

In 1934, Leonian (6, p. 36) published another key based on such characters as, growth on malt extract agar after six days, host range, growth in the presence of 1:80000,000 malachite green, production of reproductive bodies by the mycelium when transferred from pea broth to water, nature of antheridia and sporangia and formation of sporangia, and chlamydospores. In the same paper, he merged a number of other species like P. jatrophae and P. parasitica

into one group called P. palmivora. The reasons he gave for merging these species together was, that the whole group had only a few characters in common while the variations shown by the individual members of the same species were so great that it was more convenient to bring this whole group under one name. This merger of several species was not generally accepted by other workers.

Tucker (9) published his monograph of the genus Phytophthora in 1931. The key he gave was based on more or less the same criteria that were used by Leonian, but in addition Tucker used growth on corn meal agar at 35°C, the production of oospores on lima bean agar, and oat meal agar and in different natural fruits.

Frezzi (2, p. 47) in 1950, published his studies on the species of Phytophthora and gave a key for the species found in Argentina. He did not use host range as one of his criteria for the classification in his key, but laid more stress on the morphological characteristics such as, size and nature of the sporangia and oospores, and the nature of antheridia. Two other criteria which he used were, growth on malachite green at concentrations of 1:4000,000 and 1:8000,000 and growth at 35°C.

Waterhouse and Blackwell (11) published their key to the species of Phytophthora recorded in the British Isles in 1954. The characteristics they used were, nature

of the sporangiophore (branching), nature of the wall of the oogonium, nature of the sporangia, and host specificity.

In a genus like Phytophthora, where the members of the same species vary so much in their characteristics, no single key is adequate to place a particular member of Phytophthora satisfactorily. However, by using a number of criteria in different keys certain species can be satisfactorily identified.

METHODS AND MATERIALS

Media

A number of media were used in the course of study of Phytophthora parasitica. Potato dextrose agar was used to maintain the stock cultures. Since the fungus grows rapidly in pea broth, pea broth was used for growing the fungus prior to the transfer to water for sporangia and zoospore production. Dung infusion media, using different dungs at three different concentrations, were used for stimulating the production of oospores.

The following is the description of the procedures used for preparing media.

Pea broth. One hundred fifty grams of dried split peas in 500 ml. of tap water were autoclaved for 3 minutes. The supernatant liquid was decanted and the volume was brought up to 1000 ml. The broth was autoclaved for 15 minutes.

Oat meal agar. Thirty-five grams of oat meal were brought to boiling, steeped 30 minutes and strained. Fifteen grams of agar and 15 g. of dextrose were then added and autoclaved for 15 minutes.

Corn meal agar. DIFCO corn meal agar was used and was made according to DIFCO directions.

PDA. Two hundred grams of potatoes and 20 g. of dextrose mixed with 20 g. of agar were put in 300 ml. of

water in 2 different containers and autoclaved for 15 minutes. The potato supernatant was strained through cheese cloth and was mixed with the solution of melted agar and dextrose. The volume was brought up to 1000 ml. and the mixture was autoclaved for 15 minutes.

Dung infusion agars. Oven dried cow, sheep and horse dungs were used at concentrations of 50 g., 100 g., 200 g. per liter. Five grams of dextrose and 20 g. of agar was added to each liter of strained and autoclaved infusion and the mixture was autoclaved again for 15 minutes.

Plant media. Four types of plant media, namely V-8 juice agar, lentil agar, fuchsia decoction agar and split-pea agar were used. These media were prepared by adding 100 g. of lentils, 100 g. dried split peas, 150 g. of fuchsia foliage and 125 ml. of V-8 juice respectively, to one liter of tap water and heating it at 90°C. for three-quarters of an hour. After filtering the mixtures through cheese cloth, restoring the filtrate to 1000 ml., and adding the autoclaved mixture of 15 g. of dextrose and 15 g. of agar, they were autoclaved for 15 minutes again.

Culture

All cultures were maintained on PDA. The stock cultures were maintained on PDA slants at 25°C. and were transferred to new slants after every 2 weeks. On PDA

the fungus produced good vegetative growth and a few sporangia occasionally.

Isolation

Isolations from stems and roots were made by the tissue transfer technique (4, p. 290). Diseased plant parts were surface sterilized in 10 per cent commercial Clorox for 3 minutes after which small portions of stem and root tissue were removed with a sterile razor blade and transferred aseptically to PDA plates. The plates were then incubated in a 25°C. chamber. Within 24 hours the fungus grew out of both the stem and root sections. Stem and root sections were also placed in petri plates containing sterile distilled water and incubated at 25°C. Within 48 hours abundant sporangia were seen on the hyphae which grew out of the pieces of stem and root tissues.

Isolation of Phytophthora parasitica from infested soil has been reported by several workers and was also tried in the course of this study. The apple test (9, p. 190) and the lemon trap test (8, p. 23) both failed to isolate P. parasitica from the infested soil. In this respect it may be noted that Dastur (1, p. 191), who originally established the species, was unable to isolate this fungus using apples.

Inoculation

The best method found for obtaining sufficient fungal growth for the preparation of inoculum, was to grow the fungus in pea broth at 30°C. Petri plates containing 15 ml. of pea broth each, were inoculated with P. parasitica and were incubated at 30°C. A number of small colonies appeared after 24 hours of incubation. After a total incubation of 36 hours the colonies were washed twice with sterile distilled water, were covered with distilled water and were incubated at 25°C. for 24 hours. This procedure resulted in abundant sporangial production which in turn gave hundreds of zoospores in the medium. The contents of all petri plates were transferred to an Erlenmyer flask and the mycelial colonies were slightly macerated with a glass rod. This suspension was used as the inoculum.

Inoculations were made by boring a hole with a cork borer near the periphery of the clay pot. Two such holes were made on either side of the plant, and 10 cc. of inoculum were added in each hole. The holes were filled with greenhouse soil and the pots were watered heavily. Features of the experimental plants are described in the section concerning pathology of P. parasitica.

IDENTIFICATION OF THE FUNGUS

Identification of Phytophthora species is always a difficult task because many of the described species are highly variable and few reliable criteria are available to divide the genus.

Tucker (9, p. 190) used host specificity and growth of the fungus on different media as his criteria to divide the genus into different species. Leonian (5, p. 493) divided the genus on the physiological relationships of the fungus with respect to different media and sources of nutrition, while Frezzi (2, p. 47) based his key on such characters as growth of the fungus at 30°C., size of sporangia, chlamydospores, oospores and the nature of antheridia.

Despite the above attempts there is still much more to be done and understood before a good key to the species of Phytophthora can be developed. Morphological characters such as size of sporangia, chlamydospores and oospores are deceptive due to the variation in size of these structures which is often seen. Differences such as host specificity and nature of antheridia, though inadequate to divide this complicated genus, are of value in distinguishing certain species. The division of the genus entirely on the basis of physiology, merges species of Phytophthora which have

distinct differences in the morphological characteristics and hence is not satisfactory.

The fungus which was pathogenic to fuchsia plants was identified by making use of two keys and by direct comparison with a known culture of Phytophthora parasitica. Comparisons were made by growing the known and unknown organisms on different media and then measuring the rate of growth, observing gross morphology, the size and shape of sporangia and growth at 25°, 30°, and 35°C.

In order to make use of the different keys, one needs information on the morphology and life cycle of a fungus. Most of this information was obtained by studying the fungus but since all attempts to stimulate sexual reproduction failed, no information regarding the nature of antheridia and oospores could be obtained. However, by making use of Tucker's (9, p. 190) and Frezzi's (2, p. 47) keys, the pathogen was identified as Phytophthora parasitica. Widely spreading growth on malt extract agar, profuse growth on corn meal agar, absence of oospores on lima bean, potato dextrose, corn meal and oat meal agars and growth on corn meal agar at 35°C., together with the papillate character of sporangia, were the criteria used by Tucker to classify Phytophthora parasitica. All the above criteria were fulfilled by the species of Phytophthora isolated from Fuchsia plants.

Frezzi's criteria namely, growth in presence of Malachite green at a concentration of 1:4000,000, growth at 35°C. and the size and shape of sporangia and other spores were also fulfilled by the pathogen. Thus even though the type of antheridia was not determined, with the help of other characters the fungus could be identified.

GROWTH ON PLANT MEDIA

Growth studies on Phytophthora parasitica have been made in the past by using only a few standard media and only one or two incubation temperatures. It is known that the rate of growth or the general appearance of a particular fungus could be changed by altering the substrate on which it is grown. Experiments were therefore carried out using five different types of plant media in order to see the changes that take place with the alteration of the medium.

Organic materials used were, lentils, dried split peas, fuchsia decoctions, V-8 juice, and potatoes. The media were made as previously described. Four petri plates containing 20 ml. of each medium were inoculated by putting an agar disk of 4 mm. in diameter in the center of the petri plate. These agar disks were cut from an 8 day old culture of P. parasitica grown on PDA at 25°C. The incubation temperatures used were, 5°, 15°, 25°, 30°, and 35°C. Colony radii were measured every day for 5 days. The first reading was taken 48 hours after inoculation.

The following table shows the average increase in radius per day on each medium at 5 different temperatures.

TABLE 1

The influence of temperature on rate of growth of *P. parasitica* on different plant media.

Temperature °C.	Average increase in radius (mm.) per day.				
	Fuchsia	Peas	Potatoes	Lentils	V-8 Juice
35	0.73	4.0	4.0	3.2	5.0
30	5.1	4.5	4.6	4.0	5.2
25	4.7	4.0	3.5	5.6	6.2
15	2.5	2.4	2.0	1.8	4.2
5	0.0	0.0	0.0	0.0	0.0

On fuchsia decoction dextrose agar, pea dextrose agar, and potato dextrose agar, the highest increase per day occurred at 30°C. while on lentil dextrose agar and V-8 juice dextrose agar, it was highest at 25°C. In case of potato, pea and fuchsia dextrose agar media, the increase per day increased with increasing temperature up to 30°C. In case of lentil and V-8 media it increased with increasing temperature up to 25°C. and decreased as the temperature increased to 30°C. and 35°C. Although the rate of growth at 35°C. decreased on all media the sudden drop in case of fuchsia dextrose agar is very interesting to note. There is a possibility that some of the contents of fuchsia dextrose agar might have an inhibitory effect on the growth of the fungus at 35°C.

EFFECT OF pH ON GROWTH OF PHYTOPHTHORA PARASITICA

Knowledge of the effect of hydrogen ion concentration on the growth of a soil pathogen is an important aid in predicting the capacity of the pathogen to grow at different soil pH levels.

Media of different pH levels were prepared by combining appropriate quantities of .1 molar HCl and NaOH with pea broth.

The pathogen was grown in this medium of different pH levels at 30°C. for 3 days. Growth occurred from pH 3.4 through pH 9.4. Where fungal growth occurred, a shift of pH units toward the acid side was observed.

The results show that the pathogen can grow at initial pH values of as low as 3.4 and as high as 9.4.

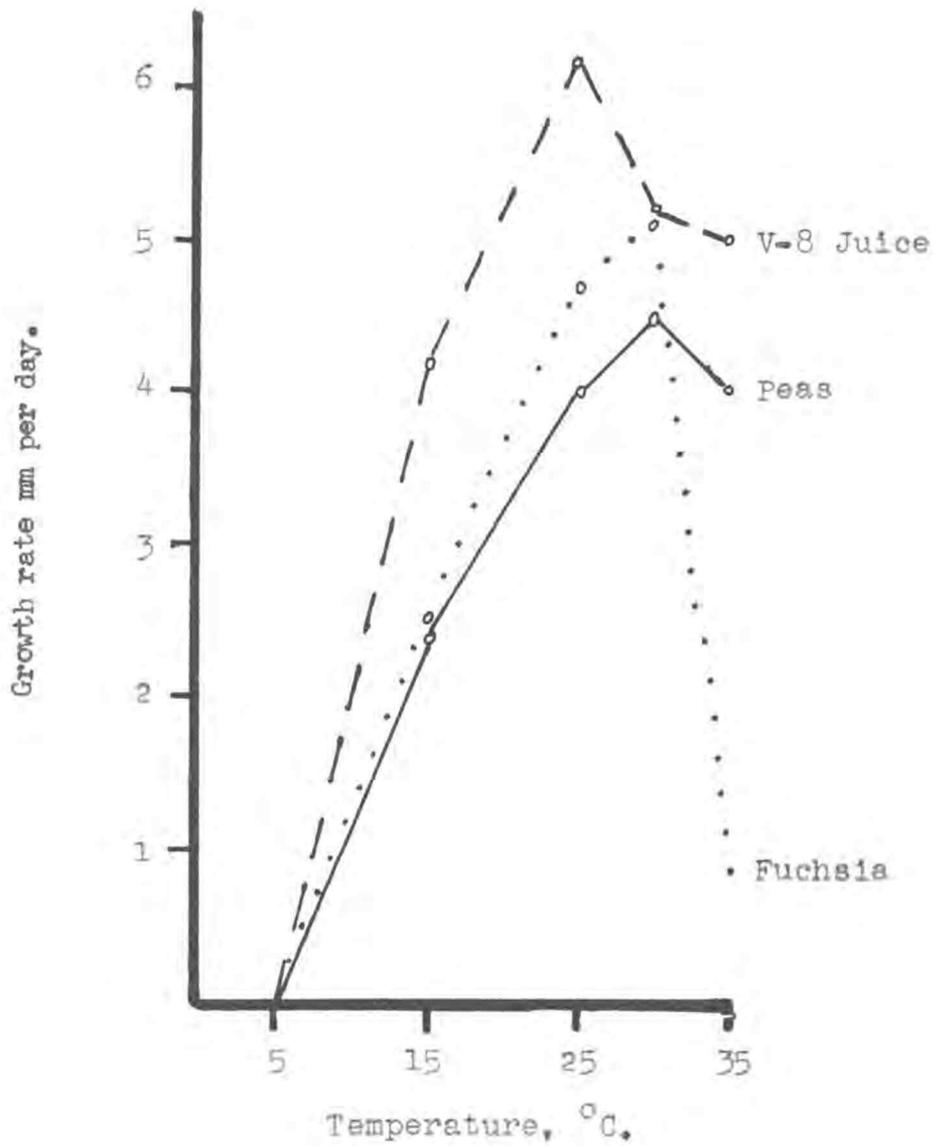


FIGURE 1

The influence of temperature on rate of growth of *P. parasitica* on different plant media.

SPORULATION IN CULTURE

It has been reported by Dastur that P. parasitica produces sporangia, zoospores, chlamydospores and oospores. However, in this particular organism oospores were not seen on any of the regular media or the special media that were used to stimulate the oospore production. Chlamydospores were produced with relative ease on milk agar, corn meal agar or dung infusion agars. Sporangia were produced on all media except PDA. The conditions under which sporangial production takes place being most important, from the standpoint of understanding the disease, it was decided to study the effect of temperature on the production of sporangia. It can be noted here, that zoospores are probably the primary infectious units which are responsible for infection and their production is dependent on the sporangial production. Hence the study on the effect of temperature on the sporangial production would be of value in understanding the disease development.

Formation of Sporangia

Of the media used, pea broth proved to be the best initial culturing medium. When the colonies from pea broth are transferred to distilled water, they produce abundant sporangia at room temperature. The effect of temperature on the rate of sporangial production was

studied by using the colonies grown in pea broth and transferring them to distilled water. Four replicate petri plates containing small mycelial colonies in non-sterile distilled water were kept at 5°, 10°, 15°, 20°, and 25°C. Observations made after incubation of 24 and 48 hours are shown in Table 2.

TABLE 2

Influence of temperature on sporangial production.		
Temperature °C.	Sporangial production after	
	24 hours incubation	48 hours incubation
5	0	0
10	+	+
15	++	++++
20	+++	++++
25	++++	++++
30	+++	+++
35	+	+

The above results indicate that production of sporangia was good at 15°, 20°, 25°, and 30°C. and it was moderate at 10° and 15°C. There was sparse sporangial production at 35°C. and none at 5°C.

Formation of Oospores and Chlamydo spores

Chlamydo spores were produced on corn meal agar, milk agar and on dung infusion agars. The formation of oospores was not observed at all. The sexual stage of the fungus is very important from the standpoint of identification

of the fungus and an effort was made to stimulate the oospore production in P. parasitica.

Dung infusion agars were made as described in the section on Methods and Materials. Three replicate plates were inoculated and were incubated at 20°, 25°, 30°, and 35°C. Gough et al. (3, pg. 13) originally used the dung infusion agars to stimulate the germination of oospores of P. parasitica. No oospore production was noticed during one month in which the observations were made.

Another experiment in which autoclaved leaves of fuchsia were inoculated with P. parasitica also failed to produce any oospores. Petri plates containing autoclaved leaves were put in a dish containing water and a Bell jar was placed over the dish to prevent drying. In still another experiment in which pieces of sterile fuchsia stems were put in sterile water agar and the plates were inoculated with P. parasitica, oospores were not produced.

PATHOLOGY OF P. PARASITICA

Before describing the studies regarding the pathology of Phytophthora parasitica, it would not be out of place to give a short description of the disease symptoms.

Infected plants, at temperature favorable for disease development, start showing gradual wilting of leaves. Leaves on the lower part of the plant wilt first and in a few hours all the leaves droop down due to loss of turgor in the petioles. As the disease advances, the leaves wither and fall off and the plant becomes dry and dies (Figure 2). Diseased plants have a much reduced root system and the infected areas of the roots show dark colored patches or areas. Within a few hours after infection the fungus could be detected in the lower one inch of the stem.

Pathogenicity Studies

The mere fact that Phytophthora parasitica was isolated from diseased fuchsia plants could not be used as proof that the fungus was the cause of the disease. This could be established only by artificially inoculating the plants with a pure culture of the organism and producing disease symptoms. In order to ascertain whether Phytophthora parasitica was pathogenic to fuchsia plants, pathogenicity experiments were conducted in the greenhouse.

Temperature tanks were used to hold the plants at different temperatures to study the effect of temperature on the development of disease.

The Fuchsia plants used for the experiments were approximately 3 month old plants, which were grown from cuttings. These plants were potted in clay pots using a light sandy loam greenhouse soil that contained 1 part of peat to 3 parts of soil. Temperature tank cans were covered from the inside and outside with polyethylene sacs and were filled 3/4 full with sand. Clay pots with plants were then placed in the sand, which was used as a heating medium, leaving the margins of the pots above the sand level.

Inoculations were made as previously described under Methods and Materials except that distilled water without inoculum was used in case of control plants.

In all 18 plants were inoculated. Six of these inoculated plants, with their 6 control plants, were placed in 65°F. temperature tank. Similar groups were put in 75°F. and 85°F. temperature tanks.

On the seventh day after inoculation all 6 plants in 85°F. temperature tank began to show wilting (Figure 3). The disease symptoms showed by these plants were identical with those showed by the plants which were brought from the nurseries near Portland. In a few days (4-5) the plants had dried completely and were dead. The control



FIGURE 2

General symptoms of Fuchsia root rot showing wilt, defoliation and reduced root system.



FIGURE 3

Effect of temperature
on development of Fuchsia root rot.

Inoculated plants in front, checks in the rear. Note dead plant at 85°F and absence of symptoms at 65°F and 75°F.

plants at 85°F. were at the same time very healthy and were growing rapidly. The inoculated plants as well as the controls kept at 65°F. and 75°F. did not show any symptoms and there were no apparent differences between the growth of the inoculated plants and that of the controls. Isolation results from the plants which were kept at 75°F. were negative during the first week after inoculation but Phytophthora parasitica was recovered from them 10 to 14 days after inoculation. After 22 days the inoculated plants kept at 65° and 75°F. showed no symptoms of disease. These plants were then divided into two groups. One group consisting of three inoculated plants and three controls from the 65°F. series, and a similar group from 75°F. series were transferred to 85°F. and were heavily watered. The remaining plants in 65° and 75°F. temperature tanks were left in the respective tanks to use in repeating the experiment.

These plants began to show typical disease symptoms on the 16th day of their transfer to the 85°F. temperature tank. No differences regarding the time of symptom production or their nature were noticed between the group transferred from 65°F. and that transferred from 75°F. Following appearance of disease symptoms three of the plants showing symptoms were transferred to 75°F. temperature tank within about 10 hours after the appearance of the disease symptoms. The object in doing this was to see whether the

plants showing symptoms would recover if transferred to a lower temperature within a few hours after the symptoms appeared. None of the three plants which were transferred to the lower temperature recovered.

The results obtained in this experiment were duplicated by using the other plants remaining at 65° and 75°F. The only difference between the results produced this time and the ones that were produced previously was that these transferred plants began to show symptoms on the 19th day after their transfer instead of on the 16th day as was done by the plants in the first group.

Nature of the Influence of Temperature

Another experiment was conducted to ascertain whether high temperature influences infection principally, or both infection and disease development. In this experiment 21 plants were inoculated and kept at 85°F. Five control plants were also maintained at the same temperature. Twenty-four hours after inoculation 6 plants were taken out of the 85°F. temperature tank. Three of these plants were put in a 75°F. temperature tank and the remaining 3 were taken out of the clay pots, washed and brought to the laboratory for isolations. A similar procedure was followed after 48 and 80 hours.

In order to avoid the possibility of getting false results due to germination of zoospores on the surface of

the roots, the entire plants were sterilized in 10 per cent Clorox for 15 minutes. This insured the emergence of the fungus from the tissue only if it had penetrated into the tissue. From each plant, 3 long roots having secondary and tertiary branches, which had dark colored areas on them, were selected for isolation. The surface sterilized roots were cut into pieces with a sterile razor blade and put on PDA plates. After 12 hours of incubation at 30°C. the plates were inspected for fungal growth. Isolations showed that infection had occurred regardless of whether the isolations were made 24, 48, or 80 hours after the inoculation of the plants.

TABLE 3

Time required for infection and disease development.

Transferred from 85° F. to 75° F. x hours after inoculation	Infection at time of transfer	Disease development after transfer to 75° F.
24	+	-
48	+	-
80	+	+

The transfer of inoculated plants from 85°F. to 75°F., which was done correspondingly with the isolations resulted in the following. Plants which were transferred 24 and 48 hours after inoculation did not produce any symptoms, but the ones which were transferred 80 hours after inoculation began to show symptoms on the 7th day. This 7 day period

coincided exactly with that of the plants which were left in the 85°F. temperature tank and which were inoculated at the same time as the transferred ones. This proved that if the inoculated plants were kept at 85°F. for 80 hours, the disease developed to such an extent that the transfer of these plants to lower temperature (75°F.) did not stop the disease development.

DISCUSSION

In view of the fact that this disease had not been reported, primary emphasis was placed on establishing the cause of the disease and investigating the influence of temperature and other factors on the disease development. The influence of temperature was of particular interest since the disease developed extensively in plants of all ages during the summer of 1958, which was unusually warm. The high optimum temperature for growth of Phytophthora parasitica further suggested the possibility of high temperature being a most important factor in the development of this disease.

Investigations of the effect of temperature on the formation of sporangia indicate that sporangia are formed at all temperatures except at 5°C. Sporangia were abundant after 48 hours at 15°, 20°, and 25°C. Therefore production of sporangia apparently is not a limiting factor in disease development since the disease does not develop at temperatures below 25°C. in spite of abundant sporangial production at lower temperatures. It is assumed here that the production of sporangia in the soil is about the same as in distilled water. The results regarding the effect of pH on growth of the fungus show that the fungus can grow over a wide range of pH and therefore soil pH would not be a limiting factor in the production of the disease.

The studies on pathogenicity show that P. parasitica is the cause of fuchsia root rot and that temperature is the principal limiting factor in the development of the disease. When inoculated plants were incubated at 65°, 75°, and 85°F., the plants which were kept at 85°F. were the only ones which showed disease symptoms. Other considerations, such as, soil pH, sporangial production and the effect of temperature on the germination of zoospores may be of minor importance in disease development.

Greenhouse inoculation studies using soil temperature tanks also helped reveal the nature of the role of temperature in the disease syndrome. All plants which were inoculated and then kept at 85°F. for 24, 48, and 80 hours were infected as shown by root isolations, but when plants were transferred to a 75°F. temperature tank after 24 or 48 hours at 85°F. the disease did not develop. This means that although infection occurred within the first 24 hours, the transfer of the plants to 75°F. after 24 hours and 48 hours prevented disease development. Plants that were kept at 85°F. for 80 hours and then transferred to 75°F., developed the disease. This shows that the 48 hours period is not enough for the fungus to produce disease symptoms, even though infection occurred in the first 24 hours after inoculation. However, the 80 hour period proved to be enough for the fungus to develop in the plant to such an extent that the transfer of the plants to lower temperature at

this stage did not prevent the death of the plants. Thus, it can be seen that high temperature is very important in the production of the disease.

Isolations from plants inoculated and kept at 75°F, showed that they were infected in about 10 to 14 days. This proves the important fact that infection may occur at lower temperatures but the disease does not develop. Thus, lower temperature does not prevent infection but does prevent disease development. High temperatures speed up infection and favor disease development in infected plants.

Finally, an attempt should be made to explain the outbreak of this disease in the summer months of 1958 since this disease had not been observed on fuchsia plants in previous years. The widespread outbreak of disease in young plants and in old stock plants, many of which were in hanging baskets and located in such a way that introduction of inoculum would be difficult, indicates that some of the plants had latent infections. At summer temperatures that normally prevail in the Willamette Valley, infection might occur but temperatures would not be high enough to favor disease development. Therefore a high percentage of plants in a planting might be infected without any obvious development of disease. A comparison of the temperature data for June, July, and August of 1958 with temperature data for 1955, 1956, and 1957

shows that high temperatures in 1958 could have been responsible for disease development (Table 4). Data in Table 4 were recorded by the U. S. Weather Bureau at the Portland Airport Station (10).

TABLE 4

Temperature data for June, July, and August 1955, 1956, 1957, and 1958				
Year	Month	Average °F.	Highest °F.	No. of days 90° F. or above
1955	June	60.7	95	2
	July	63.0	86	0
	August	65.5	90	1
1956	June	59.0	81	0
	July	68.0	102	4
	August	65.4	93	1
1957	June	62.2	79	0
	July	65.6	87	0
	August	66.6	86	0
1958	June	65.2	95	4
	July	70.0	102	8
	August	70.0	95	3

The pathogenicity studies conducted in this investigation showed that if the inoculated plants were incubated at 85°F. for 80 hours, the disease developed even though plants were subsequently moved to a lower temperature unfavorable for disease development. However, when plants were incubated for 48 hours or less at 85°F. then moved to 75°F., disease did not develop. Therefore it would be necessary for plants to be maintained for a period of more than 48 hours at a temperature above 75°F. before disease

would develop. The temperatures in nature fluctuate widely during day and night, and it is very hard to interpret these data in terms of results obtained in the greenhouse under controlled temperature conditions. Since the average temperatures in June, July, and August 1958 were higher than those in previous years, and since there were many more days with temperatures above 90°F. it is likely that the high temperatures in 1958 were responsible for disease development. Soil temperature does not fluctuate to the same extent as the atmospheric temperature and the frequency with which higher temperatures were attained in the summer months of 1958 might have kept the temperatures higher for a long enough period to favor disease development. Disease development may have been favored also by a cumulative effect of high temperatures. It is also possible that a few plants may have been diseased in years prior to 1958 but may not have been detected or reported.

SUMMARY

1. In the summer of 1958, a root rot disease of fuchsia species was observed in the nurseries near the Portland area.
2. The cause of the disease was found to be Phytophthora parasitica Dastur, which produces disease in infected plants at high temperature.
3. Laboratory studies showed that P. parasitica from fuchsia species grows over a wide range of temperature and pH and produces sporangia in distilled water between 10°F. and 35°F.
4. Oospores were not observed in any of the media on which the fungus was grown. Attempts to stimulate oospore production were unsuccessful.
5. Soil temperature tanks were used to hold the plants at different temperatures and to study the effect of temperature on infection and disease development.
6. In pathogenicity studies on plants kept at 65°F., 75°F. and 85°F. infection occurred in 24 hours at 85°F., symptoms appeared on the 7th day and the plants died subsequently. Transfer of plants to 75°F. after symptoms appeared did not prevent death of the plants.

7. Inoculated plants that were incubated at 85°F. for 24 and 48 hours respectively, and then transferred to 75°F. did not show disease symptoms, but plants transferred to 75°F. after 80 hours of incubation at 85°F. developed disease symptoms on the 7th day and died eventually.
8. High temperature was thus shown to speed infection and development of disease.
9. The outbreak of the disease in Portland area only in 1958 was attributed to high temperatures during the summer months of 1958.

BIBLIOGRAPHY

1. Dastur, J. F. Phytophthora parasitica n. sp. A new disease of castor oil plant. India. Department of Agriculture. Memoirs. Botanical series. 5:177-231. 1916.
2. Frezzi, M. J. Las especies de Phytophthora en la Argentina. (The species of Phytophthora in Argentina.) Revista de Investigaciones Agrícolas Buenos Aires 4:47-133. 1950.
3. Gough, F. J. et al. Germination of Oospores of Phytophthora infestans. Phytopathology 47:13. 1957.
4. Klotz, L. J. and H. S. Fawcett. Isolation of Phytophthora species. Phytopathology 29:290-291. 1939.
5. Leonian, Leon H. Physiological studies on the genus Phytophthora. American Journal of Botany 12:446-498. 1925.
6. Leonian, Leon H. Identification of Phytophthora species. 1934. 36 p. (West Virginia. Agricultural Experiment Station. Bulletin 262.)
7. Mehrotra, B. S. Physiological studies on some Phytophthoras III, carbon requirements. Lloydia 14:122-128. 1957.
8. Tsao, Peter H. Serial dilution method for estimating disease potentials of citrus Phytophthoras in the soil. Phytopathology 48:398. 1958.
9. Tucker, C. M. Taxonomy of the genus Phytophthora de Bary. 1933. 208 p. (Missouri Agricultural Experiment Station. Bulletin 153.)
10. U. S. Weather Bureau. Climatological Data. Oregon. v. 61, no. 6-8. June-Aug. 1955; v. 62, no. 6-8. June-Aug. 1956; v. 63, no. 6-8. June-Aug. 1957; v. 64, no. 6-8. June-Aug. 1958.
11. Waterhouse, Grace M. and E. M. Blackwell. Key to the species of Phytophthora recorded in the British Isles. Kew, Surrey, 1954. 9 p. (Commonwealth Mycological Institute. Mycological papers no. 57.)