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PHYTOPHTHORA ROOT ROT OF GUAYULE

A root rot of guayule (*Parthenium argentatum*) Gray has been observed in all of the principal areas in the Southwest where guayule has been under cultivation.

In many plantings, especially those on heavy, poorly drained soils the disease caused losses of 5 to 10 per cent of the stand. At Glendale, Arizona more than 90 per cent of the plants were killed by this disease in test plots that were flood irrigated during the summer of 1943.

The symptoms on vigorously growing plants are a wilting of the leaves and drooping of the succulent stems and flower stalks followed by death of the plant, usually within a week after the first signs of distress are noted. Plants in a less active state of growth when affected usually do not wilt but the leaves develop a yellowish-green color and gradually die back from their tips. Several weeks, or even months, may elapse before these plants are completely overcome. The lesions usually develop 2 to 6 inches below the soil surface but may also occur at the root-crown and bases of the main branches. The lesions are generally slightly sunken and firm in texture.

The cultural characteristics and temperature-growth relationships of the Phytophthoras isolated from diseased specimens collected in various locations in California, Texas and Arizona were determined. On the basis of Tucker's classification of the species of Phytophthora all of the isolates, with the exception of two obtained from fields near Soledad, California were identified as *P. drechsleri* Tucker.

The Soledad isolates were identified as *P. cinnamoni* Rands. That both species were pathogenic to guayule, producing identical symptoms, was demonstrated by inoculation of healthy plants and isolation of the respective species from the lesions thus produced.

Guayule transplants growing in waterlogged gravel cultures inoculated with a suspension of swarm spores of *Phytophthora drechsleri* developed root lesions in aerated as well as in nonaerated cultures. Wilting occurred 2 to 3 days earlier in the nonaerated cultures, presumably because of the deleterious effect of the nonaerated condition on the normal physiology of the root system. That excessive moisture is conducive to rapid development of the disease was demonstrated experimentally.

Infection of the roots in soil inoculated with *Phytophthora drechsleri* was shown to be dependant upon soil temperatures above 60°F.

Measures likely to reduce or prevent loss from Phytophthora root rot in existing or future plantings, based upon field observations and experimental results are given. These include the selection of well drained soils for future plantings, limiting irrigation on heavy poorly drained soils to one thorough application early in the season before the temperature at the 6 inch depth reaches 60°F., and avoiding saturation of the upper foot of soil for periods in excess of 24 hours at times when soil temperatures are above the minimum for the rapid growth of the fungus.

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PHYTOPHTHORA ROOT ROT OF GUAYULE

Introduction

Guayule, *Parthenium argentatum* Gray is a rubber-producing shrub, native to certain sections of the Chihuahuan desert. In 1912 the Intercontinental Rubber Company transferred its developmental activities with this plant from Mexico to California. As a result of the war, interest in guayule as a domestic source of rubber was renewed. In 1942 the United States Government purchased all private holdings within this country and launched an expanded production program. By June 1944 more than 30,000 acres of guayule were under cultivation in field plantings and indicator plots established on a variety of soil types and under widely differing climatic conditions in California, Arizona and Texas.

A root rot of guayule has been observed in all of the principal areas where this crop has been planted in California and the Southwest. In 1941 Smith (5)[1] reported that guayule "is rather susceptible to excess moisture and often succumbs to a rotting of the roots when the soil becomes too wet." The specific cause of the disease was not determined; however, it is likely that the rot described by Smith was caused by Phytophthora. The possibility of

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[1]Figures in parenthesis refer to literature cited in bibliography.

root rot developing under conditions of excess moisture in the absence of Phytophthora will be discussed later in this paper. Campbell, Leach, Presley and Snyder (1) reported a root rot from which they consistently cultured a species of Phytophthora. This paper presents the identification of the causal organism and determination of its pathogenicity, field observations and the results of experimental studies on this disease.

DISTRIBUTION AND EXTENT OF LOSS

Phytophthora root rot caused losses of 5 to 10 per cent of the guayule in many of the experimental plantings in Texas and Arizona during 1943. At Glendale, Arizona, more than 90 per cent of the plants were killed by this disease in several test plots that received flood irrigations during the summer of 1943. In California Phytophthora root rot was most prevalent in the Tracy-Newman District (San Joaquin Valley) where many of the plantings were on clay or clay loam soils with poor internal drainage. In September, 1943, the disease was observed in 22 of the 25 fields surveyed in the Tracy-Newman District of California (Table 1).

Table 1. Losses caused by Phytophthora root rot in 25 fields in the Tracy-Newman District. September, 1943.

Loss	Fields surveyed	Acreage Surveyed	
Per Cent	Number	Acres	Per Cent
0	3	694	19
Trace-1	16	2178	58
1-5	2	248	7
5-10	3	465	12
25	1	160	4
Totals	25	3745	100

Losses of 5 per cent or more of the plants were recorded for 625 of the 3745 acres included in the survey. Losses in other planting districts of California were, for the most part, restricted to small groups of plants at the ends of rows or low areas in the field where water accumulated (Fig. 1A) and usually represented less than one per cent of the field stand.

SYMPTOMATOLOGY

On vigorously growing plants, the first above ground symptoms of the disease are wilting of the leaves and drooping of the succulent stems and flower stalks. Permanent wilting soon occurs and usually the plant is killed within a week after the first signs of distress are noted. Plants in a less active state of growth when affected usually do not wilt. The leaves of these plants develop a yellowish-green color and gradually die back from their tips. With the latter sequence of symptoms, several weeks, or even months, may elapse before the plant is killed. The dead leaves usually remain attached to the plant for some time.

Phytophthora lesions on guayule roots (Fig. 1B, C) are usually slightly sunken and firm in texture. The infected tissue is dark brown or black and is sharply delimited from the healthy tissue. The woody portion of the root beneath the affected bark is also discolored. Older lesions are generally invaded by secondary organisms which usually produce a spongy, honey-combed texture.

The lesions generally develop 2 to 6 inches below the soil surface but may also occur at the root-crown and bases of the main branches. On older plants, the lesions that cause wilting are generally 2 to 5 inches in length and often involve the entire distal portion of the tap root as well as portions of the adjacent secondary roots. However, lesions less than one-half inch in length may cause wilting and death of recently transplanted guayule. Apparently healthy plants were found which had well developed lesions on their secondary roots. Only those lesions which girdle the tap root sufficiently near the crown to seriously impair the functioning of the root system are effective in causing wilt and subsequent death of the plant. A few cases have been observed in which the development of secondary roots above the tap root lesion enabled the plant to recover even when the greater portion of the tap root was rotted. However, these plants generally had a stunted, unthrifty appearance.

The development of pronounced above ground symptoms does not always closely follow the girdling of the tap root by Phytophthora lesions. Well-developed lesions were frequently found on apparently healthy plants growing in root rot areas. Plants of this kind were generally found only after growth had been retarded by low temperatures. In April, 1944, plants with well-developed lesions on their tap roots were observed in several plantings in the San Joaquin Valley. The affected plants had symptoms characteristic of plants that became infected when in a partially dormant condition. The root lesions on these plants were usually swollen rather than depressed and had a somewhat spongy texture. New infections were not observed in any of the plantings in this area until several weeks later. The foregoing explains why individual plants may die long after the existence of conditions favorable for infection.

FIELD OBSERVATIONS OF FACTORS INFLUENCING THE SEVERITY OF THE DISEASE

Moisture

Garrett (3) states "when soil moisture content falls below a certain value, infection by phycomycetous fungi and other organisms which infect the host by means of free-swimming zoospores is usually completely inhibited." Infection of guayule by Phytophthora likewise appears to be dependent upon a high soil moisture content. In areas where the fungus was present, flood irrigation or impounding of water in low spots in the field invariably caused high mortality.[2] During the summer months, new infections

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[2]The killing of guayule in waterlogged areas where the soil temperature at the 6-inch depth was above 90°F. has been frequently observed. Isolations made from the roots of such plants never yielded Phytophthora nor was any other fungus consistently recovered. In these instances, death was attributed to suffocation of the root system. Injury produced by the extended waterlogging of the soil could usually be distinguished from Phytophthora root rot by the nature of the root injury and by the extent of mortality within the affected area. In areas where plants were killed by waterlogging of the soil usually all of the plants were dead; whereas, in typical Phytophthora root rot areas (Fig. 1A), occasional plants remained unaffected. Greenhouse experiments showed that the duration of the waterlogged soil condition necessary to result in the death of the plant was inversely proportional to the soil temperature. At a soil temperature of 90°F., flooding of the soil for 3 days killed guayule plants growing in pasteurized soil, while at 100°F., flooding of the soil for 24 hours produced the same results.

developed after each irrigation in the infested plantings on the heavy soils. Usually the greatest mortality occurred during the week following the application of water.

It seems highly probable that new infections occur only during the period immediately following the application of water, although many plants develop symptoms and die long after this time. The subsequent development of above ground symptoms is dependent upon the location of the lesion, the rate at which it develops, the extent of the development of secondary roots above the lesion, and the condition of the plant at the time of infection, as well as on the diverse environmental factors governing the water relations of the plant.

Soil Type

Phytophthora root rot was most prevalent on the heavy soil types, especially those with impeded internal drainage. On light, well-drained soils, infection could usually be traced to an unusually heavy rainfall or an excessive irrigation during the period when the soil temperature was near the optimum for the development of the fungus. This relationship is to be expected with a fungus which is dependent on a high soil moisture for its development. The heavy soils take longer to return to field capacity; consequently, on these soils conditions favorable for infection extend over a longer period than on light soils.

Temperature

In California the disease is most active from May through September, the greatest loss occurring in June, July and August. In 1944 the first signs of current season infections in the Tracy-Newman District appeared in late April. Soil temperatures (clay soil) at the 6-inch depth were below 60°F. during the first half of April but were consistently above 60°F. and exceeded 65°F. for a short time on several days during the latter part of the month. The results of a greenhouse experiment in which infection of guayule by *Phytophthora drechsleri* was shown to be dependent upon soil temperatures in excess of 60°F. are presented later in this paper.

THE CAUSAL FUNGUS

Isolation

In general, the causal fungus was more readily isolated from the lesions on younger plants than from the lesions on older plants, probably because the former could be detected at an earlier stage of development. Cultures made from the advancing edge of newly developed lesions were seldom contaminated with growth of secondary invaders.

Recovery of Phytophthora from the rotted roots was accomplished by various techniques. Tissue plantings were made from the margin of the lesions to prune agar, potato dextrose agar and water agar. On prune agar rapid growth of Phytophthora was obtained, usually without bacterial contamination. Shallow irrigation, in petri dishes, of small pieces of washed tissue taken from the margin of the lesion yielded sporangia which readily developed pure cultures of the fungus when transferred to potato dextrose agar. Another method that proved very satisfactory was the inoculation of apples with small pieces of the infected tissue. After 4 days lesions developed about the point of inoculation. Transfers made from the margin of the apple rot yielded pure cultures of Phytophthora, and only rarely other fungi, when plated on potato dextrose agar. Bacteria did not contaminate the isolations made by this technique. The recovery of Phytophthora could usually be determined by the type of rot produced on the apple. Phytophthora has been isolated from root lesions on plants collected in the Sacramento, San Joaquin, and Salinas Valleys of California, the Gila and Santa Cruz Valleys of Arizona, and the Mesilla Valley of Texas.[3]

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[3]Isolations of Phytophthora from specimens collected in Arizona and Texas were made by J. T. Presley.

Identification and Cultural Characteristics

Representative isolates of the causal organism (as determined by the initial inoculation experiments) were sent to Dr. C. M. Tucker who identified them as *Phytophthora drechsleri* Tucker. Comparative tests were then conducted at Salinas with these and the Phytophthora isolates from various locations in Texas, Arizona and California. All of the isolates, with the exception of those from Soledad, California,[4] were found to be *P. drechsleri* as determined by their conformance with the characteristics defined for this species by Tucker (6).

The isolates of *Phytophthora drechsleri* and *P. cinnamomi* made excellent growth with pronounced concentric zonation on potato dextrose agar at 25°C. (Fig. 2). No oogonia were produced on potato dextrose agar, corn meal

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[4] The isolates of Phytophthora obtained from root lesions on guayule from two fields at Soledad, California, differed from *P. drechsleri* mainly by their failure to make growth on corn meal agar or potato dextrose agar when maintained at 35°C. for 96 hours (Fig. 3). When returned to 25°C. for 96 hours these cultures made no growth; whereas, those fitting the description of *P. drechsleri* resumed growth at 25°C. even after having been maintained at 40°C. for 96 hours. The Soledad isolates did not develop sporangia in Petri's mineral solution as abundantly as the *P. drechsleri* isolates. According to Tucker's classification for the genus, these isolates are considered to be *P. cinnamomi* Rands.

agar, steamed corn meal or oatmeal agar after two months at room temperatures. The isolates of P. drechsleri made good growth on corn meal agar or potato dextrose agar after 4 days at 35°C. (Fig. 3). The sporangia of P. drechsleri developed in Petri's mineral solution were non-papillate, ovate, pyriform to obpyriform, 37-53 x 28-37µ (average of 75 sporangia 43.7 x 32µ).[5] The sporangia of P. cinnamomi were non-papillate, ovoid to ellipsoid, 42-88 x 25-42µ (average of 75 sporangia 62 x 35µ). Sporangia were rarely developed by either species on potato dextrose agar but were abundant on mycelium transferred to Petri's mineral solution. All isolates were pathogenic to wounded apple fruits and potato tubers. Swollen irregular vesicles, superficially resembling chlamydospores, were produced in abundance on the hyphae on agar media.

Effect of Temperature on Rate of Growth in Culture

The critical temperatures for growth of 8 cultures of Phytophthora isolated from guayule were determined. The isolates were grown in petri plates on potato dextrose agar. The inoculum was obtained from cultures grown on

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[5] Tucker gives the following measurements for the sporangia of P. drechsleri developed in Petri's mineral solution: 24-38 x 15-24µ (average of 50 sporangia 31.4 x 21µ). For the sporangia of P. cinnamomi, Tucker gives the following measurements: 38-84 x 27-39µ (average 57 x 33µ).

oatmeal agar for 12 to 16 days. Disks of agar approximately 5 mm. in diameter, covered by mycelium of the fungus, were cut from the periphery of the cultures and transferred to the center of each plate. The plates were all incubated at 22°C. for 6 hours before exposing them to the various temperatures. The plates were incubated 96 hours at 5, 10, 15, 20, 25, 27.5, 30, 35, 40 and 45°C. The average diameter of the growth in each plate was measured after 24, 48, 72 and 96 hours.

In Table 2 the average diameters of growth in the plates after 96 hours incubation at each temperature is given for 8 of the Phytophthora isolates obtained from guayule. Repeated trials gave similar results. The cultures for which the data are presented were selected as representative of the type isolated from root lesions on plants collected at the locations cited. _Phytophthora cinnamomi_ did not grow at 35°C.; whereas, _P. drechsleri_ made good growth at this temperature (Fig. 3). Temperature growth curves for isolates Nos. 3 and 6 (_Phytophthora cinnamomi_) and Nos. 5 and 10 (respectively, the least and most rapid growing of the _P. drechsleri_ isolates) are shown in Fig. 4. The physiologic difference between _P. cinnamomi_ and _P. drechsleri_ as well as the similarity of behavior of the conspecific isolates is demonstrated.

Table 2. Average diameters of mycelial growth on potato dextrose agar in plate cultures after 96 hours incubation at various temperatures of 8 isolates of Phytophthora isolated from guayule. (Average of 3 replicates.)

Culture No.	Location	Average diameter of mycelial growth in mm. Temperatures expressed in degrees Centigrade 5°	10°	15°	20°	25°	27.5°	30°	35°	40°	45°
1	Westley, Calif.	2	16	27	41	60	64	65	51	2	0
3	Soledad, Calif.(1)	5	16	29	49	58	47	31	0	0	0
5	Vernalis, Calif.	4	17	28	44	59	62	60	45	3	0
6	Soledad, Calif.(2)	2	10	26	34	50	48	39	0	0	0
7	Salinas, Calif.	3	16	32	47	61	62	58	52	7	0
8	Canutilla, Texas	7	18	28	49	63	64	61	50	3	0
10	Sacaton, Arizona	10	18	28	48	68	72	72	63	9	0
38	Lamont, Calif.	11	17	26	52	66	68	66	60	1	0

EXPERIMENTAL STUDIES

Proof of Pathogenicity

The ability of Phytophthora drechsleri to produce lesions on guayule was demonstrated by inoculation experiments.

In the first experiment plants growing in pasteurized soil in 8-inch clay pots were used. Each pot contained 5 healthy plants. The soil was carefully removed from one side of the tap root of each plant and the periderm was slightly scarred with a sterile scalpel one inch below the ground line. Three plants in each of 4 pots were inoculated by placing a small piece of potato dextrose agar containing mycelium of *P. drechsleri* (isolated from root lesions on plants collected at Westley, California) in contact with the injured periderm. The remaining 2 plants in each pot were similarly treated with sterile agar to serve as checks. The soil was replaced, and thereafter, as before, the plants were watered only as needed. The first wilting occurred on the 5th day, and on the 7th day the leaves of 10 of the 12 inoculated plants had wilted. After 14 days all 12 inoculated plants were dead while the checks remained healthy. Examination of the roots of the inoculated plants revealed the presence of well-developed lesions (Fig. 1D) similar to those developed in the field (Fig. 1B). The roots of the check plants were unaffected. *P. drechsleri* was recovered from the inoculated plants.

In subsequent experiments, employing this inoculation technique, the pathogenicity of the *P. drechsleri* isolates from Vernalis, Calif., Salinas, Calif., Lamont, Calif., Sacaton, Arizona, and Canutilla, Texas, was demonstrated. *P. drechsleri* was cultured from the lesions thus induced. In a like manner, the pathogenicity of the isolates of *P. cinnamomi* obtained from two plantations near Soledad, Calif., was demonstrated and the organism reisolated from the lesions.

In a later experiment, several pieces of potato dextrose agar containing mycelium of *Phytophthora drechsleri* were placed 2 inches below the surface of the soil in two 8-inch pots each containing 5 healthy plants. The tap roots of these plants were not disturbed. Two control pots were treated similarly except that sterile agar was substituted for the inoculum. These pots were kept in the greenhouse and watered in the usual manner. After 4 weeks 2 of the 5 plants in one of the inoculated pots were wilted. Examination of the roots showed the presence of lesions girdling the roots of 4 of the plants. After 8 weeks 3 of the 5 plants in the remaining inoculated pots were dead. Examination revealed well-developed lesions on the roots of all 5 plants including the 2 plants that had not wilted. No lesions were produced on the roots of the 10 check plants.

Effect of Moisture and Aeration upon Infection and the Subsequent Development of Symptoms

Procedure.--

Two hundred 8-month-old, hardened guayule plants were dug from a bed in the Alisal Nursery at Salinas, California. The plants were carefully washed and all of the feeder roots were removed by pruning. The tops were pruned to within one inch of the crown and all but 4 inches of the tap root was removed. One hundred plants of uniform diameter were planted in gravel contained in 1 qt. glazed crocks, one plant per crock. The crocks and gravel had previously been treated with a formaldehyde drench and flushed several times with tap water. Each plant received 100 cc. of Hoagland's complete nutrient solution daily. Tap water was added as needed to keep the plants from wilting. After 2 months most of the plants had considerable top growth and numerous flower stalks. Seventy-two plants of uniform size and state of development were selected for use in this experiment.

The plants were separated into 18 sets of 4 plants each. Inoculation of 36 plants was accomplished by introducing 50 cc. of a tap water suspension of swarm spores[6] of Phytophthora drechsleri into each crock. Into each of the remaining 36 crocks 50 cc. of tap water was poured.

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[6]Sporangia of Phytophthora drechsleri were produced in abundance from small disks of oatmeal agar containing mycelial growth after several days in Petri's mineral solution. Abundant swarm spore evacuation was obtained by cooling the cultures to 5°C. for 30 minutes and then subjecting them to room temperatures for 10 to 20 minutes.

The plants were then divided into 9 lots each containing 4 inoculated and 4 non-inoculated plants. The treatments imposed on each lot were as follows:

I. Control series (Watered only as needed)

II. Waterlogged-nonaerated series

1. Duration 12 hours.
2. " 24 "
3. " 48 "
4. " 96 "

III. Waterlogged-aerated series

1. Duration 12 hours.
2. " 24 "
3. " 48 "
4. " 96 "

Aeration of the solution was obtained by air from a compressor unit entering the solution through a capillary tube inserted through an opening at the base of the crock. The velocity of the air stream was regulated by means of stop-cocks. The rising air bubbles created slight currents in the solution thus further enhancing aeration.

Temperature and relative humidity data.--

During the course of the experiment air temperatures in the greenhouse ranged from 72° to 88°F. Relative humidity varied from a low of 42% to a high of 82%. The temperature in the root zone of the control and waterlogged-nonaerated series varied from 69° to 77°F. and in the waterlogged-aerated series from 67° to 77°F.

Results.--

A. Inoculated plants.--

I. Control series.-- The plants which were given water only as wilting of the leaves indicated that watering was necessary did not develop above-ground evidence of root infection throughout the course of the experiment. On the 14th day, when examination of the root systems was made, a Phytophthora lesion approximately one inch in length girdling the tap root 2 inches below the crown was found on one plant. The tap roots of the other three plants were free of lesions. The feeder roots of all 4 plants had a glassy, unhealthy appearance. Isolations from the tap root lesion and from the feeder roots yielded Phytophthora drechsleri.

II. Waterlogged-nonaerated series.-- The inoculated plants subjected to the waterlogged condition in the absence of aeration for 48 and 96 hours began to wilt on the afternoon of the 2nd day even though the root systems were submerged in water. The plants receiving this treatment for 12 and 24 hours did not show signs of wilting until the 3rd day when one plant in the 12-hour set and 2 plants in the 24-hour set wilted. On the 4th day all inoculated plants in the waterlogged-aerated series were wilted. On the 5th day all inoculated plants in this series were wilted beyond the stage where recovery could be expected. Examination of the root systems on the 14th day disclosed marked injury of the tap roots and feeder roots of all plants in this series. Although the development of wilting was directly proportional to the duration of the waterlogged condition there was no correlation between the duration of the waterlogged condition and the extent of root infection at the time when the roots were examined. *Phytophthora drechsleri* was isolated from the lesions.

III. Waterlogged-aerated series.-- Wilting of the inoculated plants in the waterlogged-aerated series first occurred on the 4th day. On the 5th day all but one inoculated plant in this series had wilted. Wilting of all of the inoculated plants in this series beyond the stage where recovery was likely did not occur until the 8th day. The tap roots and feeder roots of all the inoculated plants in this series were extensively rotted. In the aerated cultures, the duration of waterlogging did not appear to influence the rate of wilting nor the extent of root injury. *Phytophthora drechsleri* was isolated from the tap root lesions.

B. Noninoculated plants (check plants).--

I. Control series.-- The check plants in the control series remained healthy throughout the course of the experiment and no lesions were present on the roots.

II. Waterlogged-nonaerated series.-- The check plants waterlogged for 12 and 24 hours respectively in the nonaerated series did not wilt and examination of their roots on the 14th day showed no visible detrimental effect from the treatment. Two check plants waterlogged 48 hours in the absence of aeration wilted on the 2nd day and had not recovered by the 14th day. Although the tap roots of these plants did not appear to be affected the feeder roots were discolored. The other two plants of this series did not wilt. The roots of these plants appeared normal. Three of the check plants waterlogged 96 hours were wilted on the 3rd day and by the 8th day all 4 plants were wilted beyond recovery. Examination of the roots on the 14th day showed discoloration of the tap roots of 3 of the plants and excessive reddening of the feeder roots of all 4 plants. Isolations made from the tap root lesions did not yield Phytophthora.

III. Waterlogged-aerated series.-- All check plants in the waterlogged-aerated series remained healthy throughout the experiment. After 14 days there was no visible difference between the roots of the plants in this series and those of the control series.

Representative plants from each lot as they appeared seven days after the start of the experiment are shown in Fig. 5.

Interpretation of results.--

Under the conditions of this experiment infection of guayule by *Phytophthora drechsleri* was shown to be favored by the presence of excess moisture at the surface of the root. Although aeration of the solution retarded the development of wilting it did not materially affect the pathogenicity of the fungus nor the susceptibility of the host.

Inoculated plants of the waterlogged-nonaerated series developed symptoms more rapidly because of the additional deleterious effect of the nonaerated condition upon the plant. This effect would have been expressed even in the absence of the pathogen as shown by the wilting of noninoculated plants in the waterlogged-nonaerated series. The significance of this experiment from the standpoint of control will be considered later.

Effect of Temperature on Infection

Procedure.--

Guayule transplants rooted in pasteurized soil in 2" x 4" plant bands were transplanted to 6-inch pots containing pasteurized soil previously inoculated with a pure culture of *Phytophthora drechsleri*. The soil was inoculated by mixing one part of oat-fungus culture with 20 parts of sand. After a 5-day incubation period one part of the inoculated sand was mixed thoroughly with 20 parts of pasteurized soil. Six replicates and 2 checks were maintained at 60°, 75°, and 90°F. in constant-temperature tanks, patterned after those developed at the University of Wisconsin (2). A high soil moisture content was maintained by watering twice daily with water brought to the temperature of the series to which it was applied.

Results.--

The first wilting occurred on the 4th day in the pots maintained at 75° and 90°F. On the 9th day the leaves of 3 plants at 75°F., and 4 plants at 90°F. were completely dried. On the 12th day 5 plants in inoculated soil at 75°F. series were dead as were all 6 plants of the 90°F. series. All 6 plants of the inoculated series at 60°F. were severely wilted on the 12th day. On the 12th day the plants maintained at 75° and 90°F. (Fig. 6) had numerous lesions along the entire length of the tap root. Those kept at 60°F. all had lesions at the ground line but no lesions whatsoever on the rest of the tap root (Fig. 6). A record of the surface soil temperature of the pots kept at 60°F. showed that the temperature at 0-1/2" depth ranged from 60°-70°F. daily.

Interpretation of results.--

On the basis of this experiment it appears that

Phytophthora infection is inhibited at soil temperatures of 60°F. or lower. This temperature relationship is in agreement with conclusions drawn from field observations.

DISCUSSION

Field observations indicate that Phytophthora root rot is likely to be serious only on heavy soils, on soils with poor internal drainage, or in areas where excess soil moisture is provided or retained at times when the temperature of the upper 6 inches of soil is above 60°F. Only those lesions sufficiently near the crown of the plant to inactivate the greater part of the root system cause wilting and subsequent death of the plant.

That excessive moisture is conducive to rapid development of the disease was demonstrated in the aeration-waterlogging experiment, since none of the plants in the inoculated control series wilted and only one plant developed a small lesion at the lower end of its root. In the presence of a waterlogged-nonaerated condition wilting and death of the inoculated plants occurred two to three days earlier than in the waterlogged-aerated series. Infection of the roots in inoculated soil was shown to be dependent upon soil temperatures above 60°F.

In view of the foregoing observations and experimental results the following measures are suggested to reduce or prevent loss from Phytophthora root rot in existing or future plantings of guayule.

CONTROL

Where Phytophthora is known to be present heavy soils or those with poor internal drainage should be discriminated against for guayule plantings, especially in areas where summer rains are frequent and heavy.

Field observations indicate that only those lesions sufficiently near the crown of the plant to inactivate the greater part of the root system cause wilting and subsequent death of the plant. Therefore, in plantings where the fungus is known to be present, care should be taken to avoid keeping the upper foot of soil that is in contact with the crown and tap root above field capacity for more than 24 hours, especially when soil temperatures are near the optimum for the development of the fungus (70° to 90°F.). Hunter and Kelley (4) show that a heavy and prolonged irrigation of a silty clay loam, wetting the soil to the "Field capacity" to a depth of at least 8 feet, during the first week of April stored sufficient water for good shrub development during the following three months. This practice would make possible the irrigation of soils know to be infested with *Phytophthora drechsleri* at a time when the soil temperature at the 6-inch depth is below 60°F., thus decreasing the possibility of infection by this organism.

Flood irrigation in areas where Phytophthora is known to be present should be practiced with extreme caution. In row irrigation flooding of low areas in the field should be avoided.

Figure 1. A. A typical Phytophthora root rot area in a two year-old field planting of guayule showing the plants in various stages of wilt and defoliation and the remaining healthy-appearing plants which distinguish Phytophthora root rot areas from those killed by drowning. B. Root lesions caused by natural infection by *Phytophthora drechsleri* on guayule transplants during their first year in the field. C. A typical Phytophthora root lesion on a two year-old plant on which the above-ground symptoms had only recently developed. A thin layer of the bark has been removed to show the sharp line of demarkation at the margin of the black sunken lesion. D. Root lesions produced in the greenhouse on guayule transplants by inoculation with pure cultures of *P. drechsleri* isolated from guayule.

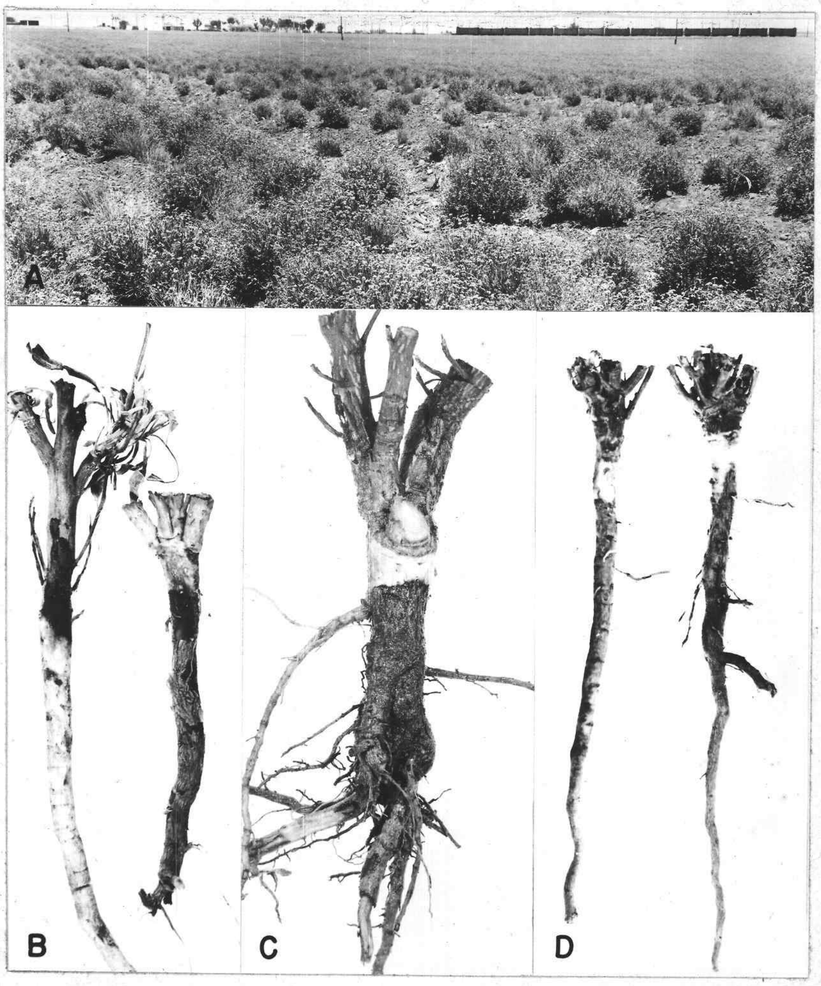

A
B
C
D

Figure 2. Characteristic growth of cultures of Phytophthora isolated from guayule on potato-dextrose agar after 96 hours at 25°C. Cultures 1, 5 and 7 are _Phytophthora drechsleri_ Tucker; Cultures 3 and 6, _Phytophthora cinnamomi_ Rands.

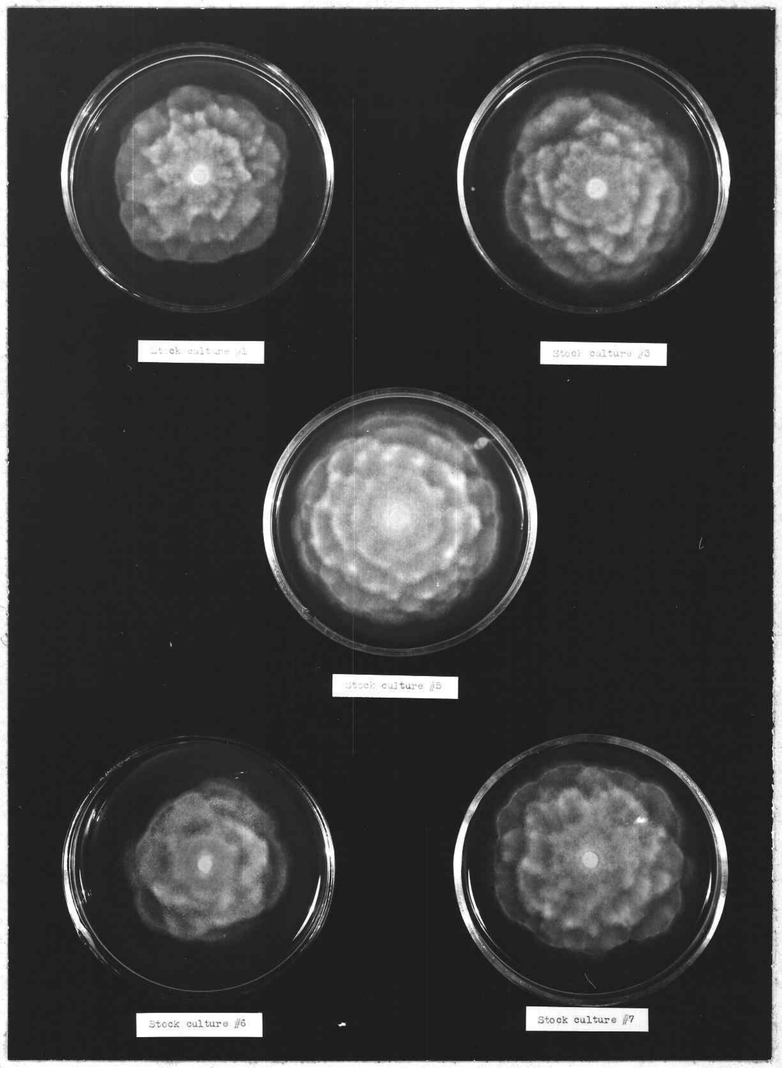

Stock culture #1
Stock culture #3
Stock culture #5
Stock culture #6
Stock culture #7

Figure 3. Characteristic growth of cultures of Phytophthora isolated from guayule on potato-dextrose agar after 96 hours at 35°C. Cultures 1, 5 and 7 are *Phytophthora drechsleri* Tucker; Cultures 3 and 6, *Phytophthora cinnamomi* Rands.

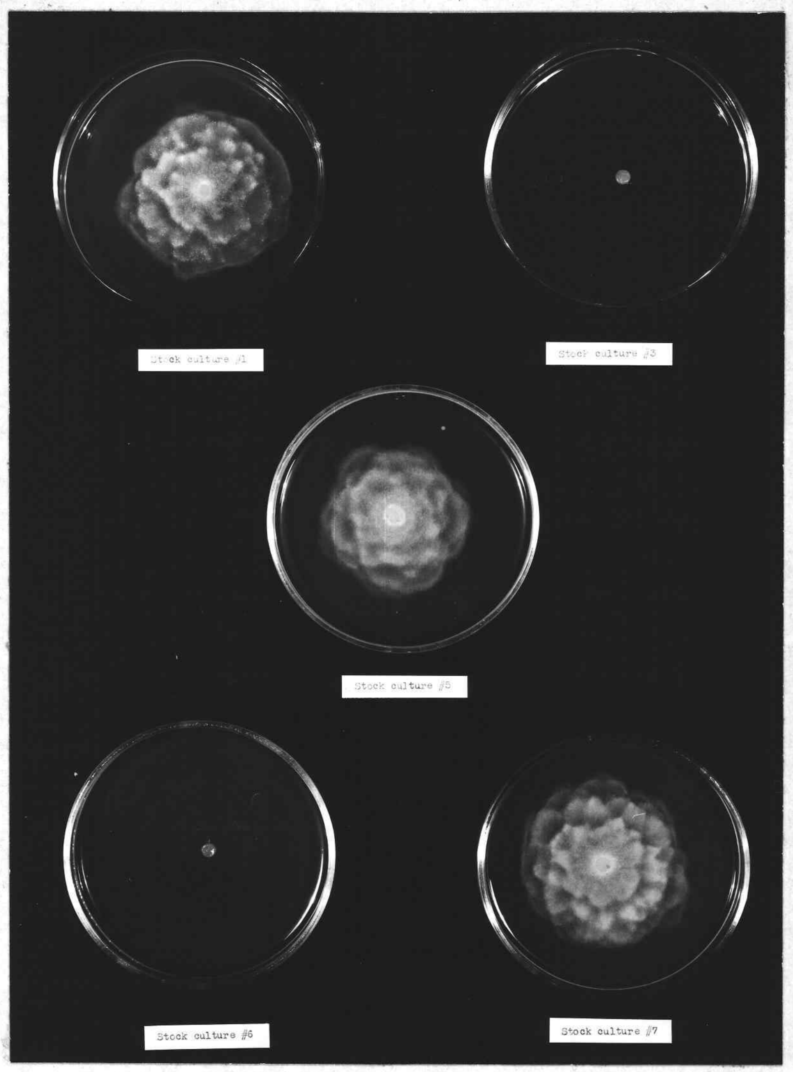
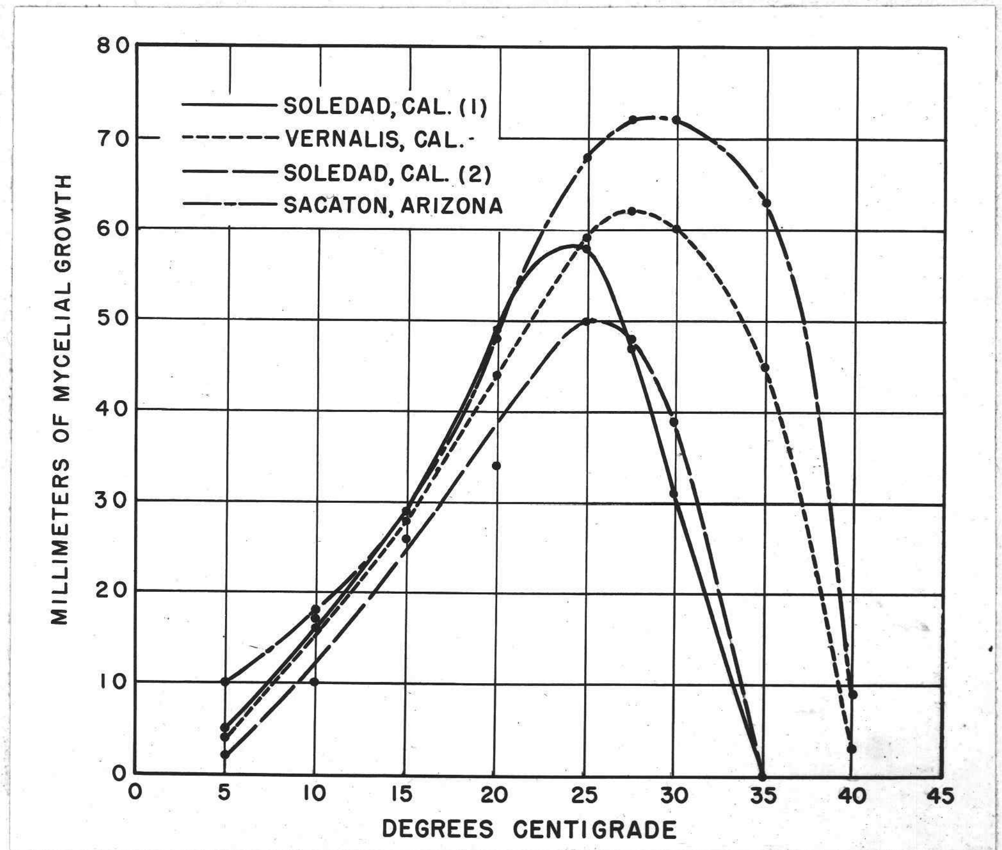

Stock culture #1
Stock culture #3
Stock culture #5
Stock culture #6
Stock culture #7

Figure 4. Temperature-growth curves for Phytophthora cinnamomi and P. drechsleri isolated from guayule. Millimeters of mycelial growth after 96 hours on potato dextrose agar in plate cultures maintained at various temperatures: (3) P. cinnamomi (Clark lease; Soledad, Calif.); (6) P. cinnamomi (Soledad Ranch Co. lease; Soledad, Calif.); (5) P. drechsleri (Vernalis, Calif.); (10) P. drechsleri (Sacaton, Arizona).



80
70
60
50
40
30
20
10
0
MILLIMETERS OF MYCELIAL GROWTH
SOLEDAD, CAL. (1)
VERNALIS, CAL.
SOLEDAD, CAL. (2)
SACATON, ARIZONA
0
5
10
15
20
25
30
35
40
45
DEGREES CENTIGRADE

Figure 5. Representative plants from each lot of the aeration-waterlogging inoculation experiment. Photographed 7 days after the start of the experiment.

INOCULATED WITH PHYTOPHTHORA DRECHSLERI

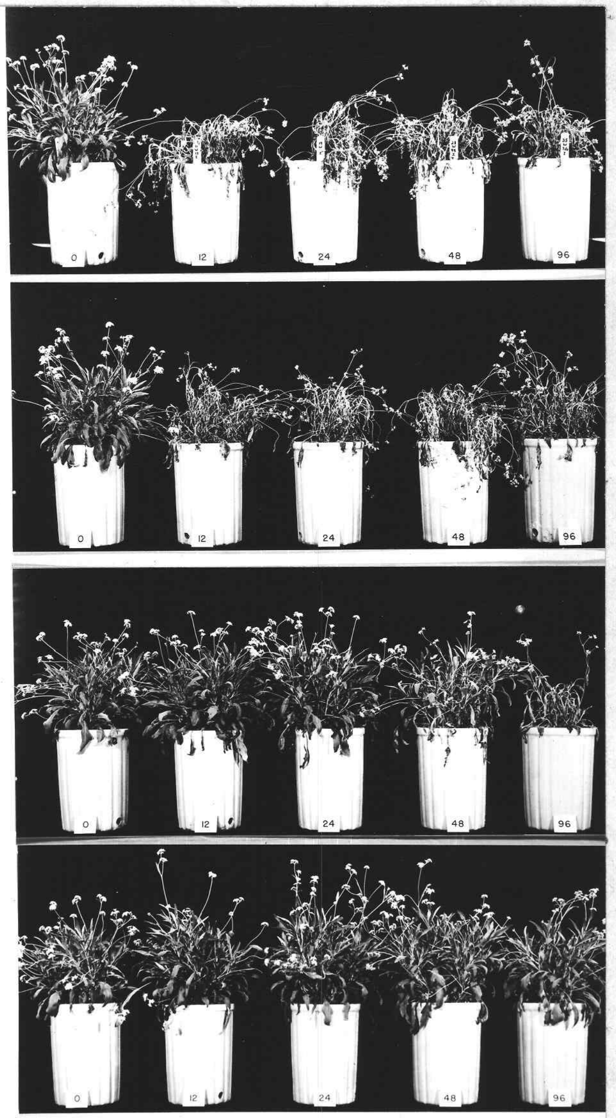
Nonaerated

Aerated

CHECKS

Nonaerated

Aerated



Numbers designate time (in hrs.) that roots were submerged in water.

Figure 6. Guayule transplants grown in soil inoculated with pure cultures of Phytophthora drechsleri and maintained at 60°, 75° and 90°F. respectively. The plant grown in soil maintained at 60°F. developed a lesion only at the ground line where the temperature reached 70°F. during the daytime; the lower part of the root is unaffected. Those grown at 75° and 90°F. show numerous lesions on the tap root.


60°F
75°F
90°F

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