FACTORS INFLUENCING DEVELOPMENT AND CONTROL OF PHYTOPHTHORA FRAGARIAE HICKMAN, THE CAUSE OF RED STELE DISEASE OF STRAWBERRIES

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INTRODUCTION

The strawberry is the most widely grown small fruit in the United States (35, p. 63). In Oregon the strawberry today is the most valuable of all fruit crops grown in the state. With a cash farm value estimated in 1960 at $12,000,000 (123), strawberries exceed the cash value of such crops as pears, snap beans, onions, hops, peppermint, sugar beets, oats, and all small fruits. Receipts from strawberries exceed the combined receipts from apples, cherries, peaches, plums, prunes, filberts, and walnuts. Wheat, barley, and potatoes surpass strawberries in cash value (73, p. 1-43; 75, p. 1-103; 123).

Strawberry production has been a major horticultural enterprise in Oregon for over 60 years. However, the main development has been only since the establishment of the processing industry. Over 90 percent of the state's crop goes to processors for canning or freezing (98). Production has risen from 5.8 million pounds in 1899 to a record 91.5 million pounds from some 18,500 acres in 1957. Top farm cash value of the crop was $13,144,000 received in 1955 (75, p. 22). Only California consistently markets more strawberries than does Oregon (73, p. 34).

With rising costs of production and increasing competition from other areas, profitable strawberry production in Oregon is dependent upon high yields. In this state, where strawberries ripen in late spring and early summer, growers cannot benefit from the higher prices or the restricted competition of the winter or early spring fresh fruit markets. Any factor which reduces quality or yield of strawberries, or which increases cost of production, will reduce the competitive ability of the region.

Red stele, caused by Phytophthora fragariae Hickman, is an extremely important fungus disease causing a root rot of strawberries. Infected plants bear poorly, become very drought-susceptible, and die. This may occur to such an extent that strawberry production is not profitable. The Willamette Valley, where over 95 percent of the strawberries produced in Oregon are grown, provides an ideal environment for this fungus. The potential importance of the disease in Oregon is obvious.

The strawberry red stele disease was first observed in Oregon in 1937 (181) although it may have been present as early as 1934 (164). The fungus may have been brought into the state with diseased planting stocks from other areas. Spread within the state was rapid and by 1939 the need for red stele-resistant strawberry varieties was acknowledged. Breeding for red stele resistance became a

major objective, along with the original goals of improved freezing and canning qualities (164). By 1941 the disease had spread to nearly every county in the Willamette Valley and was especially common in the commercial strawberry areas. At that time many people considered red stele disease more important than all of the other strawberry diseases combined (181).

No practical means are known for eradicating the fungus from the soils in which it has become established. Use of resistant plant varieties in infested areas has enabled strawberry production to continue. No varieties exist, however, which are resistant to all races of Phytophthora fragariae. This fungus is capable of adapting to varieties which previously were resistant. Many growers have been able to avoid the disease by planting red stele-free strawberry planting stock in disease-free soil. No chemical or cultural treatments are known that will assure normal crops in infected plantings.

The purposes of these investigations were to study the influence of the cultural environment on mycelial growth, production of zoosporangia and release of zoospores by P. fragariae, and to determine the value of certain Douglas fir bark by-products for control of red stele disease.

REVIEW OF LITERATURE

Description of Disease

Strawberry red stele disease is caused by invasion and destruction of roots by the fungus Phytophthora fragariae Hickman (14, p. 19; 68, p. 94). Infected plants are reduced in size and show general symptoms of starvation (4; 14, p. 12; 166, p. 3). The fungus, P. fragariae, which is most active during cool wet weather (4; 68, p. 94) may invade and seriously damage a major portion of a plant root system before above ground symptoms are apparent. Young, white, fibrous roots are first invaded, then the fungus grows up through the larger roots (14, p. 12; 68, p. 104-7). Roots more than a year old are not affected (14, p. 12). Growth of the fungus is usually limited to the roots (4; 8,. p. 415, 417; 13; 14, p. 12), although Hickman (68, p. 94, 105) has reported isolation of P. fragariae from the crown, petioles (but not leaves), and stolons of certain very susceptible varieties in England.

The fungus grows in the central core or vascular region of the root, producing a characteristic red or brown color while leaving the surrounding cortex white (2; 3; 13; 14, p. 19; 68, p. 93). This distinctive feature provides both a name and a symptom for

positive diagnosis. No other strawberry disease has this feature (68, p. 93, 105).

Invasion of roots by P. fragariae allows entry of other soil organisms (14, p. 12; 68, p. 105). Infected rootlets decay, frequently leaving short rat tail-like remnants of main roots devoid of adventitious roots: another symptom typical of the disease.

The most dependable evidence of red stele disease is the microscopic observation of the Phytophthora oospores in diseased roots (14, p. 15). The continued association of these large and distinctive oospores with the disease was first discovered in 1926 (4) and has since been confirmed by many.

Above ground symptoms associated with this root disease are slow in developing and, in the field, most often occur in two-year-old or older plants (8, p. 413). At first the older leaves may have a browning of the edges of the laminae, then may wilt and die (68, p. 93). As disease development progresses new leaves become smaller, have short petioles, and are bluish-green (40). Plant size decreases gradually as the number of functional roots is reduced. Heavily infected plants are unable to produce fruit or runners (14, p. 12; 68, p. 93; 166; 168).

The cool, rainy periods which favor red stele disease development also retard plant growth processes (35, p. 3-4). Water

requirements are low and may be supplied by the limited root system of the diseased plants. When growth processes are stimulated by abundant warm weather the higher amounts of water required cannot be supplied by the crippled root system, especially if the rains cease and the soils become dry. Severe wilting of the plant usually results (13; 14, p. 15). Whether death occurs depends upon the extent to which the root system has been destroyed, on the influence of weather conditions upon plant water requirements, and on the ability of the plant to produce new roots (14, p. 13-15; 68, p. 93).

History and Geographic Distribution

This root rot disease was first noted as a "failing" of strawberry plants in Lanarkshire, Scotland, in the early 1920's. The cuase of the failing was not then known but the disease was recognized as being new and distinguishable from other strawberry diseases recognized at that time (3; 4; 122; 166; 167; 168). "Lanarkshire Strawberry Disease" was the first name used to identify the disease (166; 168).

Wardlaw (168) attributed the disease to unfavorable soil conditions such as acids, lack of humus, bad drainage, and too heavy a soil type, causing poor root systems and starved plants susceptible to fungal and other root diseases. However, he did find a Pythium

species which in inoculation experiments was capable of producing the disease. He thought the problem was more agricultural than mycological. Fungal attack was only an important accessory to the main problem, which was health of the plant.

O'Brien and M'Naughton (122) disagreed with Wardlaw and pointed out that the disease could be serious even on good soils, well manured and well drained. They suggested that the disorder was due primarily to parasitism by endotrophic mycorrhiza.

Alcock (2; 3) found a pathogenic Phytophthora species, identified by oospores and sporangia, consistently associated with this new disease. The Phytophthora was thought to be a new species, and the disease then was often known as the Phytophora Disease of Strawberry. Alcock proposed the name "red core root" for the disease. This was soon shortened to "red core" and today this name is commonly used for the disease in many countries.

Red stele disease was not observed in strawberry fields in England until 1935 when it was reported by Wormald (180) and Hickman (68, p. 90). However, Hickman (68, p. 90) states that Alcock in 1931 and 1932 found Phytophthora in strawberry roots sent to Scotland from England. The disease has since been reported from many localities in England (120), and is now common throughout the British Isles.

A "black stele" root rot, similar to the Lanarkshire disease of Scotland, was recognized in the United States by H. W. Anderson in Illinois in 1930 (8, p. 412) and reported in 1935 (6). Demaree and Darrow (39) report that E. J. Anderson found the same disease in Maryland in 1935. One farmer in Maryland is reported to have observed the disease regularly since 1925 (39).

Red stele was soon found in other states, and has now been reported in nearly all the states included in the triangle bounded by Maine, Arkansas, and Wisconsin, as well as in Colorado, California, Oregon, and Washington.

The disease is found in Canada in the Fraser Valley (25) and Vancouver Island (99; 100) of British Columbia, in New Brunswick (27), the Niagara peninsula (28), and Nova Scotia (26).

In New Zealand red stele of strawberries apparently is an old, well-established disease (12, p. 1; 147; 177), although positive identification was not reported until 1959 (147). It has recently been reported in South Australia (90). Other countries in which the disease has been recognized are France, where it is called apoplexy (121; 156), Austria (142), and the Pushkin District of Russia (105) where it is called a blight of strawberry.

Anderson (6), the first United States investigator to report the disease, used the name black stele root rot. Terms used by

succeeding investigators included red core, red-core root rot, black stele, brown stele, brown-stele root rot, Phytophthora disease, brown core and similar designations. Demaree and Darrow (39) proposed the name red stele as more descriptive of the disease. This name was used in all their and other United States Department of Agriculture publications on the disease. State experiment station investigators followed this example and "red stele" is the common name now used in the United States and Canada. Red core, the term developed in Scotland and England, is used throughout most of the rest of the world. In this thesis the designation red stele will be used.

Nomenclature and Morphology

Bain and Demaree (13) in 1937 isolated from red stele-diseased roots a fungus which they identified as a Phytophthora, possibly a new species. Hickman in 1938 isolated an identical fungus in England and in 1940 described it as Phytophthora fragariae n. sp. Proof of pathogenicity, using pure cultures of the fungus, was demonstrated by Bain and Demaree in 1938 (13) and Hickman in 1940 (68, p. 97).

Bain and Demaree (14, p. 12) and Smith (147) showed that the American and the New Zealand red stele Phytophthoras are identical with P. fragariae Hickman. Hickman (72) later presented an

excellent general discussion of the genus Phytophthora and its many species, their distribution, host range, survival and spread, and physiologic specialization. Species determinations in Phytophthora are treated by Frezzi (59) and Waterhouse and Blackwell (171). No comprehensive taxonomic treatments have been published since the monograph by Tucker (157) and the species key by Leonian (94). Fitzpatrick (50), Blackwell (19), Bessey (17), and Middleton (107) treat classification problems above the species level.

Excellent descriptions, drawings, and measurements of hyphae, zoosporangia, zoospores, oospores and related structures have been provided by Alcock (2; 3), Hickman (68), Bain and Demaree (14), Smith (147) and McKeen (102).

Dissemination of Phytophthora fragariae

Scottish investigators early reported that red stele root rot was spread by the distribution of infected strawberry plants. Howells (3; 77) traced the disease development in Scotland through distribution of plants, originally obtained in England, from one farm in the Braidwood district to the Lanarkshire district and from there to other areas. In 1922 and again in 1926 the disease reached epiphytotic proportions (166). Hickman observed the disease become important in the Kent, England, area by distribution of affected

runners from field to field. Similar observations have been reported in America (8, p. 419; 40; 153) and New Zealand (147).

Spread of the fungus within a field by movement of zoospores in moving surface or soil water has been reported by Alcock (4), Demaree and Bain (40), Hickman (68), Temple (153) and many others. It has also been suggested by some (3; 68, p. 92; 77; 153) that P. fragariae may be spread during cultivation, by blowing soil, on farm implements, and on the feet of men, animals and wading water fowl.

P. fragariae persists in the soil in a viable condition for a long time, presumably as oospores in infected strawberry roots in the soil (72, p. 7). Hickman (72) has reported survival of the fungus in infected soil, kept free of strawberry plants, for over 13 years.

Conditions for Disease Development

Disease development is dependent upon several interrelated factors, among them being adequate soil moisture, favorable soil characteristics, cool temperatures, and susceptible plants.

In the previous section the importance of soil water in spread of the fungus was noted. Free soil water is also necessary for initial disease establishment. There are many reports connecting disease

severity with wet soil conditions (3; 4; 39; 67; 68; 76; 103; 131; 136; 147; 167). Hickman (68), Hickman and English (69), and Ali (5) stress the necessity of high levels or frequent applications of soil moisture for disease establishment. Dryness is the chief factor limiting disease development in spring or summer (103).

Ali (5), Hickman and English (69) and others have shown adequate moisture levels for high disease incidence must be associated with cool temperatures (from 5-15° C.). Unavailable soil moisture, whether caused by lack of rain, good soil drainage, snow or frozen soil, is a limiting factor in development of disease. Craig (33) has attributed insignificance of the red stele disease in Nova Scotia to cold winter conditions.

It has often been suggested that heavy, compact, poorly drained soils are most conducive to disease development (68, p. 107). However, Hickman and English (69), comparing disease incidence in clay and in sandy soils, demonstrated that, in general, the lighter the soil texture the greater the amount of infection, provided the sandy soils receive frequent applications of water. Better distribution of zoospores in loose soils was suggested as probably accounting for the difference. This agrees with the observations in Scotland that disease was most severe on light rather than heavy soils (3).

Soil pH has been observed to influence amount of infection in

plants growing in the field (68, p. 111-2). Amount of infection has been shown, in pH-adjusted soils to decrease with rise in soil pH to the alkaline side (69). However, both Wooley (179, p. 31-2), and Hickman and English (69), indicate the relationship between disease and soil pH may not necessarily be a strictly limiting factor for the occurrence of P. fragariae since the disease is severe in some soils of high pH (69; 179, p. 31).

Investigators early observed that certain strawberry varieties displayed high resistance to red stele disease (6; 33; 39; 68, p. 106-108; 134; 153). At present no varieties are known which are immune to all races of P. fragariae (29; 31; 115; 137, p. 60). The fungus has been found to adapt itself to varieties which had previously been resistant (135; 152, p. 26; 165).

Hosts

Strawberries were the only known host for P. fragariae until recently. O'Brien and M'Naughton in 1928 (122) thought there was evidence that the fungus could infect other plants such as grasses and clovers. Alcock (4) made many attempts to isolate this fungus from weeds and cultivated plants but never found it and had no reason to suppose that P. fragariae could occur in any other host.

Hickman (68), Bain and Demaree (14, p. 26-7), and McKeen

(102) grew various kinds of plants in infested soils and attempted innoculations of many plants and plant tissues with pure cultures of P. fragariae. They tested common field weeds as well as plants or plant tissues which other Phytophthora species were known to attack. Hickman, and Bain and Demaree, did not infect any living tissue or plant species outside the genus Fragaria. McKeen (99; 101; 103) reported loganberry and Cascade berry both susceptible to P. fragariae and was able to isolate the fungus from loganberry. Bain and Demaree tested four species of Fragaria and found all highly susceptible to P. fragariae.

In 1955 a report from Canada mentions P. fragariae attacks seedlings of strawberry, spinach, tobacco, and tomato grown aseptically and placed on agar cultures of different strains of the fungus (23, p. 37). Whether this means the different seedlings are possible hosts was not stated. Tomato (14, p. 26-27; 102) and spinach (102) have been investigated by others and found not to be hosts.

Other Phytophthoras on Strawberry

There are many references to Phytophthora cactorum Schroet. on strawberry fruit but only a few mention this species on strawberry plants. Beaumont in 1938 found in one location in Scotland

that P. cactorum caused decay of leaf bases and adjoining parts of the crown and wilting of strawberry plants (16, p. 32). Hickman is reported to have isolated P. cactorum from some of these plants (51, p. 5). The root system was not invaded as it is in red stele disease. Fleetwood-Walker (51) used one of Hickman's P. cactorum isolates for nutritional comparison with P. fragariae.

Oyler in 1938 (124, p. 50) reported that an isolate of P. cactorum from heath induced local infection in strawberry.

A more recent report from Canada (23, p. 37) states that when aseptically-grown strawberry seedlings were placed on agar cultures of the fungus some isolates of P. cactorum were more pathogenic than P. fragariae.

Bain and Demaree (14, p. 27) attempted inoculations of strawberry using six species of Phytophthora. All species except P. fragariae failed to infect strawberry roots. P. cactorum was not included in these tests.

Host-Parasite Relations

P. fragariae causes red stele disease in strawberry plants. How it does so is not well understood. This fungus has been found, with certain exceptions (68, p. 105; 99; 101; 103), to invade and cause red stele disease to develop only in young strawberry roots.

It has been suggested (68, p. 105) that P. fragariae is the primary invader, but that decomposition of the root tissues is brought about by secondary invaders. Species of Pythium, Rhizoctonia, Cylindrocarpon, Phoma, Septomyxa, Fusarium and other fungi are commonly found in red stele-diseased roots. Pythium, and less commonly Rhizoctonia, follow P. fragariae very closely in the vascular tissue and are the most persistent contaminants when trying to isolate P. fragariae (2; 13; 68; 167).

Entrance into and growth within a root has been traced by several researchers. Hickman (68, p. 89-118), Wooley (179, p. 33-38) and Goode (62) have published noteworthy accounts. As reported by Goode (62) germinated zoospores of P. fragariae swim about and eventually contact a root. They have been observed to encyst readily on roots of immune, resistant, and susceptible strawberry plants, on strawberry roots killed by boiling water, and on roots of non-host plants, but rarely on wool or fine glass rods. Encystment takes place in the root hair zone and at the root tips. Germination of cysts in the root hair zone is haphazard with germ tubes growing in all directions. At the root tip, however, germ tubes penetrate directly into the tissue. Penetration beyond the epidermal layer occurs only in susceptible strawberry varieties. After penetration into the root tips of strawberry plants susceptible to P. fragariae the hyphae

grow both intra- and intercellularly (62; 68, p. 104-107) through new cells and directly toward the differentiating vascular cylinder within which they are then confined. Goode did not observe anatomical differences in root-tip structure which would explain differences in susceptibility and concludes that resistance to P. fragariae is physiological in nature. The mycelia grow upwards in the pericycle, the phloem, and the central parenchyma, and less frequently in the protoxylem elements. In the older parts of the root, in which secondary thickening has taken place the fungus is confined to the phloem and pericycle. Fungus location there must be purely for nutrient reasons since strawberry roots have a comparatively small amount of phloem tissue in proportion to the amount of xylem and size of the root (117, p. 633).

Oospores are produced close behind the advancing edge of the fungus, are limited mainly to the phloem but also are found in the pericycle (4; 62; 68, p. 104-107; 179, p. 33-38).

P. fragaraie has been reported to extend into the crown only by Hickman (68, p. 105) who identified the fungus in crowns, petioles, and stolons by zoosporangia produced from diseased tissues as well as by isolating pure cultures from petioles. Hickman also reported the fungus travels downwards into healthy roots from an infected rootstock, but only rarely did he observe this and then in one variety

only.

As the fungus mycelium advances so does the reddish discoloration of the invaded tissue. The red pigment is not formed in advance of the fungus, nor can P. fragariae be found in advance of the red color (68, p. 104-7; 179, p. 33-38). Hickman thought the color might be due to some toxic substance produced by the fungus in the roots. Wooley (179, p. 33-38) on the other hand thought it impossible that toxic compounds are formed and translocated in advance of this fungus, killing cells and causing red discoloration. Alcock and Foister (3) state that the reddening of the central cylinder is probably due to the presence of oxidase. Jarvis (80; 83) and Ali (5) report that the pigment may be melanin.

No evidence of plugging by mass action of the fungus, by tylose formation or by waste products resulting from a disturbed metabolism of the host was observed by Wooley (179, p. 69). Wilting of diseased plants was believed to be the result of water deficiency in aerial parts of the plant rather than a toxic reaction. No inhibition or stimulation of respiration of strawberry roots or tomato petioles was noted by Wooley (179, p. 39-43) in experiments testing filtrate from lima bean broth cultures of P. fragariae or autolysates from P. fragariae fungal mats indicating to him that toxins are not produced by the fungus.

Ali (5) in histochemical studies of susceptible and resistant strawberry varieties found that susceptible varieties had no cellulase or lignin while resistant varieties had appreciable amounts of both. The susceptible variety also had less reducing sugar than the resistant variety.

Jarvis, in Scotland, investigated host-parasite relations of physiologic races of P. fragariae with the object of elucidating the disease-resistance mechanism. Preliminary work suggested that retardation or inhibition of the terminal oxidase systems is important and histological investigations showed an increase in phenolic compounds and their oxidation products, melanins, in diseased roots as compared with healthy roots (80). In uninfected roots the terminal oxidase was cytochrome oxidase with little polyphenol oxidase activity.

Growth in culture of different physiologic races of the fungus was indistinguishable except when certain phenolic compounds, including analogues of strawberry root constituents, were added to the media. There were marked differences between the races in growth rate on most of the polyphenol sybstrates (81). However, Jarvis found that even though isolates of P. fragariae have widely differing tolerances to a given set of polyphenols (83) there was no correlation between three strawberry root phenols (80) and resistance to pathogenic races of P. fragariae (82). Differences found appeared

to him to be of little phytopathological significance (83).

McKeen (102) obtained a slight indication that spraying sucrose on the leaves tended to increase resistance of strawberry plants to red stele infection. Vaughan, et. al. (159) found that heavy applications of nitrogen to soil significantly increased the development of red stele. Plants receiving the most nitrogen had the greatest incidence of disease.

Early Scottish and American workers quickly recognized that some strawberry varieties were more resistant than others (4; 9; 39; 76; 168). Breeding programs to develop resistant varieties were initiated in Scotland in 1933 (132; 134), and in the United States in 1937 (163). These programs produced selections suitable for use in red stele-infested areas.

Soon it was observed that after remaining resistant for a number of years these varieties could, under certain conditions, become severely infected. Reid (132;134) and Waldo (164) have reported this loss of resistance in the field. Anderson and Colby (9), and Reid (131), observed breakdown of resistant varieties to occur in very wet, rainy years. Further implications were not made. Since 1948 there has been growing realization of the importance of this phenomenon in relation to breeding resistant strawberries (72). Once a resistant variety becomes susceptible infection may be

severe, root destruction extensive, and the resulting damage comparable with that of the older susceptible varieties (137). It was soon demonstrated that new physiologic races of the pathogen were being developed and that no source of resistance to all races of P. fragariae was available (31; 135; 137, p. 60; 165). Races which developed in the field were observed to have a high degree of specialization, and arose with relative frequency (134). Red stele resistance is now considered only a matter of degree (164). This new development greatly complicates the breeding programs.

Additional evidence of the multiplicity of races was that some strawberry varieties were resistant in some areas but not in others (143). United States, Scottish, and English workers using pure culture techniques soon proved the existence of many races (70; 86; 111).

Today physiologic specialization in P. fragariae is well known. However, it was many years after the disease became important before this phase of the host-parasite relationship became obvious.

The number of races recognized is increasing steadily. In 1950 in the United States two physiologic races were known (143); today five races are reported (29). In Britain three races were known in 1951 (70) while today 12 (72, p. 9) or 13 (115) are

recognized. In British Columbia McKeen has found at least 6 races (102).

Determination of physiologic races originally was performed using several varieties of strawberries grown in infested soils, either in field or greenhouse. Today the method has been refined to use of differential varieties artificially inoculated with pure cultures of P. fragariae, and grown in sand in the greenhouse under controlled conditions for prescribed periods of time (70; 114). Unfortunately, strawberry varieties and selections used in these tests are not standardized throughout the world. Even the numbers of indicator selections vary. In America Converse, et. al. (29), in recent years use five varieties for their determinations while McKeen (102) used 12 varieties. In Britain Hickman has used three and eight indicator varieties (70; 72, p. 9) while Montgomerie has used four and eight varieties (109; 111; 115). A standard international set of genotypes and procedures for determining and designating races is needed (29; 102; 114) to make results obtained in one country comparable with those obtained by another worker in a different country.

A further complication in the determination of races, but of interest in host-parasite relations, is the recent finding of Montgomerie (113; 114) that runner plants from the same clone may

Page misnumbered due to typing error.

not react alike when inoculated with the same P. fragariae isolate. She suggests that this behavior between runners from the same clone may make any permanent classification of physiologic races virtually impossible.

McKeen (102) found no correlation between pathogenicity and morphology of the six races he identified in Canada. Tweedy (158, p. 21) provided some evidence in support of determining races by use of different media. He thought such a method would require less time and probably be more accurate than the present intricate, complicated, space requiring process of inoculating different test varieties. Further support for such a proposal can be inferred from studies of Fleetwood-Walker (51, p. 15) who compared two isolates of P. fragariae and found definite morphological and physiological differences between the isolates. Davies (37) showed some small physiological differences between three isolates. However, the findings of Jarvis (80; 83) would indicate any such systems would need extensive testing before they could be adopted. Physiologic races determined by nutrient requirements might be entirely different than physiologic races as determined by their varietal pathogenic capabilities (83). A rapid method for distinguishing strains of the strawberry red stele fungus has been reported (23, p. 37). Aseptically-grown seedlings of strawberry, spinach, tobacco, and tomato were

placed on agar cultures of fungus isolates. The strains of P. fragariae differed in the extent to which the various seedlings were attacked.

Culture of Phytophthora fragariae

Successful isolation and culture of P. fragariae was not accomplished for many years after the red stele disease was well recognized. Pythium species were consistently isolated from red stele-diseased plants by the early workers (2; 167; 168; 169) and there are some reports that Pythium was the causal agent of the disease (167; 168). An example of the persistence researchers showed in trying to isolate casual organism of this disease is afforded by the reports of Alcock (3; 4) who made more than 4, 000 attempts, using 30 different kinds of media, to isolate the Phytophthora she knew and had essentially proved (2; 3; 4) was the cause of the disease, but was unsuccessful in all attempts. Later Bain and Demaree (13), Demaree and Bain (40), Hickman (67; 68, p. 94) and others (99; 147) were able to isolate the fungus in pure culture, and proof of pathogenicity was demonstrated.

P. fragariae is a comparatively slow-growing fungus (68, p. 95; 147) and selective as to media upon which it can be grown (14, p. 11-15; 68, p. 97-101). Oatmeal, French-bean, lima-bean, and

kidney-bean agars, steamed bean pods and pea decoction are some of the more favorable media, while the fungus grows poorly or not at all on such common laboratory media as corn-meal, potato-dextrose, and malt-extract agars.

Hickman (68, p. 100-101), and more recently Tweedy (158, p. 21) and Felix (48), have reported growth of P. fragariae on certain seeds. Hickman grew the fungus on hemp seeds. Tweedy, testing five races, found that two grew well on hemp seeds in water and three grew not at all. Felix, using one race of the fungus, found growth was best on Austrian pea and barley seeds, but good growth also occurred on wheat, oats, corn, soybean, and lima bean seeds. On seeds of Sudan grass and Chinese cabbage growth was very slow. No growth occurred on hemp seeds in his experiments.

McKeen in 1956 (100) reported autoclaved potato-dextrose agar (PDA) would not support growth of P. fragariae while potato agar, Seitz-sterilized PDA, or PDA prepared by autoclaving the dextrose separately from the potato extract and mixing when cool, would support growth. Testing 12 reducing carbohydrates and 15 non-reducing carbohydrates in the same manner, substituting them individually in place of dextrose, he found the reducing carbohydrates, but not the nonreducing carbohydrates, when autoclaved with potato decoction produced a toxin inhibitory to growth of P. fragariae.

Autoclaving was necessary for this reaction. Similar results were obtained when glycine or three amino acids were substituted for the potato decoction in his experiments.

Optimum temperature for mycelial growth has been found to be between 18 and 22° C. No growth takes place below 4° and sustained temperature of 30° or higher are lethal (14, p. 11-20; 68, p. 100-101; 103). The most favorable pH range for growth is between 6.0-7.2 with 6.8 the optimum. P. fragariae has not been found to grow at pH values below 4.0 and above 7.6 (14, p. 17-18; 37). Tweedy (158, p. 22) found P. fragariae cultures raised pH of solid media, and growth ceased at pH 8.0. He used buffered media to extend the viable period of P. fragariae cultures. On the other hand Fleetwood-Walker earlier (51, p. 5) had found, using liquid cultures, that at the concentrations required to stabilize pH level during autoclaving the several buffers tested had a depressing effect on growth of P. fragariae.

Synthetic culture media supporting good growth of P. fragariae have been reported by Fleetwood-Walker (51, p. 49-87) and Davies (37). P. fragariae, like other species of Phytophthoras (47; 60), is heterotrophic for thiamin alone (51, p. 46) for its vitamin requirement. Preferred carbohydrate sources (37; 51, p. 24, 45-47) were found to be dextrose, fructose, a mixture of the two, sucrose,

maltose and dextrin. Both investigators found nitrogen requirements could be met only by use of yeast extract, casein hydrolysate, or certain amine acids. There appeared to be no correlation between the structures of amino acids and their utilization (37). Fleetwood-Walker (51, p. 74) and Davies (37) have shown calcium in quantities characteristic of macro- rather than micro-nutrients is required for growth of P. fragariae.

Growth of P. fragariae in liquid cultures was found by Fleetwood-Walker (51, p. 5) to be greater in static or "non-shake" than in agitated or "shake" cultures. Mycelial homogenates were found by Davis (37) to be a more satisfactory inoculum for liquid cultures than agar discs cur from colonies of P. fragariae grown on solid agar media. The use of agar discs for inoculum in liquid media was found to have several disadvantages, including high coefficient of variation between replications and an erratic lag phase (sometimes up to 10 days) between inoculation and start of active growth (37).

Waterhouse in 1930 (170), and Blackwell and Waterhouse in 1931 (18), surveyed the literature on asexual reproduction in species of Phytophthora. Sporangium production is affected by several factors, the most important being moisture and temperature. Maximum production of sporangia occurs, in all species, in the presence of free water, while optimum temperature varies with the

species. Type of medium on which the fungus is grown also influences sporangium production, and in some cases oxygen concentration and light intensity are also known to influence this process. Age of a culture is a factor: older cultures often do not produce zoospores. Instead the contents of sporangia are extruded as an unorganized protoplasmic mass (68, p. 101).

Temperatures optimum for sporangial production of P. fragariae are slightly lower than for optimum mycelial growth. Hickman (68, p. 100-101) reports maximum production of sporangia at temperatures of 5-15° C, with numbers of sporangia decreasing with increase in temperature to 20°. Felix (48), however, reported 20° was most favorable for sporangium and zoospore production. Bain and Demaree (14, p. 19) report that sporangium production was most vigorous and abundant at 14°C, slightly less at 18°, fairly abundant at 10° and 22°, and only a few sporangia were produced at 25°C. Confirmation of these findings is found in the experiments of Fleetwood-Walker (51, p. 14), Goode (62) and McKeen (103). McKeen (103) also found sporangia formed at low temperatures released zoospores most rapidly if the termperature was raised slightly.

With P. fragariae, sporangium formation was found by Hickman (68, p. 100-101) to occur best in liquid cultures, no sporangia being

produced on solid media except sparingly so on Petri's solution agar. The type of liquid in which the culture is immersed is important for asexual reproduction in this species. Hickman (68, p. 100-101), Fleetwood-Walker (51, p. 13-14), Hickman and Goode (71), Goode (62) and others report that pond water or soil water stimulates sporangial production much more than the same water sterilized by autoclaving or filtering, while sterile distilled water inhibits production of sporangia. Tweedy (158, p. 21), on the contrary, found sterile distilled water and tap water gave best sporangial induction while pond water was very inferior for this purpose. McKeen (103) reported autoclaved soil water was equally as effective as the same water sterilized by Seitz-filtration. He did not compare these treatments with non-sterile soil water, however rain water and melted snow were excellent for inducing production of sporangia (14, p. 20; 158, p. 20). Goode (62) has investigated pH requirements for P. fragariae sporulation using non-sterile pond water at 13-14°C. She reports the most favorable range to be 6.0-6.8°C.

Pond water and soil leachates have been commonly used to stimulate sporangial production and zoospore release in pure cultures of P. fragariae. However, in Scotland, Montgomerie (114) obtained more consistent results by adding to water a small amount of "Mullard's Nutrient Powder", a preparation for feeding plants grown

in vermiculite.

P. frageriae sporulates well when grown on certain seeds. Hickman (68, p. 100-101) found hemp seed cultures of the fungus placed in Petri's solution produced abundant sporangia at proper temperatures. Felix (48), in experiments using one fungus isolate, found that P. fragariae grown on lima-bean agar consistently failed to induce sporangia. The same isolate grown on seeds which had been autoclaved in tap water then inoculated with the fungus supported abundant sporangial production. Many sporangia were produced by cultures growing on sunflower, squash, pea and soybean seeds. Fewer sporangia were formed by cultures growing on seeds of barley, oats, wheat, Austrian pea, corn and Chinese cabbage.

Sporangial production of P. fragariae in vitro has been found to be inconsistent, erratic, and unpredictable. Montgomerie (109) studied effects of light, temperature, aeration and substrate on sporangial production. She reports clear results were not obtained owing to the large variation within treatments. Fleetwood-Walker (51, p. 6-7) and Davies (37) preferred using zoospores rather than mycelium as inoculum for their nutrition studies of P. fragariae. However, they found zoospore inoculum to be impractical since a sufficiently dense suspension of zoospores could not be produced under aseptic conditions, different isolates showed marked differences in ability to sporulate under identical conditions, and the

sporulation capacity of each isolate varied over a period of time.

Much remains to be learned concerning sporangial production, especially the factors limiting sporangial formation by the several physiologic races.

Sexual reproduction in P. frageriae is but little understood. Location of oospores in roots has been studied by many and the process of fertilization is well described. However, other studies are limited. Although the fungus produces oospores abundantly in plant tissues the ability to do so is much reduced (63), or lost (103), when grown on artificial media. Fleetwood-Walker has observed racial differences in this respect (51, p. 15). Gregg (63) found membranes, such as bean or oat tissue fragments or artificial membranes such as cellophane, celloidin, or paraffin wax-vaseline mixtures, provided a mechanical stimulus for production of reproductive organs. However, viability of the oospores produced was not tested. Accounts concerning germination of P. fragariae oospores are meager. Presumably the fungus lives through the summer or through other unfavorable periods in the oospore stage. Probably new physiologic races develop through sexual procedures although direct evidence is lacking. Much needs to be learned concerning the factors affecting dormancy and germination of P. fragariae.

Control

Measures to eradicate or, failing this, mitigate strawberry red stele disease have been sought since this disorder was first recognized. The destruction is so great that in strawberry producing areas where the fungus is not present measures to prevent its introduction have received much study.

Control of this disease by improving soil conditions was one of the first practices recommended. Wardlaw (166; 169) suggested avoiding heavy, poorly drained, clay-like soils that are subject to waterlogging or hard-pan formation, which, he believed, were the main factors causing the disease in strawberry plants. Application of these recommendations did not alter the course of the disease. Other researchers investigated cultural methods and it soon was obvious (3) that no marked improvement followed the adoption of any special method of cultivation.

In more recent times cultural practices have been shown to decrease red stele symptoms and reduce severity of the disease (44; 56; 58; 66; 118; 147; 179, p. 61). The most successful practices have been deep cultivation to loosen soil and improve drainage (147), and growing strawberry plants on the tops of ridges plowed in the field (44; 56; 58; 66; 118). Improved cultural methods appear to

reduce disease severity only slightly and, while they may be desirable, will not eliminate red stele disease. They may be of practical value when used in conjunction with other control measures.

Chemical control of the disease has been disappointing. Early investigations were reported by Alcock, et. al. (3; 4). Temporary control was obtained by spraying plants with lime-sulfur, while a partial control was achieved by soil sterilization with cresylic acid. In Russia spraying with one percent Bordeaux at the beginning of bud stage has been recommended (105). Control of the disease with Nabam (disodium ethylene bis-dithiocarbamate) was claimed in 1951 in Connecticut (150, 151), but attempts to duplicate the results have been unsuccessful (30; 57; 179, p. 57-60). Wooley (179, p. 44-60), Jeffers (87), Lembright (92), and Converse (30) have reported investigations with several chemicals. Apparently no material applied to the soil or to the plants themselves provides adequate control of the disease. However, techniques used for field-testing chemicals have been questioned (30) and some promising materials may have been erroneously evaluated.

Because 90 percent of strawberry roots have been reported to be in the upper six inches of soil (dry weight basis) (15) it might seem cultural or chemical methods should provide an adequate control. However, strawberry roots extend as deep as 40 inches (65).

Wooley (179, p. 64-66) has noted that the fungus occurs to a depth of at least 20 inches. Therefore the possibility of eradicating the fungus from the soil by presently-known chemical or cultural treatments is very slight.

Rotation of strawberries with other crops will not free a soil from the red stele fungus within a reasonable period of time. At the present time use of resistant varieties appears to be the only practical method for control of strawberry red stele disease.

Early surveys resulted in the discovery of two major sources of resistance. In Scotland resistance was obtained from an obscure, highly resistant variety known as Number 52, probably a strain of the Frith variety (132). In the United States the variety Aberdeen was found to have a high degree of resistance (7; 84). Combining these sources yields progeny with resistance higher than either parent alone (132; 162). Resistance is inherited as a dominant character although probably more than one genetic factor is required to achieve a high degree of resistance (84; 132). Other sources of resistance are being investigated. Certain clones in Fragaria chiloensis Duchesne, F. virginiana Duchesne, and F. ovalis Rydb. have shown high degree of resistance (30; 31; 115; 163; 164).

Although commercially acceptable resistant varieties have

been developed, the need for new varieties continues. Development of new physiologic races of the fungus (132; 164) as well as the threat of spread of existing races requires that new varieties, resistant to these races, be continuously available. No source of resistance to all races is available (135) and there are no grounds for expecting that commercial varieties immune to all races of P. fragariae will be obtained (137).

In Oregon selections originating from F. chiloensis and F. ovalis have been found to have remarkable vigor as well as red stele resistance (164). F. chiloensis especially, when crossed with standard varieties, yields progeny with remarkable vigor. Some of these selections, when infected with P. fragariae, have only part of the root infected. The plants put out new roots rapidly in the spring and in fertile soil with ample moisture grow as rapidly as fully resistant selections (164). Similar results are reported from Scotland (138) where such plants grow satisfactorily in the field for years despite the infection. Instead of complete immunity, ability of a selection to live with red stele, known as "field resistance" or "survival value" perhaps will become the object of breeding.

Testing for red stele resistance in the strawberry breeding programs originally was done in naturally-infested field plots. Field testing is still retained for selections found resistant. In order to

screen more seedlings at younger ages and to provide more unifrom soil, moisture and temperature conditions, testing for resistance was moved into greenhouses. Techniques used or evolved in the various systems have been abundandantly described (41; 69; 70; 71; 85; 114; 133; 138; 143; 152).

It was obvious to early researchers that an important means for preventing losses caused by red stele would be by avoiding the disease. Accordingly it has been recommended that red stele-free planting stock be used on land free of P. fragariae (4; 14, p. 28; 68, p. 114; 76; 147). To aid growers in obtaining disease-free plants, programs for inspection and certification of strawberry nursery stock have been instituted in many areas (24; 34; 43; 44; 54; 55; 96; 106 160). Methods for eliminating the red stele fungus from valuable strawberry breeding stocks have been worked out (1; 160).

Hot-water treatment of infested plants was found ineffective since the pathogen proved slightly more heat tolerant than the plants (14, p. 28). Chemical treatment of diseased plants is not reliable (30; 87). It is therefore essential that red stele-free stock be used on P. fragariae-free land.

P. fragariae has been reported to exist in land not previously planted to strawberries (22, p. 6; 77; 88; 179, p. 69). The need for

a method of testing a field for presence or absence of P. fragariae before going to the trouble and expense of planting the whole field to certified disease-free plants is obvious.

Legislation prohibiting the importation of red-stele-diseased plants has been enacted in several European countries where the disease has not been found (42; 53; 128). Restrictions against spread of the disease have been recommended by the European Plant Protection Organization (174).

Certain soil fertilizer and organic matter applications have been found to markedly alter the development of red stele disease. Heavy applications of stable manure (3) and nitrogen fertilizer (159) increased incidence and promoted spread of red stele disease. Straw mulch incorporated into a high nitrogen soil caused reduction in available nitrogen and increase in severity of P. fragariae (89). A four-inch mulch of Douglas fir sawdust applied to plots of strawberries was found by Vaughan, et. al., to increase the incidence of P. fragariae. Sawdust incorporated into soil also raised the percentage of plants infected. Extension of the disease was attributed to a decrease in soil temperature and an increase in soil moisture content (159). On the other hand Aspitarte (10, p. 64-70, 90), working with Douglas fir bark materials, has reported red stele disease of strawberry was decreased with additions of hot

water-extracted bark and with various ammoniated bark products.

PHYSIOLOGIC SPECIALIZATION OF PHYTOPHTHORA FRAGARAIE IN OREGON

Until recent years the commercially important varieties of strawberries grown in Oregon have been susceptible to red stele disease. The red stele-resistant variety Siletz, released in 1955 (165) has now replaced susceptible varieties in many red stele-infested soils. At the time of release the Siletz had not shown evidence of susceptibility to the race or races of P. fragariae present in Oregon (165). The following year Siletz plants in a field at Salem were severely affected by red stele. In 1960 during the search for a suitable location for field-testing certain Douglas fir bark products a planting of Siletz strawberries was found near Philomath in which the plants were very severely damaged by this disease. Inspection of numerous strawberry fields throughout the Willamette Valley in 1960 and 1961 for red stele disease showed that in a very few restricted areas Siletz plants were severely diseased. It was therefore presumed that for this strawberry variety more than one pathogenic race of P. fragariae is now present in Oregon. In general, however, Siletz plants growing in fields with severely red stele-diseased Marshall, Northwest, or Shasta plants were found to be completely free of red stele disease.

The race or races of P. fragariae pathogenic to Siletz may have

arisen quite recently. The development of new races pathogenic to hitherto resistant varieties has occurred in other areas (134) and was to be expected (165). A knowledge of the number of different races present in a given area, the prevalence of each race, and the appearance of development of new races, is essential to any breeding program for the development of resistant strawberry selections. Also a knowledge of the race or races present in an area and their pathogenecity to different strawberries is an important prerequisite for the intelligent selection of strawberry varieties to be grown in an area. Because incipient red stele infection is hard to detect and strawberry plants are regularly shipped from one part of the country to another, races of the fungus are constantly being moved from region to region. A race originating in one locality is a potential threat to other strawberry growing regions.

Wooley (179, p. 20-26) in 1951-53 attempted to determine the races of P. fragariae present in Oregon. Because of the difficulty of obtaining pure cultures of this organism he attempted race determinations by growing several strawberry varieties in soils collected from several red stele disease areas. He was not able to draw any conclusions (179, p. 26) concerning the number of races of the fungus in the soils he tested, but suggested that positive determination of races in Oregon would have to be delayed until pure cultures

of P. fragariae could be obtained from different locations and experiments conducted under controlled conditions with uniform amounts of inoculum. Determination of races by means of pure cultures is both desirable and feasible and has been recommended by Hickman and Goode (71), Montgomerie (110), and Converse, Scott and Waldo (29).

In the present investigations it was decided that any study of races of P. fragariae in Oregon should be conducted using pure cultures of the fungus. Races could then be determined by inoculating strawberry varieties or selections with standard amounts of inoculum of the various isolates of the fungus.

Isolation and Culture of Phytophthora fragariae

Isolation of P. fragariae is difficult. Although the red stele disease has been recognized since the early 1920's isolation of the causal fungus was not accomplished for many years. Alcock (2; 3) attempted more than 4, 000 isolations using 30 kinds of media but was unsuccessful in obtaining any pure cultures of the causal organism. Bain and Demaree (13) in 1938 and Hickman (68) in 1940 were among the first to isolate the fungus. Since then only a few other researchers have reported isolating P. fragariae. The number of isolates of this fungus in existence is not numerous. As late as 1960

no isolates were available from the western United States, although ten isolates have been reported from British Columbia, Canada (103).

In the present investigation initial attempts to isolate the fungus were not successful. The methods described by Bain and Demaree (13) and Hickman (68), were employed with modifications and the method which eventually was found to be most satisfactory for isolation of the fungus was as follows. Strawberry plants were selected which had new, clean, white roots which had been newly infected. The plants were kept moist and referigerated until they were brought into the laboratory. There they were washed thoroughly and the leaves, parts of the crown, and all old roots removed. The remaining crowns with attached roots were washed in running cold tap water overnight. This kept roots turgid and in good condition for dissecting, and thoroughly cleaned them. P. fragariae isolation attempts were made with short (approximately 1 cm.) root sections carefully selected and prepared so that one end was obviously red stele-diseased while the opposite end was apparently healthy, possessing no red stele symptoms. The sections were surface sterilized in 10-25 percent Clorox or Purex (5.25% sodium hypochlorite) for five to ten minutes or even longer, removed to sterile paper towels and cut with sterile implements into 2-3 mm. lengths. The one or two lengths in which discoloration of the stele was just

barely discernible were selected and the stele separated aseptically from the cortex. The dissected steles were imbedded in water agar in Petri plates and incubated at 15-25°C. Each section was inspected daily with a microscope for appearance of hyphae characteristic of P. fragariae. Sections of agar containing hyphae but not the root tissue were transferred to test tubes containing clear liquid casein hydrolysate media (37; 51, p. 101). Test tube slants of standard Difco lima bean agar (LBA) were also used but most rapid and vigorous growth of the fungus and greatest ease of detection of contaminants was provided in the clear liquid media. The isolates were transferred from the liquid media to LBA slants after it was established that they were not contaminated. Identification of the fungus was checked by hyphal measurements and by production of sporangia in nonsterile pond water or soil leachate.

Young, white, recently infected roots were more satisfactory for isolation of the fungus than older roots. With unusually clean roots it was not necessary to dissect the steles. Also, imbedding dissected steles in a drop of water agar on a cover slip and inverting over a sterile Van Tiegham cell partially filled with sterile water (13) was not as satisfactory as imbedding the sections in water agar in Petri plates.

Inclusion of 100-200 ppm (w/v) streptomycin nitrate in the

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media, before autoclaving, for inhibition of bacterial contaminants was not beneficial. In such a media P. fragariae hyphae grew out from the dissected steles, then lost their granular constituents and decomposed.

Eckert, et.al. (46) reported that use of pimaricin, penicillin, and polymyxin overcame the difficulty of isolating Phytophthora species from infected citrus roots by controlling the fast-growing or antagonistic secondary fungi and bacteria. Pimaricin was active against a large number of non-oomycetous fungi but it did not inhibit the growth of sporulation of ten Phytophthora and four Pythium species (P. fragariae was not included in their tests). Penicillin and polymyxin were reported non-inhibitory to Phytophthora but were effective against the majority of soil bacteria.

Experiments were conducted using pimaricin, penicillin G, and polymyxin B·H_2SO_4 at 50 and 100 ppm (w/v) and terramycin at five ppm. Pimaracin, pimaracin and terramycin, terramycin, pimaracin and penicillin, or pimaracin, penicillin and polymyxin were added to water agar after the media was autoclaved but before it solidified (40-45°C.). Dissected strawberry root steles prepared as described above were imbedded in the media in Petri plates. Microscopic examination revealed that pimaracin and terramycin, either alone or in combination with other antibiotics, inhibited fungal growth,

especially of P. fragariae, from the steles. Typical P. fragariae hyphae either did not develop from steles or if they did soon lost their granular constitutents and decomposed, while hyphae of P. fragariae as well as contaminating fungi developed readily from steles in untreated water agar. Inclusion of the antibacterial antibiotics reduced but did not eliminate bacterial growth from the steles. Fungal contaminants were more of a problem in isolating P. fragariae than were bacterial contaminants.

Because the antibiotics, at the concentrations tested, proved to be inhibitory to P. fragariae their use was discontinued. The most important factor in isolation of P. fragariae was the selection of freshly infected roots in which the fungus was in an actively growing condition.

In the spring of 1960 six isolates of P. fragariae were obtained. One to two months after isolation a grey bacterium developed in four of the cultures. Attempts to free the fungus from the contaminant failed and only two of the isolates were maintained. During April, May, and June of 1961 several more cultures of P. fragariae were isolated, but again some were lost to the same contaminant. The final collection consisted of the following 14 isolates of P. fragariae from five fields and four different strawberry cultivars (Table 1).

Table 1. Isolates of P. fragariae collected in the Willamette Valley.

Code Number	Year Isolated	Host	Source
8-E	1960	Puget Beauty	Plants grown in greenhouse in soil collected from Lewis-Brown Horticultural Farm, Linn County, Oregon.
9-A	1960	Marshall	Same as 8-E.
2FS-C 2FS-D 2FS-Z	1961	Siletz	Faxon farm, south of Philomath, Benton County, Oregon.
23-A	1961	Northwest Clone 10	Isolated from plants in a shipment of Washington State Certified Strawberry Plants.
25-C 25-D 25-E 25-F 25-G 25-J 25-I	1961	Marshall	Ticknor farm, near Gresham, Multnomah County, Oregon.
27-B	1961	Marshall	Greenhouse, Oregon State University. Plants grown in a mixture of soils from Marion, Clackamas and Linn Counties, Oregon.

Propagation of Test Varieties of Strawberries

Pathologic races of P. fragariae are recognized by their ability to infect various indicator selections or varieties of Fragaria. No two investigators, however, have used the same set of differential or indicator varieties. This greatly complicates the problem of determining races since results obtained by one investigator with one set of varieties or selections cannot be compared with results obtained by other researchers with other test varieties.

In the present investigations the five indicator varieties used by Converse, Scott, and Waldo (29) at Beltsville, Maryland, the four varieties used by Montgomerie (112) in Scotland, and the Siletz, a variety becoming commercially important in Oregon and known to be resistant to some races of P. fragariae were used. Dr. E. K. Vaughan, Oregon State University, in 1960 kindly supplied a number of Maryland 683 and Stelemaster plants. Early in 1961 Dr. R. H. Converse, Plant Industry Station, U.S. Department of Agriculture, Beltsville, Maryland, provided a few plants each of the varieties Aberdeen, Blakemore, Del Norte, Maryland 683, and Stelemaster, which he had used to determine races, as well as Perle de Prague, Auchincruive Number 11, Climax, and Aberdeen, the varieties used by Montgomerie. Mr. G. F. Waldo furnished several plants of

Del Norte, a selection of F. chiloensis.

Several of the varieties or selections formed stolons and runners profusely. Others, especially Maryland 683, had not formed any stolons in the greenhouse at Oregon State University. A major problem, therefore, was to find a method to induce or increase stolon production.

Thompson and Guttridge (154) and Parlingis and Boynton (126) reported gibberellic acid (GA) induced stolon production in plants growing in natural short day lengths in autumn. Went (173, p. 131), working with Marshall strawberry plants found runners were formed only in long photoperiods at temperatures above 10°C. or as an effect of such treatments. He reported runners first appeared in his experiments one month after beginning of the long photoperiod. In his experiments no runners were produced in short day treatments at any temperature.

In December, 1960 large, mature Stelemaster and Maryland 683 plants were given one or two foliar sprays at two week intervals using 100, 250 or 500 µg GA per plant. Some plants in the greenhouse were exposed to natural daylight and fluorescent light supplemented with incandescent light, so that a twelve hour light period was followed by four hours of dark, four hours of light, then four hours of dark, for a total photoperiod of 16 hours daily.

Some plants of each variety or selection were placed outdoors in cold temperatures for one month while others were left in the greenhouse, exposed to either the long photoperiod or the natural short winter photoperiod. After at least one month exposure the plants were brought back into the greenhouse, repotted and fertilized frequently with a complete fertilizer (Rapid-Gro). Some plants were exposed to long photoperiods and others to natural short day lengths.

All plants which had been held outdoors under cold conditions had ceased growth and were dormant. On transfer to the greenhouse they resumed growth, were very vigorous, and eventually all plants produced many runners. Cold-treated Maryland 683 plants produced runners whether or not they had been sprayed with GA. Plants of several different selections that had been exposed for one month to cold temperatures produced a greater number of stolons than similar plants held in a greenhouse and not exposed to cold temperatures. Some selections, such as Maryland 683, produced runners only following cold-treatment. Repotting plants following cold treatment, and exposure to long photoperiods either increased number of runners produced or accellerated the initiation of runners.

Thirty to eighty plants each of Maryland 683, Perle de Prague, Auchincruive Number 11, Climax, Aberdeen, and Del Norte are now

available. Certified Blakemore, Stelemaster, and Siletz plants can be purchased commercially so greenhouse increase of these varieties is not necessary. After one more season the number of plants available should be sufficient to allow race determinations with isolates now on hand.

A New Method for Identifying Races of Phytophthora fragariae

In the present studies a new technique for determining races of P. fragariae was investigated. Aberdeen, Siletz and Northwest plants were grown in sand in tin cans 10.5 cm. in diameter and 17.5 cm. tall, lined with polyethylene bags (Figure 1). One corner of the bag was pulled through a hole at the bottom of the can and cut off, allowing the can to drain. A glass wool mat at the bottom of the can prevented loss of sand.

When several roots six to eight inches long had formed the plants were carefully removed from the sand and replanted in glass tubes (Figure 2) one-half inch in diameter and seven inches long. Each tube was fitted with a glasswool plug in the bottom and was wrapped with rubber bands near the top and bottom so that tubes held together in a bundle would not touch each other but would be separated by a 1-2 mm. gap. Three or four roots were inserted in each tube and the tubes filled to within one-half inch of the top with

Figure 1. Strawberry plants growing in sand in containers lined with polyethylene bags.

Figure 2. Strawberry plants grown for one month in sand in 1/2 in. by 7 in. glass tubes.

washed sand. Several tubes were required for each plant, the number of tubes depending upon the number of roots on the plant. Tubes containing the roots of each plant were held together with rubber bands. Each bundle was then wrapped with black polyethylene to exclude light from the roots. It had been found that roots grown in the light developed a purple pigemntation while those grown in the dark remained white.

When the strawberry plants were to be inoculated with P. fragariae a rubber stopper was inserted in the bottom of each tube and the tubes were filled with distilled water or sterile soil leachate to within 5 mm. of the top. Five agar discs from three-week old cultures of P. fragariae on LBA were added to each tube. Only one race was added to any one tube. The four races A-1, A-2, A-3 and A-4 used in these experiments were kindly supplied by Dr. R. H. Converse, U. S. Department of Agriculture, Beltsville, Maryland. The inoculated plants were incubated in a 10-15° chamber and additional distilled water or soil leachate was added as required to keep the discs covered with liquid. After two or three days the rubber stoppers were removed, and after a week the plants were moved to a 15-25°C. greenhouse. The plants were watered twice daily with tap water and once a week were irrigated with dilute Hoagland's solution.

After three weeks the roots were inspected for red stele development.

It was hoped that by using this method racial identity of several isolates of P. fragariae could be determined, and at the same time the strawberry plants used in this identification could be checked for response to several known races of the fungus. If all roots of a plant were not inoculated, plants could be used again later by putting them back in sand and letting sufficient new roots develop for future race determinations.

With modification the above technique could be adjusted for uses other than for race determinations. Influence of different inoculum levels, influence of various known races on different commercial or new red stele-resistant selections, host nutrition studies, etc., could be studies. This method would provide economy of plants as well as economy in time spent inspecting roots for disease symptoms.

Results of this experiment were suggestive and interesting. No roots on any of the Siletz plants showed red stele symptoms. This was expected since tests by Converse, et. al. (29) show this variety to be highly resistant to all four races. With the variety Aberdeen symptoms developed on a few roots in tubes to which race A-3 inoculum had been added although symptoms did not extend for more than one inch in any root. Attempts to isolate P. fragariae from

roots showing red stele symptoms were unsuccessful. Converse, et. al. (29) found Aberdeen highly resistant to races A-1 and A-4, susceptible to races A-2 and A-3. Roots inoculated with race A-2 did not develop red stele symptoms. Roots from one Northwest plant were inspected. This plant had been grown in the tubes of sand longer than the other plants tested and had developed many new white unbranched roots, which, as they developed, were guided out to different tubes and encouraged to grow down in them. This plant had been inoculated with races A-1 and A-3 only. Several of these new white roots but none of the older roots were found to have obvious, advanced red stele symptoms. These symptoms were found only on a few roots in tubes receiving A-3 or mixed A-3 and A-1 inoculum. One check (non-inoculated but flooded with water) root showed typical red stele symptoms. Apparently great care must be exercised in handling of inoculum.

Discs of agar plus fungus, used as inoculum, were periodically removed from the liquid in the tubes and inspected for production of sporangia. Although sporangia were observed their appearance was sporadic and varied considerably between discs and between races. Heavy or dense sporangium production was observed on only a few discs. The races A-4 and A-1 generally produced sporangia most abundantly. Race A-2 produced considerably fewer sporangia

than did any of the other races. These observations may explain why Aberdeen roots were not infected by race A-2 and infected only slightly by race A-3, although the variety is reported highly susceptible to these races.

More discs were cut from LBA Petri dish culture of P. fragariae. These discs were immersed in distilled water, water from rivers, streams, or gravel pits, as well as autoclaved and not autoclaved soil leachate. Discs were incubated in these solutions at 12-16°C. for three days but sporangia did not develop. After incubation for several days on a laboratory bench (20-25°C.) profuse sporangial production was observed to have occurred on many discs in several solutions. No apparent differences could be observed between the solutions tested,

In these experiments it appeared obvious that production of sporangia by the races of P. fragariae needed further investigation before the above-described method for identifying races of P. fragariae could be properly evaluated. Experiments designed to investigate sporangial production of P. fragariae were therefore conducted and are reported in a later section of this thesis.

CULTURE OF PHYTOPHTHORA FRAGARIAE IN VITRO

P. fragariae has been reported (13; 14, p. 11-15; 68, p. 100; 147) to grow on certain commonly used laboratory media but not on others. This subject was thoroughly reviewed in an earlier section of this paper. Media reported favorable for growth of this fungus contain decoctions from lima bean, French bean, oatmeal, kidney bean, bean pods, and immature peas. Media prepared with malt-extract, corn meal, maize, potato-dextrose and certain other materials have been found unfavorable.

Fleetwood-Wlaker (51, p. 49-87), in work later confirmed by Davies (37), reported the development of a synthetic liquid medium which supported excellent growth of P. fragariae. The medium consisted of dextrose, $MgSO_4$, KH_2PO_4, minor elements, organic nitrogen, thiamine, and calcium. The nitrogen requirement was supplied by certain of the amine acids found in casein hydrolysate, such as dl-alanine, l-asparagine, dl-aspartic acid, dl-leucine, l-praline, l-histidine, l-glutamic acid, or dl-serine. No single amino acid, however, produced as good growth of P. fragariae as did the hydrolysed casein. Yeast extract also was a good nitrogen source for growth of P. fragariae (51, p. 22). Preferred carbohydrate sources were reported by these investigators (37; 51, p. 48) to

be sucrose, glucose, fructose, dextrin, maltose, raffinose, and mannose. Glucose, maltose, sucrose and dextrin supported better growth of the fungus than the other materials tested.

Methods of preparing certain media have been found to be important to subsequent growth of P. fragariae. McKeen in 1956 (100) found that P. fragariae did not grow on potato agar autoclaved with any of nine reducing carbohydrates, including glucose, mannose, and maltose. However, the fungus did grow on the potato agar autoclaved alone or with any of 15 nonreducing carbohydrates, including sucrose, dextrin and others. Carrying his investigations further, McKeen found that a toxin or fungistatic substance was formed through the interaction of certain amino acids and a reducing carbohydrate. He found the amino acids dl-alanine, l-(l)-leucine, Bacto asparagine, and glycine when autoclaved with dextrose all produced a substance that was toxic to P. fragariae. Dextrose and l-cystine, when autoclaved together were nontoxic to P. fragariae.

Davies did not have problems with toxin production since he autoclaved the carbohydrate sources separately and added them to the bulk of the medium when cooled. Fleetwood-Walker (51, p. 5), however, autoclaved all constituents together. The fungus grew well even when she used dextrose and either casein hydrolysate or yeast extract, good sources of the amino acids which McKeen found reacted

with dextrose to form toxic or fungistatic substances. She did report that two races of P. fragariae differed in their ability to utilize dextrin autoclaved with the casein hydrolysate (51, p. 97). Davies, using media autoclaved separately then combined, showed three races used dextrin equally well. Tweedy (158, p. 21), using five races of P. fragariae, found that although four races grew poorly on autoclaved potato-dextrose agar, one race grew well. Apparently races of P. fragariae differ in their response to the heat-produced toxins.

In preliminary experiments some races produced dense, compact colonies on agar media while others produced abundant aerial mycelium. Growth form of a particular race also varied with composition of the medium on which the fungus was grown. For example, growth of all races on French bean agar was different from that of the same races on French bean-sucrose agar. Verbal descriptions of the differences are awkward and cumbersome. Use of agar to solidify media also has certain disadvantages. Agar contains inorganic elements at a concentration sufficient to influence growth (95; 97; 130). It is a complex polysaccharide sulfate ester (127, p. 637) and has physiologically active impurities (38, 95, 140). Several fungi make limited growth on water agar (130; 175). Measurements of colony diameters are not reliable for comparison of

growth rate of different races on various media. Liquid media have the advantage of allowing accurate measurement of fungus growth by mycelial dry weights. For these reasons, liquid media were used to investigate certain factors influencing the growth of P. fragariae.

General Procedures

General methods are presented below. Techniques peculiar to a particular investigation are given during the discussion of that study. Isolates of P. fragariae used were the races A-1, A-2, A-3 or A-4 as determined by Converse, Scott and Waldo (29) and kindly furnished by Dr. R. H. Converse, U.S. Department of Agriculture, Beltsville, Maryland.

Media. Media used in these studies were potato-dextrose broth (PDB) and a synthetic medium, "medium HC" developed by Fleetwood-Walker (51, p. 49-87, 101) and later used by Davies (1959).

PDB was prepared as follows: Infusion from 200 grams of potatoes, brought to 500 ml. volume with distilled water, was dispensed into culture tubes, 10 ml. of solution per tube, and autoclaved. Twenty grams of dextrose was dissolved in 500 ml. distilled water, dispensed into separate culture tubes, 10 ml. of

solution per tube, and autoclaved. After autoclaving, the media were cooled to 35°F., then a tube of the dextrose solution added to each tube of potato infusion. This procedure avoided toxin production as mentioned by McKeen (100).

Medium HC was composed of: vitamin-free salt-free casein hydrolsate 2.5 gm., glucose 5.0 gm., KH_2PO_4 0.5 gm., $MgSO_4 \cdot 7H_2O$ 0.25 gm., Ca (as $CaCl_2$) 40 mg., thiamine (as hydrochloride) 1.0 mg., Fe 0.2 mg., Zn 0.16 mg., Cu 0.04 mg., Mn 0.02 mg., Mo 0.014 mg., and distilled water to 1,000 ml. The KH_2PO_4, $MgSO_4 \cdot 7H_2O$, calcium, micro-nutrients and thiamine were added from stock solutions stored at 35°F. Fresh thiamine solution was prepared each month. In early experiments the carbohydrate source and the Ca salt were autoclaved separately and added to the bulk of the medium when cold. Thiamine was added to sterile media through sterile filters. Media were adjusted to an initial pH of 6.8, after autoclaving, using NaOH, then dispensed aseptically, 20 ml. per flask, to sterile 125 ml. Erlenmeyer flasks capped with aluminum foil or cotton plugs. In later experiments all components were added together before autoclaving. After the pH was adjusted, the medium was dispensed into Erlenmeyer flasks, then autoclaved. A faint white precipitate formed during autoclaving, redissolved upon cooling of the medium.

Preparation of inoculum: Small discs cut from the edge of an actively growing colony on agar medium were used by Fleetwood-Walker (51, p. 5-6) as inoculum. This technique was reported (37; 51, p. 40) to suffer from a number of disadvantages such as high coefficient of variation between replicates and also an erratic lag phase, sometimes of ten days, between inoculation and the start of active growth. Zoospores, although the desired inoculum, have been reported by both Fleetwood-Walker(51, p. 5) and Davies (37) to be an impractical inoculum. They reported that the isolates they used showed market differences in their ability to sporulate under identical conditions and also the sporulating capacity of each isolate varied erratically over a period of time. A sufficiently dense suspension of zoospores could not be produced under aseptic conditions to provide a standard inoculum (51, p. 6).

Mycelial homogenates, modified after a system used by Davies (37), proved to be a suitable inoculum. Drained mycelial mats from three to five three-week old cultures grown in 20 ml. of medium HC were aseptically suspended in 50 or 80 ml. of sterile distilled water and blended for 10 seconds in a sterilized semi-micro monel metal container (360 ml. capacity) of a Waring Blendor. The resulting homogenate was transferred to a sterile Erlenmeyer flask. Inoculations were made at the rate of 1 ml. of suspension per flask.

In some cases where mycelial homogenates were not used inoculum was 4.5 mm. discs of agar cut from the edge of three-week old colonies of P. fragariae growing on standard Difco lima bean agar.

Incubation and measurement of growth: The fungus was grown in 20 ml. of liquid medium in 18 by 150 mm. or 25 by 200 mm. culture tubes, 125 ml. Erlenmeyer flasks, or other containers described in the individual experiments. They were capped with cotton plugs or aluminum foil or both. All treatments were replicated three, four, or five times, and all cultures were inoculated at 20°C. Non-shake culture was used because of the large number of containers used in the experiments, the number of stock cultures maintained and the length of time cultures were incubated. Fleetwood-Walker (51, p. 6) had reported P. fragariae reacted unfavorably to the conditions of submerged shake-culture. Dry-weight determinations of the fungus mycelial mats usually were made after 21 days incubation. The dry weights were determined by filtering the mycelial mats onto glass fiber filter papers in a Buchner funnel, rinsing with distilled water, drying at either 35° or 100° C. for 24 hours in a circulating air oven, cooling in a dessicator and weighing. When final pH determinations of the medium in which the fungus grew were desired, they were obtained with a glass-electrode pH meter.

Experimental

Heat-treating media: Potato infusion and dextrose solution were autoclaved separately, then combined after cooling. PDB, in 18 by 150 mm. culture tubes, was immersed in water baths or steam chambers adjusted to the following temperatures for the indicated time intervals: none, 37° C for 30 minutes, 60° C for 10 minutes, 60° C for 30 minutes, 100° C for 10 minutes, and 100° C for 30 minutes. PDB, in 25 by 200 mm. culture tubes was autoclaved (121° C., 15 lbs. pressure) for the following periods: none, 5, 10, 15, 20, and 45 minutes. All treatments were replicated five times. After heat-treating, the media were cooled, pH's determined, inoculated, incubated, and fungus mycelial dry weight determined. The results are tabulated in Tables 2 and 3.

Mycelial dry weight of P. fragariae grown in PDB heated at 100° C. for 30 minutes was less than in other media. This reduction, although consistent, was statistically significant only in the one case where race A-4 was incubated 6.5 months before mycelial dry weights were determined. Mycelial dry weights of P. fragariae grown in PDB heated to 60° C. consistently exceeded those in PDB heated to 100° C. or PDB not heated (Table 2). Autoclaving PDB for five minutes or longer (Table 3) altered the medium so that subsequent

Table 2. Mycelial dry weights of P. fragariae cultures grown in PDB given varying amounts of heat prior to inoculation with the fungus.

Treatment *	pH after heat treatment	Race A-1** (each figure average of 5 replications)	Race A-3**	Race A-4**
		(mg.)	(mg.)	(mg.)
Control (no heat)	5.8	9.5	5.5	38.3
37° C 30 min.	5.8	11.3	5.1	42.0
60° C 10 min.	5.8	12.0	4.2	53.4
60° C 30 min.	5.8	12.7	9.5	40.1
100° C 10 min.	5.8	9.5	3.3	29.9
100° C 30 min.	5.7	8.7	3.7	11.2
$LSD_{.05}$		2.7	3.4	15.7

* Size of culture tubes = 18 by 175 mm.

** Races A-1 and A-3 incubated 3 weeks; race A-4 incubated 6.5 months.

Table 3. Mycelial dry weights of P. fragariae cultured in PDB autoclaved for prescribed intervals before inoculation with the fungus.

Time autoclaved *	pH after autoclaving	Race A-4** (each figure average of 5 replications)
(minutes)		(mg.)
0	5.8	17.0
5	5.8	5.9
10	5.7	4.8
15	5.7	7.1
20	5.7	8.4
45	5.5	4.9
$LSD_{.05}$		2.3

* Size of culture tubes = 25 by 200 mm.

** Determined after 3 weeks incubation at 20° C.

growth of P. fragariae was retarded.

Changes in pH resulting from heat-treatment of the PDB (Tables 2 and 3) were not sufficient to account for the differences in growth of the fungus.

One lot of medium HC containing all components was adjusted to pH 6.8 and 20 ml. subsamples were dispensed to 125 ml. Erlenmeyer flasks and autoclaved. The pH after autoclaving was 6.7. In a second lot, the glucose was autoclaved separately and added to the other components after cooling. The pH was adjusted after autoclaving to 6.8 and subsamples were tranferred to sterile 125 ml. Erlenmeyer flasks. All media were autoclaved at one time in the same autoclave to avoid differences in time, temperature or pressure.

The media were seeded with mycelial homogenates of race A-1 of P. fragariae, incubated for three weeks, and mycelial dry weights of the fungus determined. Since mycelial dry weights in medium in which glucose was autoclaved separate from the other constituents were not significantly greater than in similar medium in which glucose was autoclaved with the other constituents of the medium (Table 4), it was concluded that autoclaving glucose with the other ingredients of medium HC did not produce a toxin which significantly inhibited growth of race A-1 of P. fragariae.

Table 4. Effect on growth of P. fragariae race A-1 of autoclaving glucose with and separate from the other ingredients of medium HC.

Treatment	Mycelial dry weight * (means of five replications)
	(mg.)
Glucose autoclaved separate from bulk of medium	45.5
Glucose autoclaved with other ingredients of medium	44.7
$LSD_{.05}$	4.5

* Determined after 3 weeks incubation at 20° C.

In another experiment, involving an extreme period of autoclaving, heat was found to markedly alter medium HC and inhibit subsequent growth of P. fragariae. Medium HC prepared with all constituents, including glucose, incorporated before autoclaving and pH adjusted to 6.8, were dispensed into 18 by 175 mm. culture tubes, 10 ml. medium per tube. These tubes were autoclaved at 15 pounds pressure for three hours. The medium, normally clear as water, had turned dark brown in color and the pH was 5.7, Discs of agar cut from the margins of colonies of the four races of P fragariae growing on LBA were transferred to the tubes. After four weeks, races A-1 and A-3 had produced abundant mycelial growth while races A-2 and A-4 had not grown at all. To confirm these results,

agar discs cut from the edge of three-week-old colonies of the four races of P. fragariae grown on LBA were again added to fresh tubes of medium. The results were again the same. After a lag phase of two weeks, races A-1 and A-3 produced abundant mycelial growth while races A-2 and A-4 did not grow at all. Certain races of P.fragariae are able to grow profusely on medium HC in which all ingredients have been exposed to prolonged periods of autoclaving while other races are totally inhibited by it.

Influence of container size on growth of P. fragariae: P. fragariae grown in the small (18 by 175 mm.) culture tubes grew more slowly and had lower mycelial dry weights than when grown in larger (25 by 200 mm.) tubes. To determine the influence container size or shape has on growth of P. fragariae, an experiment comparing 125 ml. Erlenmeyer flasks with 4 ounce screw-cap prescription bottles was conducted. Medium HC was prepared by autoclaving the glucose and calcium salt separate from the bulk of the medium, adding together when cold, and adjusting the pH to 6.8. This was dispensed in 20 ml. samples into sterile 125 ml. Erlenmeyer flasks, plugged with cotton and capped with aluminum foil, and sterile 4 ounce prescription bottles, capped with plastic screw-caps. These caps were not screwed down tightly. An exchange of air inside and outside the bottles was therefore possible. Mycelial dry weights of

P. fragariae race A-1 were determined after three weeks. Cultures grown in Erlenmeyer flasks were significantly heavier than similar cultures grown in prescription bottles (Table 5).

Table 5. Mycelial dry weights of P. fragariae race A-1 cultures grown in two different containers.

Container	Approx. surface area of medium	Approx. depth of medium	Approx. volume of air above medium	Mycelial dry weights* (means of five replications)
	($cm.^2$)	(cm.)	(cm^3)	(mg.)
Erlenmeyer flasks (125 ml.)	10	0.9	120	49.3
Prescription bottles (4 ounce)	7	1.5	130	35.2
	$LSD_{.05}$			6.9

* After incubation for 3 weeks at 20° C.

Containers of eighteen different sizes or shapes were selected for investigation of suitability for use in these experiments. Medium HC, prepared by autoclaving all constituents together, was used in this experiment. Final pH after autoclaving was 6.8. Marked differences were found in mycelial dry weights of P. fragariae cultured in the different containers after three weeks' incubation. Final ph's, in general, could be correlated with mycelial dry weights

and were closely correlated with container sizes (Table 6). Final pH determinations may be a more accurate measure of growth than mycelial dry weight. Variation in mycelial weights and in final pH's was less in larger containers which had greater exposed surface and where depth of the medium was not great. Small containers such as culture tubes, where the medium was an inch or more deep with little surface exposed to air, had the lowest mycelial dry weights and the lowest final pH values, but had the greatest variation between replicates in mycelial weights or pH values. There were no significant differences in the mycelial dry weights obtained in any of the larger containers. Mycelial dry weights of fungus cultured in culture tubes were significantly less than from the larger containers.

The 125 ml. Erlenmeyer flasks were chosen for future experiments because they largely eliminated variation between replicates in mycelial dry weights or final pH values, were light and fit easily into wooden containers built to fit into an autoclave, seldom cracked during autoclaving, and the opening (mouth) was large enough to facilitate removal of mycelial mats. A disadvantage of these flasks is that they require cotton plugs or aluminum foil caps which are not as handy for repeated opening of the container as are screw cap containers.

Table 6. Influence of size and shape of container on mycelial dry weights of P. fragariae race A-1 and on final pH values of medium.

Description of container	Surface area of medium	Depth of medium	Volume of air above medium	Final pH* of medium	Mycelial dry* weights (average of three replications)
	(approx. cm^2)	(approx cm.)	(approx cm^3)		(mg.)
500 ml. Erlenmeyer flask	16	0.7	545	7.7	40.6
250 ml. Erlenmeyer flask	13	0.8	250	7.8	40.4
125 ml. Erlenmeyer flask	10	0.9	120	7.9	41.0
50 ml. Erlenmeyer flask	7	1.5	50	7.4	45.9
25 ml. Erlenmeyer flask	4	2.7	5	4.6	23.3
8 oz. French square bottle	10	0.9	235	7.6	45.5
4 oz. French square bottle	7	1.5	110	5.0	34.0
2 oz. French square bottle	6	2.0	50	5.0	43.4
8 oz. Round bottle	9	1.0	230	7.5	46.6
4 oz. Round bottle	8	1.2	130	6.1	52.0
2 oz. Round bottle	6	2.0	50	4.8	26.2
1 oz. Round bottle	5	3.0	18	5.1	24.6
8 oz. Prescription bottle	9	1.1	230	7.4	47.6
2 oz. Prescription bottle	6	2.5	42	5.4	42.1
1/2 pint Milk bottle	9	1.0	225	6.8	49.8
25 x 200 mm culture tube	7	5.0	60	4.6	12.2
18 x 175 mm culture tube	5	8.5	15	4.6	10.2
18 x 175 mm culture tube (screw cap)	5	9.0	12	4.6	6.0
$LSD_{.05}$				0.2	10.8

* = After incubation for 3 weeks at 20° C in 20 ml. medium per container.

Effect of IAA upon growth of P. fragariae: Influence of auxins upon growth of P. fragariae has not been reported. Marked inhibition of P. fragariae by low concentrations of auxins might explain why strawberry varieties are susceptible or resistant to different races of the fungus. Two experiments were conducted to determine effect of various concentrations of IAA (indole-3-acetic acid) added to medium HC upon growth of P. fragariae.

Medium HC for these experiments was prepared by autoclaving the glucose and calcium salt separate from the remainder of the constituents and combining when cool. Thiamine was added to the sterile medium through a sterile filter. The medium was adjusted after autoclaving to pH 6.3 in the first, and pH 6.7 in the second experiment. Mycelial homogenates of race A-1 were used as inoculum in both experiments. The normal method of inoculum preparation (General Methods section) was modified to include washing the homogenized mycelia by centrifugation in three changes of sterile distilled water, then resuspending in sterile distilled water.

In the first experiment, inoculum (1 ml. of mycelial homogenate) was added at the rate of 2.1 mg. (dry weight basis) per flask. In that experiment IAA was added to flasks immediately prior to inoculation. Since IAA is insoluble in water, it was added

dissolved in 0.7 ml. of 95% ethyl alcohol per 20 ml. of medium.

In the second experiment 1.4 mg. (dry weight basis) of inoculum were added per flask. IAA was added four days following inoculation. Average dry weight of fungus per flask at the time of IAA addition was 3.7 mg. In that experiment the water-soluble sodium salt of IAA was used (prepared by adding the IAA to an equivalent weight of Na_2CO_3 in a sterile solution).

After incubation at 20° C. for three weeks and four weeks respectively mycelial dry weights of the fungus cultures were obtained. It was found in Experiment 1 that as IAA concentration increased from zero to 10^{-9} M, fungus mycelial dry weights increased (Table 7). This increase was approximately 10 percent over the untreated control. Between 10^{-9} and 10^{-6} M concentrations fungus weights decreased consistently but not significantly. Above 10^{-6} M concentration IAA markedly inhibited growth of P. fragariae.

A similar pattern was observed in Experiment 2. Increase in IAA concentration from zero to 10^{-5} M caused a significant increase in mycelial dry weights of about 14 percent. Above 10^{-5} M concentration IAA almost completely inhibited fungus development (Table 8). It should be remembered that in Experiment 1 IAA was added to the medium immediately prior to inoculation while in Experiment 2 the IAA was added four days after inoculation. Giving the fungus a

Table 7. Effect of IAA added prior to inoculation upon growth of P. fragariae race A-1. Fungus harvested 21 days after incubation. Media initially adjusted to pH 6.3.

Concentration of IAA (added in ethyl alcohol)	Final pH of media (means of five	Mycelial dry weights replications)
		(mg.)
Control-no ethyl alcohol	5.8	89.4
Control-with ethyl alcohol	6.5	64.6
10^{-10} M	6.4	67.0
10^{-9}	6.2	71.8
10^{-8}	6.3	70.3
10^{-7}	6.5	68.5
10^{-6}	6.4	67.2
10^{-5}	5.9	2.9
10^{-4}	6.5	0.8
10^{-3}	6.1	1.5
$LSD_{.05}$	2.7	6.2

Table 8. Effect of IAA (Na salt) added four days after inoculation upon growth of P. fragariae race A-1. Fungus harvested 28 days after inoculation, 24 days after addition of IAA. Media initially adjusted to pH 6.8.

Concentration of IAA (dissolved as Na salt)	Final pH of media (means of five	Mycelial dry weights replications)
		(mg.)
Control-no Na_2CO_3	7.0	49.3
Control-with Na_2CO_3	6.8	47.7
10^{-9}	6.6	51.7
10^{-8}	7.0	48.2
10^{-7}	6.8	51.0
10^{-6}	6.9	51.1
10^{-5}	5.8	55.5
10^{-4}	4.9	5.8
10^{-3}	4.8	5.4
$LSD_{.05}$	2.3	3.9

chance to start gowing, as in Experiment 2, may account for the slight increase in concentration of IAA required for inhibition.

From these studies it is seen that high concentrations of IAA inhibit growth of P. fragariae. Lower concentrations of IAA slightly stimulate growth of P. fragariae. In general, studies of the effect of IAA on growth of fungi have shown that growth is inhibited but only rarely stimulated (139). However, Phycomyces blakesleeanus Burgeff, a fungus closely related to the Phytophthoras, has been reported to be stimulated by IAA at a concentration of 10^{-4}M (139). Other studies using P. blakesleeanus (64, p. 422) report IAA completely inhibited mycelial growth at a concentration of 5×10^{-4}M, and greatly reduced growth at 5×10^{-5} M.

Growth curve of P. fragariae and pH changes during growth: Fleetwood-Walker (51, p. 96) investigated growth rate of P. fragariae and found maximum growth occurred within 21 days at 20° C. in medium HC. After this period growth, as measured by mycelial dry weights, decreased. Inoculum used in her experiments was 4.5 mm. agar discs cut from edges of seven-day old colonies of P. fragariae. In subsequent experiments she, and later Davies (37), used 21 days as a standard incubation period.

During the course of many experiments in the present investigations it was observed that growth rate of P. fragariae varied from

experiment to experiment. In some cases the differences in growth rates were only slight while in others these differences were pronounced and quite obvious. Initial growth following inoculation was often rapid and vigorous while in other cases growth following inoculation was slow. It was thought these growth rate variations were due to variations in concentration of mycelial homogenates used as inoculum.

Therefore, an experiment was conducted to determine the effect on growth of four concentrations of inoculum of P. fragariae race A-1. Medium HC, prepared with all components autoclaved together, was used. The pH after autoclaving was 6.8, Flasks of medium were inoculated with a concentrated mycelial homogenate suspension and with 0.5, 0.1 and 0.01 dilutions of the suspension. Mycelial dry weights of the inoculum suspensions were found to be 3.7 and 1.9 mg./ml. for the original and 0.5 dilutions, respectively. Weight of mycelia/ml. for the 0.1 and 0.01 dilutions could not be determined. The flasks were inoculated with 1 ml. of mycelial suspension, incubated at 20° C. and once a week mycelial dry weights and pH values were determined.

Inoculum concentration markedly influenced the resulting growth rate (Figure 3). The more concentrated the inoculum the more rapid the initial growth, the sooner the growth peak was

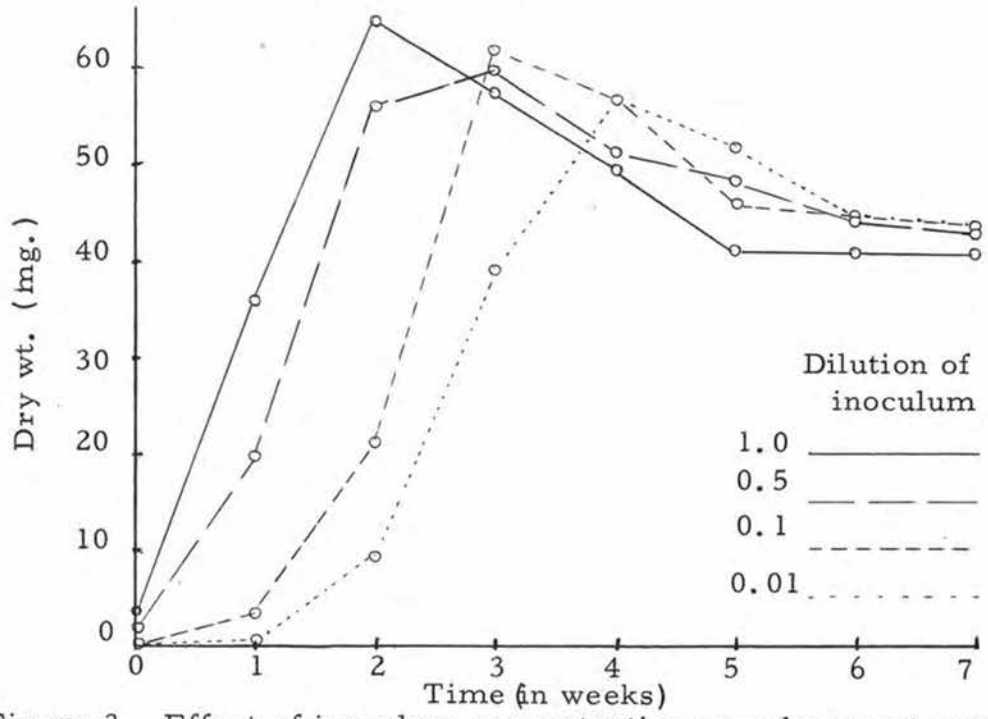

Figure 3. Effect of inoculum concentration on subsequent growth of P. fragariae race A-1.

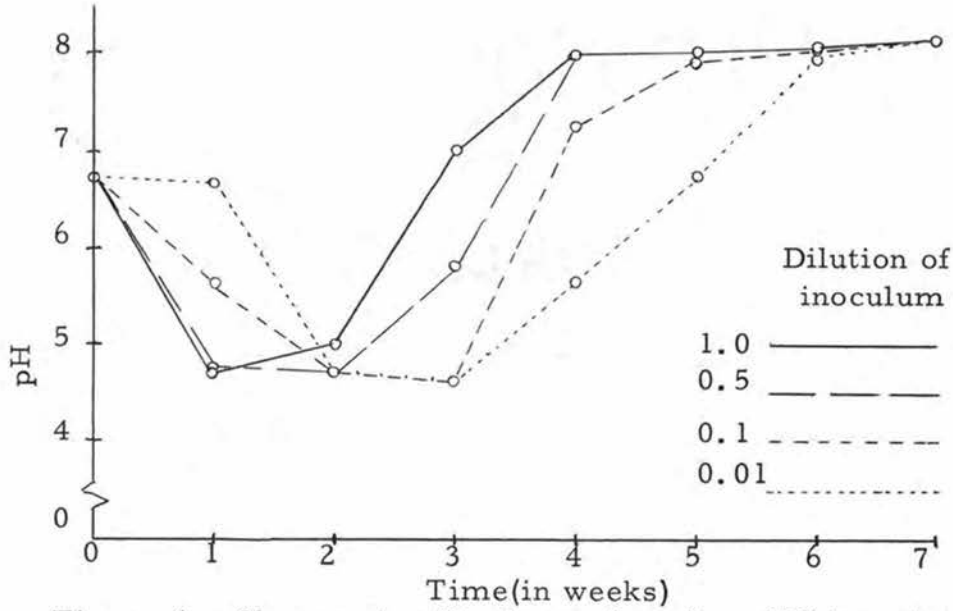

Figure 4. Changes in pH values of medium HC inoculated with P. fragariae race A-1 with four concentrations of inoculum.

reached and autolysis began. Dilution of the inoculum retarded initial growth and delayed maximum growth. In medium HC P. fragariae initially lowered the pH of the medium to a minimum of 4.6 (Figure 4). This was followed by a gradual rise to a maximum of 8.2. Decreasing the inoculum concentration slowed pH changes. Changes in mycelial dry weights of the fungus and pH values of the medium were closely correlated.

PRODUCTION OF SPORANGIA BY PHYTOPHTHORA FRAGARIAE

Factors influencing production of sporangia by P. fragariae are not adequately inderstood. It is well established, however, that sporangia form only under cool, moist conditions. Temperatures optimum for maximum sporulation have variously been found to be 5-15° (68, p. 101), 13-14° (62), 14°(14), 12-17° (103), 15-20° (51, p. 14) and 20° C. (48). Sporangia are not normally formed at temperatures below 5° or above 25° C. (14). Sporangia form more slowly at 5° than 15-20° C., but more are formed at the lower than at the higher temperatures (51, p. 14). Sporangia formed at low temperatures release zoospores more rapidly if the temperature is raised a few degrees (103). Sporangia form readily when infected roots are placed in cool water (2; 13). Pure cultures of P. fragariae growing on solid media sporulate readily when immersed in water. Sporangia have been reported by Hickman (68, p. 100) to occur only in limited numbers on solid agar not immersed in water and then only on agar medium prepared with Petri's solution (for composition of Petri's solution see Hickman, 1940, p. 100).

Waters used to induce sporulation are of critical importance. The literature on this subject is contradictory, however, . Pond waters, soil water, water filtered through soil, Petri's solution,

melted snow, rain water, and tap water to which chlorine and fluorine have not been added have all been reported to stimulate production of sporangia on P. fragariae cultures. Distilled water induced production of only limited numbers of sporangia or of none at all (14; 62; 68, p. 101; 103). Conversely, Tweedy (158, p. 20) found distilled water and tap water enhanced sporangium production more than Petri's solution or pond water.

Sterilization of pond water by heat or Seitz-filtration depresses its ability to stimulate porduction of sporangia (62). Soil water evaporated to dryness and flamed at 400° C. to an ash, then dissolved in distilled water did not stimulate production of sporangia while untreated soil water was very effective (103). Tweedy, however, found sterile distilled water induced more abundant sporangium production than did non-sterile distilled water, rain water, pond water or Petri's solution (158, p. 20). McKeen (103) found sporangia were formed as readily in autoclaved soil water as in soil water sterilized in a Seitz filter, but he did not compare these with untreated soil water. Fleetwood-Walker (51, p. 13) found autoclaved pond water supported formation of sporangia better than pond water sterilized by Seitz filtration. Alcock (2) found P. fragariae-infested root sections immersed in sterile water produced abundant sporangia and suggested that possibly the factor responsible for sporulation is

organic in nature.

Montgomerie (109) studied effects of light, temperature, aeration and substrate on sporangium production, but reported such large variation within treatments that clear results were not obtained. Felix (48) found P. fragariae grown on LBA did not produce sporangia. Several other workers used LBA for this purpose with satisfactory results (51, p. 10; 68, p. 101; 103; 158, p. 10). Goode reported sporangial production occurred most abundantly between pH 6.0 and 6.8 (62). Fleetwood-Walker (51, p. 4) and Davies (37) reported production of zoospores under aseptic conditions was erratic and variable. They were unable to consistently produce sufficiently dense suspensions of zoospores for use as inoculum for fungus nutrition studies.

A better understanding of the requirements of sporangial production is essential before certain other research on P. fragariae and red stele disease can progress.

General Methods

Races A-1, A-2, A-3, and A-4 of P. fragariae (29) used in the present studies were provided by Dr. R. H. Converse, U.S. Department of Agriculture, Beltsville, Maryland. Cultures were grown on Difco LBA at 20° C. for three weeks, at which time agar discs 5 mm.

in diameter were cut from the advancing edge of the colonies and transferred to various liquids for production of sporangia and zoospores. The liquids were contained in sterile Petri plates, 20 ml. of liquid per plate. With this amount of liquid all hyphae and agar were submerged. Petri plates were maintained at temperatures specified in the individual experiments and sporangium production was allowed to occur in the dark. Each agar disc was given a rating of 1-5 depending upon whether there were 0, 1-5, 6-15, 16-25, or more than 25 sporangia produced per disc.

Soil leachate was prepared as follows: One hundred grams of greenhouse potting soil (a soil-peat mixture) was thoroughly mixed with a liter of distilled water. Tap water was not used because chlorine and fluorine are added to the domestic water supply. When soil had settled, the water was clarified by filtration under vacuum through Whatman No. 1 filter paper on a Buchner funnel. Interposing a disc of fiber-glass window screening (145) between the paper and the pierced porcelain plate of the funnel facilitated filtration markedly. This method proved more useful for clarification of soil leachate solution than did centrifuging or allowing the suspension to settle over long periods of time. Soil leachate was prepared fresh for each experiment.

Other liquids tested in the various experiments were as follows:

Mary's River water, collected at Avery Park, Corvallis, Oregon; Oak Creek water, collected near 43rd and Harrison Streets, Corvallis, Oregon; water from Well No. 1, Botany and Plant Pathology Department Farm, Linn County Oregon (no chlorine or fluorine added); humic water from Klamath Marsh, collected from the Williams River at Kirk, Oregon; Hoagland's solution, prepared in distilled water and diluted with distilled water to 1, 10 and 100 percent concentration; V-8 juice, filtered under vacuum through Whatman No. 4 filter paper on a Buchner funnel then diluted with distilled water to final concentrations of 1, 5 and 25 percent; a solution of Superthrive (donated by Vitamin Institute, 5411-15 Satsuma Avenue, North Hollywood, California; contents as stated on label are 0.06% vitamin B_1 and 0.024% 1-napthyl acetic acid per one-half ounce; and dissolved solids 30% of total weight of liquid), prepared by adding two drops of the Superthrive concentrate to 500 ml. of autoclaved distilled water; aquarium water, from an aerated laboratory aquarium containing plant and animal populations; and distilled water. In one experiment, pH of distilled water was buffered with Na_2HPO_4 - NaH_2PO_4 to a final molarity of 0.05. Standard autoclaving procedure was 15 pounds pressure at 121° C. for 15 minutes.

Experimental

Sporulation of P. fragariae races A-1 and A-3 was compared in soil leachate, well water and distilled water. Agar discs of the fungus were examined for sporangium production after incubation at 12° C. for 24 hours. Because few sporangia had developed, the discs were incubated four days at 18-22° C. Sporangia were produced abundantly in soil leachate solution, but only sparingly in distilled water and not at all in the well water (Table 9). No differences between the two races were apparent.

Table 9. Formation of sporangia by P. fragariae races A-1 and A-3 in three liquids.

Liquid environment	Sporangia production ratings *, ** (means of 5 discs)	
	Race A-1	Race A-3
Distilled water	2.0	2.4
Well water	1.0	1.0
Soil leachate	5.0	5.0
$LSD_{.01}$	0.65	0.65

* Incubated 1 day at 12° C. and 4 days at 18-22° C.

** Sporangial production ratings:
1 - No sporangia observed
2 - 1-5 sporangia per agar disc
3 - 6-15 sporangia per agar disc
4 - 16-25 sporangia per agar disc
5 - More than 25 sporangia per agar disc

In a similar experiment using P. fragariae race A-4 (Table 10) sporangia were produced profusely in humic water, soil leachate and aquarium water, moderately in Oak Creek and Mary's River waters, but only slightly in distilled water.

Table 10. Formation of sporangia by P. fragariae race A-4 in six liquids.

Liquid environment	Sporangia production ratings * (means of 6 discs)
Distilled water	1.17
Mary's River water	3.33
Oak Creek water	3.50
Aquarium water	4.17
Soil leachate	4.17
Humic water	4.83
$LSD_{.01}$	1.24

* Incubated 1 day at 12° C.; 3 days at 15-22° C.

In a third experiment, P. fragariae race A-2 produced sporangia equally well in soil leachate, autoclaved soil leachate, autoclaved distilled water, and autoclaved Hoagland's solution. However, sporangia produced in the Hoagland's solution were conspicuously larger and a greater percentage of sporangia remained unopened than in the other solutions. These unopened sporangia had a distinct tendency to branch sympodially or develop hyphae at the terminal end of the sporangium. The sporangia which released

zoospores proliferated normally.

It was apparent that the liquid environment in which P. fragariae cultures are bathed markedly influences sporulation. The results of the first two experiments agree with the reports of Hickman (68, p. 101), Bain and Demaree (14), Goode (62), McKeen (103), and Fleetwood-Walker (51, p. 13-14), but disagree with the report of Tweedy (158, p. 20). Production of atypical sporangia in sterile Hoagland's solution observed in the third experiment has not been reported in the literature.

Results obtained with autoclaved and non-autoclaved soil leachate did not agree with published reports (51, p. 14; 62) that sterilized pond water is less satisfactory for sporulation of P. fragariae than similar water not sterilized.

In a more comprehensive experiment, ability of four races of P. fragariae to produce sporangia was compared in the following liquids: autoclaved and non-autoclaved distilled water; autoclaved and non-autoclaved soil leachate; autoclaved and non-autoclaved 1, 10 and 100% Hoagland's solutions; autoclaved and non-autoclaved 1, 5 and 25% V-8 juice; non-autoclaved Superthrive solution and buffered distilled water at initial pH values of 5.3, 6.3, 6.8, 7.3 and 7.9. Sporangium counts were made 2, 4-5, 6-7, and 9-10 days following immersion of agar discs in the test liquids. Incubation

was at 14° C. in the dark except during actual sporangium counting, when the Petri plates were maintained at 20-23° C. for a short time.

The 22 liquids were dispensed into Petri plates, five plates for each solution. Two plates were seeded with race A-1, and one each with races A-2, A-3 and A-4. Inoculum used throughout this experiment was agar discs of three-week-old P. fragariae cultures, five discs per Petri plate. Data from the two Petri plates of each liquid preparation seeded with race A-1 inoculum were used for determining statistical reliability of this experimental design.

In the above experiment it was shown that different liquid preparations markedly influence sporangium formation by P. fragariae (Figure 5), races vary in their ability to produce sporangia (Figure 6), and, contrary to expectations, autoclaving of liquids did not greatly influence their ability to stimulate production of sporangia (Table 14).

Data from this experiment were subjected to two statistical analyses. In one analysis (Table 11) the four races and 14 autoclaved liquids were compared for influence, if any, upon sporangium production while in another analysis (Table 14) influence upon sporangium production of six liquids, autoclaved and not autoclaved, and differences between races, were investigated. While total sporulation during the 10 day incubation period was greater in soil leachate than

in 100% Hoagland's solution (Figure 5), after 9-10 days of incubation numbers of sporangia produced in the soil leachate liquid were not greater, at the 1% level of significance, than those produced in 100% Hoagland's solution (Tables 12 and 15). Numbers of sporangia produced in 10% Hoagland's solution were significantly greater than in 1% Hoagland's solution (Tables 12 and 15). The other solutions tested, although differing slightly in their influence upon numbers of sporangia produced, did not support abundant production of sporangia (Figures 5 and 6). It should be noted that V-8 juice solutions supported production of sporangia better at low than at high concentrations. No sporangia were observed at any time during the 9-10 day incubation period in the 25% V-8 juice preparation. Vegetative growth of all four races of P. fragariae occurred in 5 and 25% V-8 juice solutions, and was especially heavy after ten days incubation in the 25% V-8 juice solution. Autoclaved V-8 juice appeared to be a good medium for vegetative growth of all races of P. fragariae. Some sporangia were produced in autoclaved, buffered distilled water with lower pH values, but almost none at the higher pH values (Figure 5; Table 12). Addition of buffer in the concentration used in this experiment apparently reduces subsequent sporangium development (Figure 5). Sporangium production in the Superthrive solution was negligible (Figure 5; Table 12).

Race A-4 produced significantly greater numbers of sporangia than the other races (Tables 13 and 16). Race A-1 produced a significantly greater number of sporangia than either race A-3 or race A-2. Race A-3 consistently produced more sporangia than race A-2 (Figure 6) but the differences were not statistically significant (Tables 13 and 16). There was no significant difference in the numbers of sporangia produced by race A-1 in replicates A and B.

No significant difference was found between numbers of sporangia produced after 9-10 days incubation in autoclaved or non-autoclaved liquids (distilled water, soil leachate, 1, 10 and 100% Hoagland's solution, and 1% V-8 juice solution) (Table 14).

The pH values for the 22 liquids tested in this experiment were determined at the beginning of the experiment and again after incubation for ten days with P. fragariae. Original pH values (Table 17) ranged from 4.2 (25% V-8 juice solution) to 7.6 (100% Hoagland's solution). In general, pH rose during the incubation period, except in the buffered distilled water and the 25% V-8 juice solution (Table 18). No correlation between original pH, final pH, and production of sporangia was apparent except that as already noted, more sporangia were produced in buffered distilled water at low than at high pH values (Figure 7; Table 12).

Appearance and behavior of sporangia was found to vary considerably depending upon the solution in which they developed. All races behaved similarly in this respect. Sporangia which developed in all liquids except Hoagland's solution were normal in appearance, behavior and size. The sporangial developed on long sporangiophores soon opened to release their zoospores and developed new sporangia by proliferation or by sympodial branching. In the Hoagland's solution many sporangia developed on short sporangiophores, were larger than normal, and often remained closed, retaining their zoospores. These unopened sporangia branched sympodially or developed germ tubes at the terminal end of the sporangium, or both, while opened sporangia often proliferated in the normal manner (Figure 8). The tendency for sporangia to develop atypically increased as the concentration of the Hoagland's solution increased. Also, differences in appearance of sporangia in Hoagland's and in other solutions became more obvious toward the end of the incubation period. At that time almost all normal sporangia had discharged their zoospores and were empty. Many sporangia in the Hoagland's solutions were full and in the process of developing germ tubes. In the Hoagland's solution swimming zoospores appeared to be as numerous as in other solutions despite the smaller number of opened sporangia. It must be remembered

that more sporangia were produced in Hoagland's solutions than in all the other solutions tested with the exception of soil leachate.

Race A-4 sporangia which had developed in autoclaved soil leachate, autoclaved 100% Hoagland's solution, and non-autoclaved 100% Hoagland's solution were measured. Ten sporangia were measured on each of five discs in each of the solutions. Sporangia formed in autoclaved soil leachate were significantly smaller than sporangia formed in the two Hoagland's solutions. Sporangium sizes did not vary significantly between the two Hoagland's solutions Table 18).

Structures (Figures 9a, 9b, 9c) were observed in race A-1 which were not seen with other races. They were present in all liquid treatments but were most abundant in soil leachate and the 1% and 10% Hoagland's solutions. Occurrence was slightly more common in autoclaved than in non-autoclaved soil leachate. These structures were first noticed after 4-5 days incubation, and by the tenth day of incubation were very numerous. Their identity is not known and pictures or references to similar structures were not found in literature dealing with P. fragariae. It is possible that they originated from sporangia imbedded in agar. Zoospores, released from a sporangium trapped in the agar (for a picture of zoospores trapped in agar see Figure 10) and unable to swim away,

could encyst and germinate. The germ tubes could radiate out and structures resembling those seen in these experiments would result (Figures 9a, 9b, 9c). These structures, peculiar to race A-1, were formed in all liquids including the V-8 solutions. It will be remembered that in the V-8 juice solutions, especially in the 25% solutions, sporangia were infrequently formed. Rosenbaum (141, p. 252, 254) found that Phytophthora areca, P. cactorum, and P. parasitica often released zoospores from the sporangium into a bladder or vesicle as is common in Pythium. These spores did not disperse and swim away, but germinated and developed hyphae. Rosenbaum's diagram illustrating this process (141, plate 75B) shows structures which closely resemble the structures observed in cultures of race A-1. Hickman reports old cultures of P. fragariae often do not produce zoospores. Instead the contents of sporangia are extruded as an unorganized protoplasmic mass (68, p. 101). In the present experiments such masses could have developed hyphae or germ tubes and appear as in Figures 9a, 9b, and 9c. Also, Leonian, in investigations with Phytophthora capsici, has drawings (93, p. 405, fig. 2d) of structures which he called "germinating tuberous outgrowths" which very closely resemble the structures seen in the present experiments with P. fragariae race A-1.

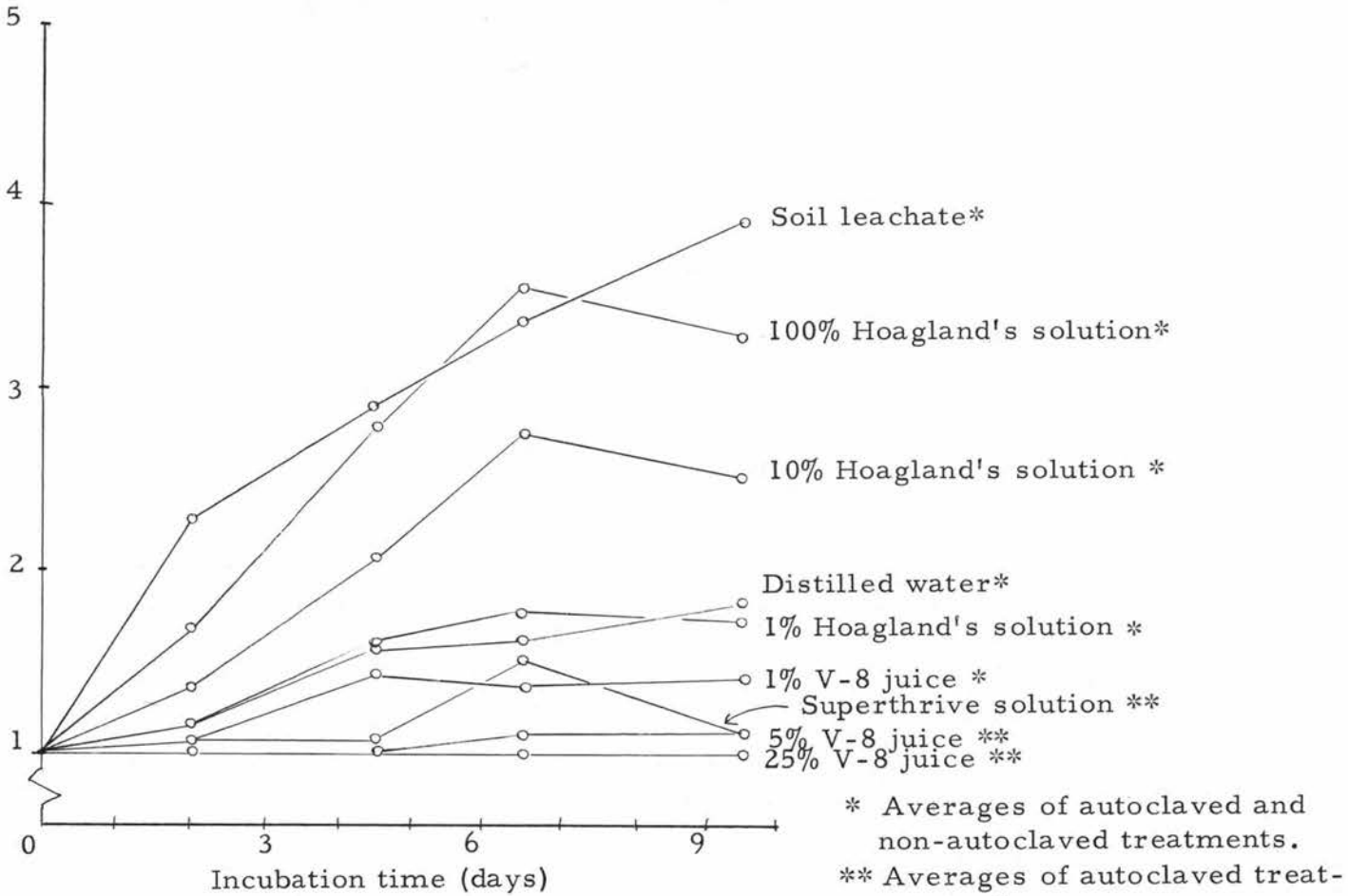

Figure 5. Effect of different liquid media upon sporangium formation of P. fragariae (averages of 4 races) incubated at 14°C. for 9-10 days.

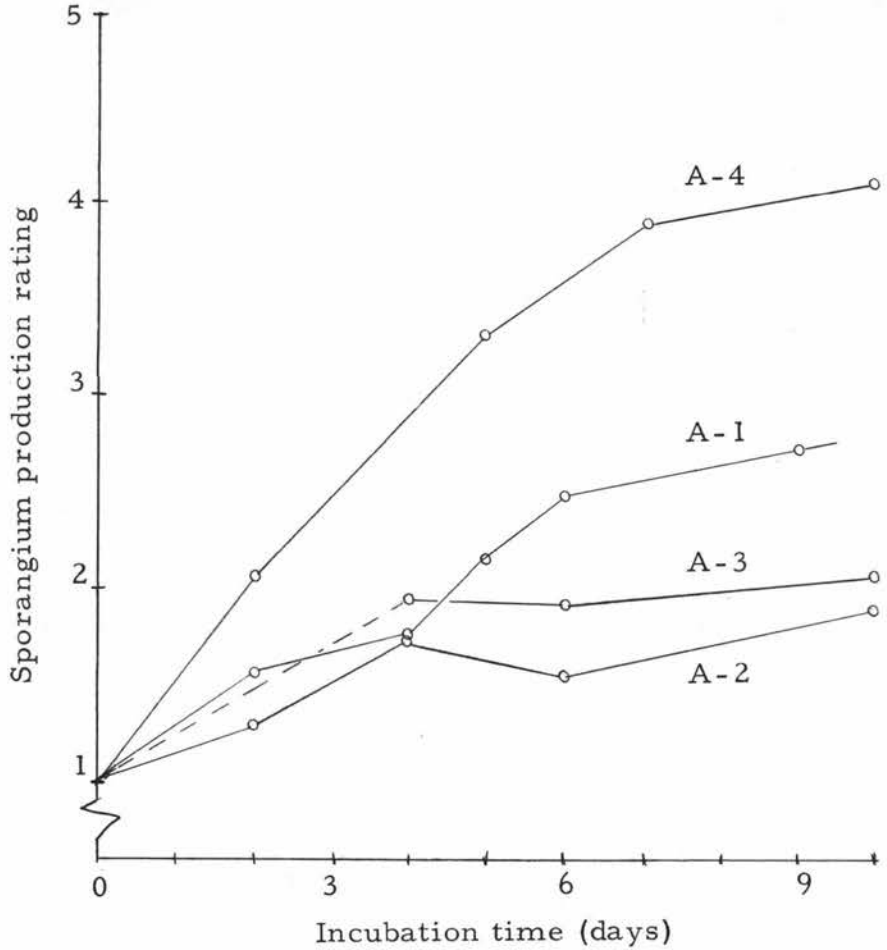

Figure 6. Formation of sporangia by four races of P. fragariae (averages of 5 autoclaved and non-autoclaved liquids) incubated at 14°C. for 9-10 days.

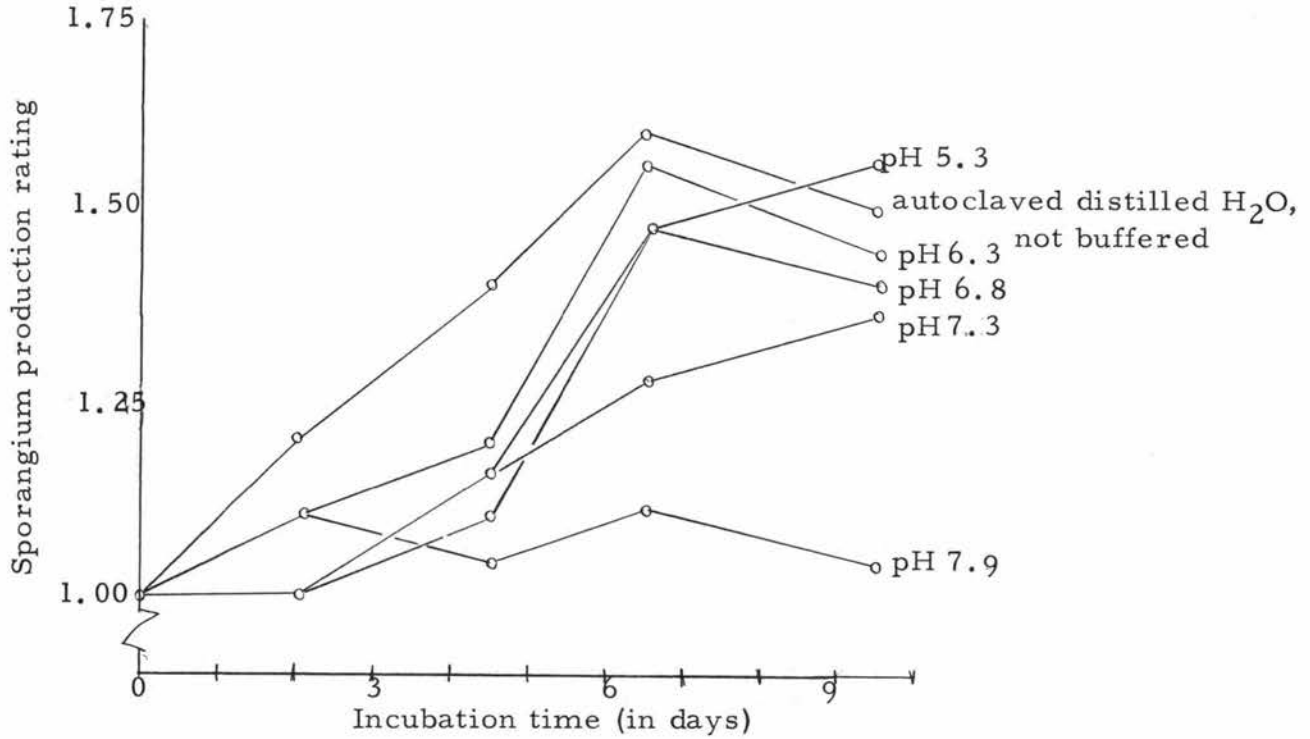

Figure 7. Formation of sporangia by P. fragariae (averages of 4 races) in autoclaved buffered (Na_2HPO_4, 0.05 M final concentration in test solution) distilled water during a 9-10 day incubation period at 14°C.

Table 11. Analysis of variance for Tables 12 and 13.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	397.374	69	5.759	14.92 **
Liquids	261.374	13	20.106	52.09 **
Races	70.617	4	17.654	45.74 **
L x R	65.383	52	1.257	3.26 **
Error	108.000	280	0.386	
Total	505.374	349	1.448	

** Statistically significant at the 1% level.

Table 12. Sporangium production by P. fragariae after 9-10 days incubation at 14° C. in autoclaved liquid environments.

Liquid	Sporangium production rating (means of 4 races)
Distilled water	1.45
Soil leachate	3.83
Hoagland's solution 1%	1.80
Hoagland's solution 10%	2.52
Hoagland's solution 100%	3.52
V-8 juice solution 1%	1.45
V-8 juice solution 5%	1.10
V-8 juice solution 25%	1.00
pH 5.3 buffered distilled water	1.63
pH 6.3 buffered distilled water	1.50
pH 6.8 buffered distilled water	1.43
pH 7.3 buffered distilled water	1.33
pH 7.9 buffered distilled water	1.05
Superthrive solution	1.05
$LSD_{.01}$	0.51

Table 13. Sporangium production of four races of P. fragariae (mean sporulation in 14 autoclaved liquids) after 9-10 days incubation at 14° C.

Race	Sporangium production rating
A-4	2.59
A-1 (replicate B)	1.74
A-1 (replicate A)	1.71
A-3	1.37
A-2	1.34
$LSD_{.01}$	0.27

Table 14. Analysis of variance for Tables 15 and 16.

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	448.9	59	7.61	12.45 **
Liquids	230.8	5	46.16	75.55 **
Races	146.9	4	36.73	60.12 **
Heat	0.2	1	0.20	0.33
L x R	44.8	20	2.24	3.67 **
L x H	8.2	5	1.64	2.69 *
R x H	0.7	4	0.18	0.30
L x R x H	17.3	20	0.87	1.42
Error	146.6	240	0.61	
Total	595.5	299	1.99	

* Statistically significant at the 5% but not the 1% level.

** Statistically significant at the 1% level.

Table 15. Sporangium production of P. fragariae after 9-10 days incubation at 14° C. in six autoclaved and not autoclaved liquids.

Liquid	Sporangium production rating (means of 4 races)
Soil leachate	3.80
Hoagland's solution, 100%	3.39
Hoagland's solution, 10%	2.45
Hoagland's solution, 1%	1.94
Distilled water	1.75
V-8 juice solution, 1%	1.38
$LSD_{.01}$	0.57

Table 16. Sporangium production of four races of P. fragariae (mean sporulation in six autoclaved and not autoclaved liquids) after 9-10 days incubation at 14° C.

Race	Sporangium production rating
A-4	3.70
A-1 (replicate B)	2.62
A-1 (replicate A)	2.38
A-3	1.87
A-2	1.73
$LSD_{.01}$	0.37

Table 17. pH of 22 liquid preparations prior to and after 10 days incubation of P. fragariae at 14° C.

Liquid preparation	Initial pH	Final pH
Distilled water, not autoclaved	6.3	7.5
Distilled water, autoclaved	6.3	7.5
Buffered(0.05M)distilled water, autoclaved	5.3	5.7
Buffered(0.05M)distilled water, autoclaved	6.3	6.3
Buffered(0.05M)distilled water, autoclaved	6.8	6.7
Buffered(0.05M)distilled water, autoclaved	7.3	7.2
Buffered(0.05M)distilled water, autoclaved	7.9	7.8
Soil leachate, not autoclaved	6.9	7.5
Soil leachate, autoclaved	6.8	7.5
Hoagland's solution, 1%, not autoclaved	6.6	7.4
Hoagland's solution, 1%, autocalved	6.6	7.5
Hoagland's solution, 10% not autoclaved	7.1	7.6
Hoagland's solution, 10%, autoclaved	7.1	7.7
Hoagland's solution, 100% not autoclaved	7.6	7.8
Hoagland's solution, 100%, autoclaved	7.6*	7.8
V-8 juice, 1%, not autoclaved	6.7	7.6
V-8 juice, 1%, autoclaved	6.5	7.8
V-8 juice, 5%, not autoclaved	5.0	7.6
V-8 juice, 5%, autoclaved	4.5	7.7
V-8 juice, 25%, not autoclaved	4.2	---**
V-8 juice, 25%, autoclaved	4.2	4.2
Superthrive solution	5.1	7.5

* A white precipitate formed during autoclaving which did not later redissolve.

** During the 10 days incubation all plates became contaminated with other organisms and were discarded.

Table 18. Measurement of P. fragariae race A-4 sporangia developed in three liquid environments during 10 days incubation at 14° C.

Liquid environment	Sporangium measurements (means of 10 sporangia per disc. 5 discs)	
	Widths	Lengths
Soil leachate, autoclaved	25.25μ	36.13u
Hoagland's solution, 100% autoclaved	33.32	52.87
Hoagland's solution, 100% not autoclaved	32.30	49.47
LSD .01	4.98	8.70

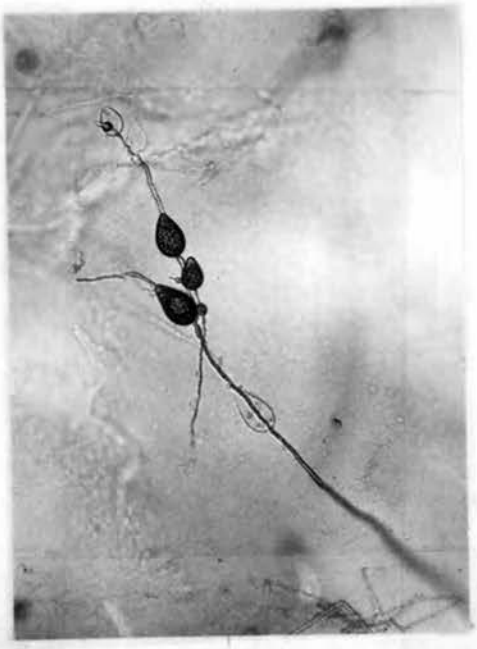

Figure 8. Sporangia of P. fragariae race A-4 formed in Hoagland's solution. Some sporangia are open and proliferate normally while others remain closed and develop germ tubes. X 100.

Figure 9a.

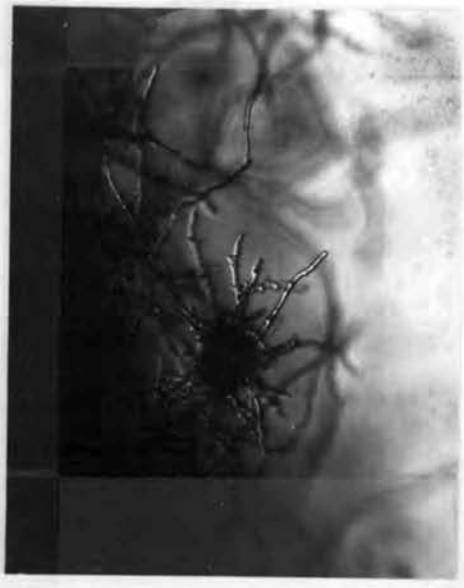

Figure 9b.

Figure 9c.

Figure 9. Structures formed by P. fragariae race A-1 after 4-5 days incubation at 14° C. (a) Several structures. X 100. (b) Hyphae radiating out from a large structure. X 100. (c) Small structure. X 100.

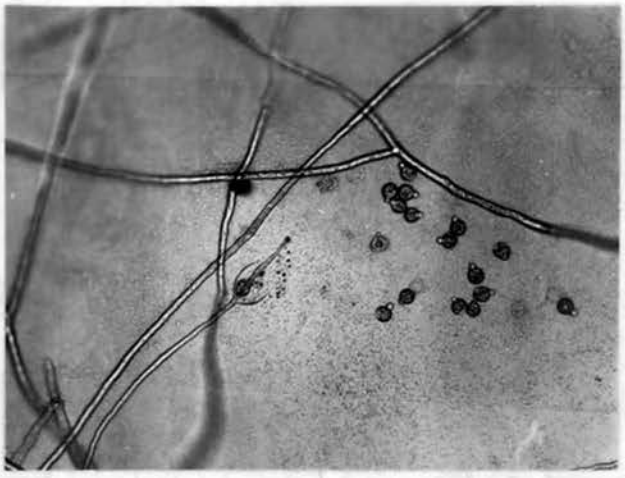

Figure 10. Sporangium and zoospores of P. fragariae race A-4 embedded in agar. X 100.

USE OF DOUGLAS FIR BARK PRODUCTS FOR THE CONTROL OF RED STELE DISEASE OF STRAWBERRIES

Incorporation of various kinds of organic matter into soil has reduced the severity of certain plant diseases (32; 36; 74; 104; 125; 146; 149; 172). On the contrary, stable manure (3; 89) and sawdust (159) incorporated into P. fragariae-infested soil have all been reported to increase incidence of red stele disease. Heavy applications of nitrogen have also been reported to increase severity of red stele disease (159). On the other hand, Aspitarte, investigating the availability of nitrogen in ammoniated bark used as a soil amendment, found certain bark additions gave good control over red stele disease while others were totally ineffective (10, p. 64-70, 90).

Aspitarte's trials were conducted at Oregon State University during the winter and spring of 1957 and 1958. Because of the striking control achieved with some of the treatments it was important to continue observations of the experiment he had started, and to verify the results, if possible, with further experiments using the same and other types of bark products.

Sources and Preparation of Bark Samples

Sources: Bark products used by Aspitarte had been prepared (10, p. 17-23) by the Oregon Forest Products Laboratory and

included whole Douglas fir bark, cork-free bark, wax-free bark, and tannin-free bark, all containing different levels of nitrogen. In the present experiments similar bark samples were again prepared by the same Laboratory, and a large supply of spent tanbark (tannin-free Douglas fir bark) was obtained from the Muir and McDonald Company, Tanners, of Dallas, Oregon. Douglas fir bark used either as whole bark or for removal of tannins or waxes, was obtained at the Larson Lumber Company, Philomath, Oregon. Trees from which the Muir and McDonald Company obtained their bark supply were second-growth Douglas fir, approximately 80 years old. Trees which the Larson Lumber Company were processing and from which bark was obtained were considerably larger and older.

Preparation: The supply of bark obtained from the Oregon Forest Products Laboratory had been prepared in the following manner. Five-hundred pounds of Douglas fir bark, obtained at Larson Lumber Company, were broken into small pieces and passed through a laboratory-sized wood chipper and a John Deere hammermill fitted with a quarter inch screen.

After air drying for several days 40 pounds of ground bark were placed in a pilot plant extractor for 16 hours with 50 gallons of hot benzene. The benzene was drained away and the bark air-dried to remove all traces of the solvent. Three additional batches

of bark, each about 50 pounds air dry basis, were extracted in a like manner. One of these was reserved for treatment with ammonia while the other two were extracted further with hot water. One of the benzene hot water-extracted batches of bark was treated with ammonia.

Hot water extraction of bark was accomplished by adding 40 pounds of air-dried bark to 150 gallons of water in a steam-heated kettle maintained at 180-190° F. and stirred continuously for ten to 15 hours. Water was drained and the bark extracted an additional two to five hours at the same temperature with a fresh 150 gallons of water. The water was again drained and the bark air dried for several days. One batch of hot water-extracted bark was tested with no further treatments; another was reserved for ammoniation.

Barks designated for ammoniation were treated with anhydrous ammonia gas (NH_3) in a pilot plant (10, p. 20-23), using about seven pounds (roughly about 20 percent of weight of the bark) of ammonia per 40-pounds of bark. The ammonia-treated barks were placed in polyethylene bags and held for several days at 90° F., 8% humidity, to remove ammonia retained in the bark moisture. From previous experience it was expected that chemically-bound nitrogen in the ammoniated barks was about two to three percent of the dry weight of the bark.

The procedures used by the Oregon Forest Products Laboratory to prepare bark samples used by Aspitarte (10, p. 17-23) were similar, except that after treatment with ammonia barks were heated to temperatures of 180 to 200° F. The high temperature evaporated most of the water and also volatized the free, and perhaps some lightly bound, ammonia (10, p. 21). Later work showed marked differences in toxicity of the two samples of ammoniated bark to strawberry plants.

Bark provided by the Muir and McDonald Company was "spent" tanbark which is much in demand as a garden mulch. It is Douglas fir bark which has been passed through a hammer mill, steamed, and extracted for tannins. In texture it was similar to the bark materials prepared at the Oregon Forest Products Laboratory. Approximately two cubic yards of this spent tanbark were obtained for use in field plot experiments. Corresponding amounts of whole bark for comparison were obtained from the Larson Lumber Company, Philomath, Oregon, and chipped and hammered to conform in texture to the spent tanbark.

Experimental

Experiment 1: This experiment was initiated to observe, for several years, the effects of various Douglas fir bark products upon the incidence of red stele disease. The experiment was

established by Aspitarte (10, p. 64-60) in 1957 and he obtained data for the first year.

Procedures used by Aspitarte in preparing the experiment were as follows. Soils from three locations with past history of red stele diseases were added to greenhouse potting soil and the composite thoroughly mixed and screened. Forty pounds of this soil mixture were mixed with bark in flats (7-1/2 in. deep, 14 in. x 16 in., hardware cloth bottom) at the rate of 100 tons per acre, with the exception of dihydroquercetin which he applied at five tons per acre. All treatments were in triplicate. Twenty Marshall strawberry plants were set in each flat in October, 1957. The flats were kept in a greenhouse until the middle of November, after which they were placed in outside cold frames. In April, 1958, the plants were removed and the roots inspected for symptoms of red stele. Numbers of healthy and infected plants were recorded. Degree of infection was not considered. Soil samples were taken for pH and microbial analysis. Findings for this first year have been reported by Aspitarte (10, p. 64-70).

The flats were retained, and for the next three years were planted to strawberries in late summer or early autumn, exposed to cool, wet conditions favorable to red stele disease development in winter and spring, and assayed for red stele development in late spring.

Soil was fallow during summer, and at some time during the summers of 1958, 1959, and 1960 each flat was emptied and a new tarpaper liner inserted over the hardware cloth bottom. The tarpaper liner retarded soil water drainage, providing the high moisture condition necessary for disease development. The Marshall variety was used in 1958-59 and 1960-61, and Puget Beauty in 1959-60.

The red stele disease observations from this experiment are summarized in Table 19. Puget Beauty strawberry plants, used in 1959-60 did not develop red stele symptoms, perhaps because of the higher red stele disease resistance of this variety.

Certain bark products gave good control of red stele disease while others were ineffective. The control afforded by even the best treatment was not permanent, however. In the first year four of the five best materials had been treated with ammonia, in the second year four of the six leading treatments were ammoniated materials, while in the fourth year the only two treatments still causing any reduction in disease incidence were ammoniated, or high nitrogen, treatments.

In the spring of 1958 Aspitarte (10, p. 67-8, 70) collected soil samples from each flat for pH and microbial analyses. He found (10, p. 67, 70) a ten-fold increase in Streptomyces and bacteria due to incorporation of the various bark materials, but little

Table 19. Effect of Douglas fir bark materials and their ammoniated forms on incidence of red stele disease of strawberry in the first, second, and fourth years after incorporation into soil, at 100 T/A rate.

Treatment	Percent of plants having red stele disease			Angles corresponding to percentages		
	1957-58*	1958-59	1960-61	1957-58	1958-59	1960-61
	(average of 3 reps.)					
Control soil (no addition)	100	91	98	90	79	86
Whole bark (0.16% N)	100	84	100	90	67	90
Ammoniated whole bark (4.27% N)	59	84	95	50	32	83
Ammoniated cork-free bark (2.29% N)	51	62	100	46	54	90
Benzene-extracted bark (0.14%)	97	56	93	84	50	78
Ammoniated benzene-extracted bark (2.58% N)	17	54	83	24	47	70 a/
Hot water-extracted bark (0.17% N)	19	2	100	26	7	90
Ammoniated hot water-extracted bark (3.03% N)	45	10	82	42	14	69 a/
Dihydroquercetin 5 T/A	100	97	98	90	84	86
$LSD_{.05}$				11.5	30.0	18.3

* 1957-58 data adapted from Aspitarte (10, p. 69).

a/ Significantly different from control at 10% level.

correlation between control of red stele and increase in microbial numbers (10, p. 67). However, the final pH of the soil was lowest, pH 5.7 and 5.6, with those treatments giving best control, while pH in treatments giving poor control was essentially the same as that of the control soil, pH 6.2.

Aspitarte also incorporated the same bark materials at 10 and 100 tons per acre rates into Willamette silty clay loam soil in pint milk bottles. Microbial determinations after four and eight months showed, at ten tons per acre all bark materials caused an increase of three to 50 times in mold counts over control soil, but at 100 tons per acre rate the mold counts increased 70 to 7,000 times, depending upon the bark product added (10, p. 56). Highest mold counts were associated with treatments containing little nitrogen. On the other hand, at ten and 100 tons per acre non-ammoniated bark did not significantly increase either total Streptomyces or bacteria (10, p. 57). With addition of the ammoniated bark, there was a large increase in bacteria and Streptomyces (10, p. 61). Soil pH measurements (in these Willamette silty clay loam soils) after four and eight months, at both the ten and 100 ton per acre rates, did not show pH differences that he found in his red stele disease experiment. Comparisons of pH data from the two experiments suggests that soil pH's are not markedly different among any of his treatments.

No correlations are apparent.

From Experiment 1 it was found that hot water-extracted bark incorporated into soil at 100 tons per acre reduced red stele disease by 80 percent for at least two years, but no reduction was apparent by the fourth year. All ammoniated bark treatments provided good initial control, but by the fourth year only two, ammoniated hot water-extracted bark and ammoniated benzene-extracted bark had any residual effect on development of red stele disease.

Experiment 2: A similar experiment was established in the summer of 1958. Soils, from seven locations with a history of red stele-diseased strawberry plants, were mixed together thoroughly. Bark products at a 100 tons per acre rate were then mixed with the P. fragariae-infested soil mixture in a cement mixer and placed in flats 7-1/2 by 14 by 16 inches with hardware cloth bottoms. All treatments were replicated ten times except the control soil and whole bark treatment which were replicated five times.

Each year certified red stele-free strawberry plants were planted, 20 per flat, in late summer or early fall. After the plants became established they were exposed for several months to cold, wet conditions. In late spring or early summer plants were dug up and roots examined for red stele. Strawberry varieties used were

Marshall in 1958-59 and 1960-61, and Puget Beauty in 1959-60. During the summers of 1959 and 1960 tarpaper liners were inserted in the flats to cover the hardware cloth bottoms and retard rapid drainage of water from the soil.

During June of 1959 fresh P. fragariae inoculum was added to one flat of each treatment. This inoculum was prepared by grinding red stele-infested roots (4-5 lbs. wet basis) in a meat grinder and mixing with ten pounds of soil collected from around roots of red stele-diseased plants. This soil and root mixture with a high red stele inoculum potential was added to builder's sand, thoroughly mixed, then equal volumes dispensed into the flats. Even distribution of this inoculum with the soils was accomplished by mixing in a cement mixer.

In 1958 the plants set in soils in which ammoniated barks had been incorporated soon showed symptoms of wilting, burning, plant collapse, and death. Almost all plants in these treatments were dead within two months. In all other soils plants were normal and vigorous. The phytotoxicity of these bark products was attributed to high ammonia levels. It will be remembered that these batches of ammoniated bark had not been heat-treated as had the similar batches used by Aspitarte, which had been exposed to temperatures of 180 to 200° F.

An attempt was made to leach excess ammonia out of the soil with heavy irrigations. Plants not dead before this heavy watering period died rapidly. New plants were set but they also died. Heavy irrigation of these flats were discontinued, the soils were allowed to dry, and in January were again replanted. At this time plants grew vigorously, appeared healthy, and soon surpassed plants in the other flats in size and vigor.

In March, 1959, a visual comparison of plants in the different treatments was made (Table 20). It should be mentioned that condition of plants grown in the absence of ammoniated barks closely resembled that of young field-grown plants during late winter and early spring.

Table 20. Compariosn of Marshall strawberry plants grown for six months in soils amended with ammoniated or non-ammoniated Douglas fir bark products.

	Potting mixture	
	Without ammoniated barks	With ammoniated barks
Leaf size	smaller	larger
Leaf condition	50% of leaves dead	few leaves dead
Color of surviving leaves	50% of leaves red 50% of leaves green	green leaves only
Plant condition	weak, spindly, smaller	vigorous, larger

Strawberries planted in autumn, 1959, in soils containing ammoniated bark again showed symptoms of toxicity. Only the plants in these soils were affected. Symptoms were not as severe as in 1958 and only a few plants died. Main symptom was a necrosis of leaf margins, black at first, then brown, progressing inward and evenutally covering the entire leaf. These symptoms which appeared first on older, then on younger leaves, were least severe on plants grown in the ammoniated benzene-hot water-extracted bark, and most severe on plants grown in the ammoniated hot water-extracted bark treatment. In 1960-61 strawberry plants in soils with ammoniated bark did not develop symptoms of toxicity. Apparently these bark materials were no longer toxic to strawberry plants.

Aspitarte (10, p. 67) did not report serious phytotoxicity in the ammoniated bark treatments, although he did record a slight reduction in numbers of surviving plants in the treatments which afforded most control of red stele disease (ammoniated benzene-extracted bark, hot water-extracted bark, and ammoniated hot water-extracted bark). Why the non-ammoniated hot water-extracted bark treatment should have reduced numbers of strawberry plants in his experiment is not obvious.

Red stele did not occur in Experiment 2 in 1958-59 because

the tarpaper liners in the flats were omitted, allowing soil to drain well and become too dry for red stele disease development. The variety Puget Beauty used in the 1959-60 season was believed to be susceptible, but proved resistant to our strain of P. fragariae. Data pertaining to effectiveness of bark products in controlling red stele disease was obtained only in the third season, 1960-61, and are summarized in Table 21.

Table 21. Effect of Douglas fir bark materials on red stele disease development in Marshall strawberry plants in the third year (1960-61) following applications to soil (bark at 100 T/A rates).

Treatment	disease incidence a/	percent of plants infested	angles correspond to percentages
Control soil (no bark)	57/63	90	77
Whole bark	40/72	56	46 *
Benzene-extracted bark	103/150	69	60
Ammoniated benzene-extracted bark	88/121	73	63
Hot water-extracted bark	128/153	84	69
Ammoniated hot water-extracted bark	13/117	11	15 **
Benzene- and hot water-extracted bark	133/158	84	78
Ammoniated benzene- and hot water-extracted bark	50/149	34	35 **

a/ Numerator - no. plants with red stele; denominator - total no. plants examined.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

In the flats to which supplemental inoculum had previously been added in June, 1959 (ten months after bark products had been incorporated) Marshall strawberry plants were severely infected during the 1960-61 season.

Several bark products or their ammoniated forms, although initially capable of markedly reducing or completely eliminating P. fragariae from the soil did not long retain this ability. Fresh inoculum added to bark-amended soils ten months after the bark was incorporated was not affected and Marshall strawberry plants were uniformly infected in all treatments.

Soil pH, determined by the water-saturation percentage method (79, p. 41-46) in flats receiving different bark products differed markedly. Soils mixed with ammoniated bark averaged pH 4.1 while those receiving the non-ammoniated forms averaged pH 5.1. These pH differences had no apparent effect on red stele disease development.

In the spring of 1961, during assay of strawberry plants for red stele disease, it became apparent that general red stele disease development within any given flat could be predicted by scrutiny of either the strawberry plants or a weed grass species which were present in all flats. Strawberry plants grown in flats in which a high percentage of the plants developed red stele disease were

small, had leaves with various hues and patterns of red intermixed with green and yellow, red petioles, and bore many ripe strawberries. On the other hand, strawberry plants in flats in which red stele disease was not severe were larger, had no red color in the leaves or leaf petioles, and only a few immature fruits. Plants in all flats were flowering profusely. Grass plants in the flats where a high percentage of strawberry plants were infected with red stele were short (4-8 inches), had sparse foliage and a small root system, and were not producing seeds. There were relatively few grass plants in such flats. Conversely, grass plants in flats in which red stele disease was not severe were tall (18-24 inches), had abundant dense foliage, a very dense, fibrous root system much intermixed with the strawberry roots and troublesome to separate from the strawberry roots, and were producing an abundance of seeds. There were many grass plants in these flats.

It should be noted that while plant appearance, both of strawberry and grass, was very consistent and uniform from plant to plant within a given flat, red stele development varied considerably between plants. Vigorous grass and strawberry plants, and a low percentage of red stele disease were most often observed in the ammoniated-bark treatments, indicating that soil fertility plays an important role in disease development.

Experiment 2 indicated that ammoniated hot water-extracted bark suppresses red stele disease development for at least three years after soil-bark mixtures have been made (Table 21). Probably most action against P. fragariae takes place during the first winter or spring since inoculum added after this time was unaffected. Ammoniated benzene- hot water-extracted bark also reduces red stele disease incidence but to a lesser degree.

Experiment 3: In July, 1959, test bark products were mixed with P. fragariae-infested soil at a 1:10 ratio (dry wt. basis) in one-gallon wide-mouth glass bottles which were partly immersed in temperature-control tanks. One Marshall and one Northwest plant were planted in each jar. Treatments were replicated three times and arranged in a randomized block design.

Ammoniated bark was phytotoxic and several replantings were necessary before plants became established. The Northwest variety was noticeably more sensitive to ammoniated bark than the Marshall variety. Once the plants became established in these soils they flourished and exceeded plants in other treatments in size and vigor. As plants became established water temperatures were lowered from 20°C. to 15°C. and were maintained at this latter temperature for three months.

In December 1959, when disease observations were attempted,

black root rot had developed so severely that if red stele was present it could not be diagnosed. Black root rot of strawberries is common in poorly drained soils in Oregon (108). Common practice, in greenhouses, to avoid black root rot disease of strawberries is to irrigate soils heavily only once or twice a week and to allow the soil to drain the rest of the time (69, 163). Such drainage was not possible in this experiment.

Observations were made on size and vigor of plants, general appearance of root development as observed through the sides of the jars during the experiment as well as when the plants were dug up and inspected, and pH of the different soils was determined. More plants developed abundant root systems in soils with non-ammoniated bark than in those with ammoniated bark. The most prolofic root systems were developed by plants growing in autoclaved soil. Vigor and size of leaves and crowns was best in soils with ammoniated bark (once plants overcame the toxic effects of such treatments), and in the autoclaved soil. Soil pH's (79) are recorded in Table 22. Again, as in Experiment 2, pH of soils, with any ammoniated bark product added was lower than that of soils without ammoniated bark products. Soils receiving bark averaged pH 4.8 while the same bark treatments not ammoniated averaged pH 5.6, approximately the difference found in Experiment 2.

Table 22. Effect of various bark treatments on soil pH. Non-drained soils incubated in glass jars six months then assayed for pH.

Treatments	Soil pH (average of 3 replicates)		
Control soil (no addition)	5.3		
Control soil autoclaved	5.6	Mean of non-	
Whole bark	5.6	ammoniated	5.6
Benzene-extracted bark	5.6	treatments	
Hot water-extracted bark	5.5		
Benzene-hot water-extracted bark	5.7		
Control soil plus nitrogen fertilizer*	4.8		
Ammoniated benzene-extracted bark	5.1	Mean of	
Ammoniated hot water-extracted bark	4.4	ammoniated	4.8
Ammoniated benzene hot water-extracted bark	4.7	treatments	
$LSD_{.01}$	0.68		0.31

* 16-20-0 fertilizer added at rate of 1/4 lb. fertilizer per 30 lbs. soil.

Experiment 4: Plots were established in a red stele-infested field of Siletz strawberries in the summer of 1960. Two kinds of bark were used: spent tanbark from the Muir and McDonald Tannery, Dallas, Oregon; and whole Douglas fir bark from the Larson Lumber Company, Philomath, Oregon. Rate of application of each material was 40 tons per acre. Soil in the field was a heavy compact clay, and although the field had underground tiles for drainage purposes, the soil was very wet and poorly drained during the winter and spring

rainy periods.

In May, an attempt was made to establish test plots, but soil was still too wet and heavy for efficient mixing of bark with soil. Therefore only one 3-1/2 by 10 foot plot with each bark material and a check plot were established.

In late July additional 3-1/2 by 15 foot plots were established. Bark was worked into the soil in accordance with the findings of Newhall and Gunkell (119), with a self-propelled rototiller having L-shaped rotary knives. Fertilizer (16-20-0) at the rate of 3-1/2 pounds per plot, was rototilled into the soil after the bark had been well mixed. Each treatment was replicated four times.

Certified red stele-free Marshall atrawberries were planted in early August. Survival was poor, largely because of hot weather, difficulties in providing adequate irrigation, and weak plants that had been stored under refrigeration for almost a year. Soil was collected from each plot, brought into the greenhouse, put into tarpaper "plant bands" and strawberry plants set into it. After two or three weeks the plants were transplanted to the field plots corresponding to the soil in which they were growing. As much soil as was possible was retained around the roots during transplanting. Tarpaper "plant bands" were removed so that strawberry roots could grow freely. Most plants survived and, when fall rains started,

grew vigorously.

In June, 1961, plants and surrounding soil were removed from the field and soaked 15 to 20 hours in tubs of water to loosen the soil sufficiently so that a water spray would wash the soil from the roots. Roots were then examined for red stele and the plants classified as either healthy or diseased.

Incorporation of either bark material into the soil reduced severity of red stele, although spent tan bark reduced incidence of disease significantly more than whole bark (Table 23). It is possible that more control of the disease was provided than is apparent from the data. Converse (30) has reported the importance, in field experiments, of transfer of inoculum from plot to plot by moving soil water. In the present experiment plots were established in one long row previously found to have strawberry plants severely red stele-diseased. If plot size had been larger or water movement from plot to plot prevented, it is possible that degree of red stele root rot control would have been greater.

Experiment 5: In late summer, 1960, several P. fragariae-infested soil were mixed. Bark products left over from 1958 and 1960 which had been stored in plastic bags, were dried at a temperature of 55-60° C. until only faint traces of ammonia gas could be detected. Soil and bark were mixed at a ratio of one part bark for

Table 23. Effect of whole bark and spent tanbark incorporated into field plots at 40 T/A upon incidence of red stele disease in Marshall strawberry plants (one year after bark materials incorporated into the soil).

Treatment	Disease incidence *	Percentage
Control soil (no additions)	49/76	64
Whole bark	20/48	42
Spent tanbark	16/59	27
LSD .05		21.3

* Numerator - no. plants with red stele; denominator - total no. of plants examined.

ten parts soil (dry wt. basis). Mixtures were placed in tin cans (17 cm. high, 15 cm. diameter), the number of replications depending upon the amount of bark available. Newly formed runner plants of Northwest variety strawberry were pinned down in this soil.

Where ammoniated bark was used, 75 to 100 percent of the original plants died and had to be replaced, often several times, before satisfactory survival was obtained.

In January when plants were established well enough to withstand cold, they were moved outdoors to a cold frame. In March, 1961, plants from several cans of each treatment were assayed for red stele development. Although the disease was present, it had not become severe and was difficult to detect (Table 24). This was thought to be because of the small size of the root systems on these plants. Plants growing in soils with ammoniated bark, although as

Table 24. Red stele disease development in Northwest strawberry plants grown in soil amended with several bark materials at a 1:10 ratio of bark to soil (dry wt. basis). Plants were established during the autumn and winter of 1960 and assayed for red stele symptoms in late March, 1961.

Treatment	Plants examined	Disease incidence*	Percentage
Control (no addition)	9	62/118	53
Whole bark (1960)	18	78/228	34
Spent tanbark (1960)	17	44/231	19
Hot water-extracted bark(1958)	24	94/278	33
Benzene-extracted bark(1958)	6	17/53	32
Benzene hot water-ext. bark "	18	72/245	29
Ammon. benzene-ext. bark "	4	0/40	0**
Ammon. benzene-hot water-extracted bark (1958)	5	0/51	0**
Autoclaved soil	6	0/68	0

* Numerator - no. roots with red stele; denominator - total no. of roots examined.

** Roots of these plants were shorter than those of other plants and had not developed the adventitious, fibrous, or lateral roots as had the other plants. Main bulk of the root system was composed of new, white roots two to five inches long.

large or larger than other plants, had the smallest root system. These roots, although numerous and healthy in appearance, were short, had few lateral branches, and had the white color of young newly-formed roots. The plants not inspected for red stele development at this time were moved to a greenhouse, allowed to grow normally and develop larger root systems for later observation.

The plants remaining in this experiment, after growing all summer, were moved on September 15, 1961, to a 15-20°C. controlled temperature chamber (natural daylength and light). The plants were watered heavily each day as well as subirrigated from saucers filled with water, following the procedures described by Hickman and English(69). Plants were removed and roots washed free of soil on November 3, 1961. Plants had therefore been exposed to moisture and temperature conditions conducive to optimum red stele development (69) for nearly seven weeks. However, no red stele disease symptoms were found in any of the plants. Why red stele disease did not develop is not known.

The plants in this experiment varied considerably in size and vigor, due to the influence of the soil-bark mixtures in which they had been growing, and not to red stele disease. If different bark treatments markedly altered plant composition, this might help explain the effect certain treatments have had on red stele disease

development in previous experiments.

Fresh weights were determined for plants grown in each treatment. Then leaves were detached, washed twice in distilled water, dried 24 hours at 70 to 80° C., and ground to a powder in a Servall high-speed Omni-mixer at 8, 000 revolutions per minute for three minutes. The resulting composite leaf tissue powders from each treatment were then assayed for nitrogen and phsophorus under the technical direction of Mr. Hamid Hussin in the laboratories of Dr. O. C. Comptom, Horticulture Department, Oregon State University. The methods used were those reported by Murneek and Heinze (116) for nitrogen, and Fiske and Subba (49) for phosphorus.

In this as in previous experiments it was evident that ammoniated barks incorporated into soil are toxic to strawberry plants. However, once the toxicity is dissipated plants grown in soils containing the ammoniated bark products, grew more vigorously and became heavier (Table 25) than plants in other soils. In this experiment, as in Experiment 3, root systems of strawberry plants grown in soils with ammoniated bark at first were smaller than those of plants grown in other treatments (Table 24). By the end of the experiment, when plants were about one year old, root systems of plants growing in ammoniated bark treatments were larger than root systems of plants in other treatments.

Table 25. Plant fresh weights and leaf tissue composition of Northwest variety strawberry plants grown in soils amended with Douglas fir bark products at ratio of 1:10 bark to soil (dry wt. basis). Soil-bark mixtures made September, 1960, plants allowed to grow without supplemental fertilization until November, 1961, when they were harvested, weighed, and assays made.

Treatment	No. of plants	Avg. wet wt. per whole plant	Percent nitrogen per gm. of leaf tissue	Percent phosphorus per gm. of leaf tissue
		(gm.)	(dry wt.)	(dry wt.)
Soil 1 (mixed sources)				
Control (no additions)	11	3.80	1.50	0.286
Whole bark (1960 supply)	12	2.29	0.92	0.150
Spent tanbark (1960 supply)	12	2.33	1.28	0.250
Whole bark (1958 supply)	11	2.14	0.93	0.150
Hot water-extracted bark (1958 supply)	15	2.80	1.26	0.220
Ammoniated hot water-extracted bark (1958)	4	8.83	2.38	0.340
Ammoniated benzene-extracted bark (1958)	14	6.46	2.30	0.380
Ammoniated benzene-hot water-extracted bark	6	6.50	2.38	0.286
Soil 2 (one farm, Philomath)				
Whole bark (1960 supply)	24	1.32	1.60	0.250
Spent tanbark (1960 supply)	19	1.56	1.92	0.326

In this experiment assay for red stele disease development in the spring of 1961 showed all bark treatments reduced incidence of red stele disease but the differences were not always statistically significant.

Nitrogen and phosphorus content of leaf tissue of plants grown in different soil-bark mixtures varied considerably (Table 25). Addition of any non-ammoniated bark reduced final nitrogen and phosphorus content of plants grown in these soils. Plants grown in hot water-extracted bark consistently contained more nitrogen and phosphorus than plants grown in whole bark. Percent nitrogen of plants grown in ammoniated bark treatments was nearly double that of control plants. Percent phosphorus in the same plants was as high as or higher than in the control plants. From this and Experiment 2 it appears investigations of correlations between plant nutrition and resistance to P. fragariae would be desirable.

Experiment 6: Ammoniated-barks incorporated into soil were toxic to Marshall (Experiments 2 and 3) and Northwest (Experiments 3 and 5) plants. In July, 1961, ammoniated benzene-extracted bark similar to that used in the previous experiments was incorporated with greenhouse potting soil at a ratio of one part bark per ten parts soil (dry weight basis). Mature Marshall, Northwest, Siletz, and Midway strawberry plants were planted in pots containing the

above bark-soil mixture. These plants were watered heavily and allowed to grow at normal warm summer greenhouse temperatures.

In this experiment no plants died as a consequence of transplanting into the ammoniated bark:soil mixture. However, typical mild water-soaked necrotic areas developed on many leaves. These symptoms were photographed in October, 1961 (Figure 11).

Figure 11. Typical mild necrosis of strawberry leaves of mature Northwest and Midway plants growing in a 1:10 mixture of ammoniated benzene-extracted Douglas fir bark and greenhouse potting soil.

Why plants in this experiment did not die as they had in previous experiments is not understood. The ammoniated bark was from the same supply used in previous experiments. The bark may have lost some of the ammonia during storage from 1958 to 1961. It was stored in plastic bags in a closed room where summer temperatures during the day often exceeded 100° F. Or, it is possible mature plants with heavy root systems, such as used in this experiment, are not as susceptible to ammonia as are younger plants (as used in Experiments 2, 3, and 5). Also, injury may be more severe under cool winter conditions than under hot summer temperatures.

Although varietal differences in reponse to ammoniated bark were not apparent in this experiment, in Experiment 3, where both Marshall and Northwest varieties were used concurrently, the Northwest plants were decidely more susceptible to injury by ammoniated barks.

If ammoniated barks are to be used for control of the red stele disease further experiments are needed to determine the effect of variety and age of strawberry plants.

DISCUSSION AND CONCLUSIONS

Certain Douglas fir bark materials and their ammoniated forms incorporated into P. fragariae-infested soils at rates of 40 or 100 tons per acre reduced the amount of red stele in strawberries grown in the treated soils. Although some bark materials afforded a high degree of red stele disease control in the first and second years the effect gradually diminished and by the fourth year incidence of disease was as high in plants grown in the treated as in untreated soils. Ammoniated bark materials usually afforded greater red stele disease suppression than non-ammoniated materials. However, in one experiment non-ammoniated hot water-extracted bark was more effective than any other material for reducing red stele development.

The mechanisms whereby bark materials inhibit red stele disease development are not known. It is possible that they exert a direct effect upon P. fragariae, the fungus causing this disease. Soils to which ammoniated barks had been added were found to have lower pH values than untreated soils or soils amended with non-ammoniated bark materials, but final pH values attained were not low enough to account for the suppression of red stele disease.

It is highly unlikely that the high nitrogen levels provided by

the ammoniated barks were directly responsible for the reduced incidence of disease since some non-ammoniated bark materials also suppressed red stele disease development. In the soils to which non-ammoniated bark materials had been added, available nitrogen was probably reduced by action of the soil microorganisms which decompose the bark. Other reports indirectly confirm the belief that ammoniated barks do not directly inhibit P. fragariae. Vaughan, et. al. (159) have shown that heavy applications of nitrogen fertilizer to P. fragariae-infested soils increased incidence and promoted spread of red stele disease. Also, Alcock, et. al. (3) found heavy applications of stable manure (a high nitrogen source) increased incidence of red stele disease.

It is improbable that bark materials may contain a leachable toxin which is inhibitory to P. fragariae. Such a substance would not be expected because hot water-extracted, benzene-extracted, and hot water and benzene-extracted barks, certainly free of any toxin leachable by cold soil water, provide better control of the disease than whole bark.

Addition of bark materials to soil may have an effect on soil moisture conditions. Any mulch reduces runoff and depresses evaporation, thus providing moist soil (91, p. 14), a condition favorable for P. fragariae. However, Aspitarte (10, p. 28-29)

found increasing the amount of nitrogen decreased the bark's water-holding capacity almost in an inverse linear relationship. Since ammoniated bark materials reduce incidence of red stele it is possible that they cause a drier soil environment, one less favorable for zoospore production and survival. Dark-colored materials, such as Douglas fir bark, added to soil absorb light and cause a rise in soil temperatures (91, p. 6-7). Light-colored materials, as Douglas fir sawdust, reflect light and cause soils to be cooler. Vaughan, et. al. (159) found that Douglas fir sawdust mulches increased incidence of red stele disease.

Bark products also may reduce red stele incidence by increasing the resistance of the host plants. It was shown that red stele-free strawberry plants grown in soils amended with various bark materials differed markedly. These differences were not due to action of P. fragariae since the disease had not developed in any of these plants. Plants grown in soils amended with ammoniated bark materials were found to be heavier (wet weight basis) and had a higher percent of nitrogen and phosphorus (per gram of dry leaf tissue) than plants grown in soils supplemented with whole bark.

The most likely way in which bark materials may affect red stele disease development is by altering the balance of soil micro-organisms to produce a biotic environment not favorable for

infection of strawberry roots by P. fragariae. It is well known that the composition of the soil microbial population is extremely complex and is determined very largely by the nature and amount of organic food available. Addition of Douglas fir bark materials supplies a source of food for the organisms capable of utilizing them. Since the bark materials were added at extremely high rates, populations of organisms capable of utilizing them also could reach high levels.

Aspitarte (10, p. 56-61) found that mold counts increased with additions of all bark materials, but Streptomyces and bacteria counts increased only with additions of ammoniated materials. It may well be that Douglas fir barks reduce incidence of red stele by favoring organisms antagonistic to P. fragariae.

Many soil organisms synthesize large amounts of compounds which are specific growth inhibitors of other microorganisms (antibiotics). IAA also is formed by certain fungi (64, p. 406; 11, p. 52-3, 57). It is possible these or other by-products may be formed and diffuse into the soil solution, thereby exerting a regulating effect on growth of other organisms such as P. fragariae. The literature contains many examples of antagonistic action of saprophytic fungi on plant pathologic fungi in the soil, resulting in reductions in disease incidence. Many of these have been reviewed

by Brian (20), Garrett (61), and Wood and Treit (176).

IAA at 10^{-4} and 10^{-5} M concentrations was found to almost completely inhibit vegetative growth of P. fragariae. It is unlikely that IAA is found in the soil in these high concentrations, but lower concentrations may interfere with production of sporangia or inhibit survival of zoospores.

Benefits in addition to red stele disease control may be realized from the addition of barks to soil. Sawdust compost has been used (129, p. 15) to counteract bad effects that follow repeated application of certain chemicals to forest nursery soils. Bark materials may be just as beneficial. Also bark may prove to be useful as a carrier of such other materials as fertilizers, insecticides, or fungicides. The use of bark in or on the soil has been suggested to aid in restoring organic matter depleted by continuous row crop cultivation.

Field observations have shown that at least two races of P. fragariae are present in Oregon. Identification of these races would be useful to the breeder of red stele-resistant strawberry varieties. To identify races present in Oregon pure cultures of isolates from many soils and strawberry varieties are required. For these purposes 14 isolates of P. fragariae were obtained from four varieties of strawberries grown in six different P. fragariae-infested soils.

A modification of the techniques for determining races is suggested which would allow for economy of plants and provide a check on whether a particular plant reaction is typical for that variety. Montgomerie (114) has noted that all plants from a given clone do not necessarily give identical responses to infection by the same race of P. fragariae.

Production of sporangia by P. fragariae is markedly influenced by different liquid environments. Soil leachate, Hoagland's solution, and water from rivers and streams were found to stimulate sporangium formation. Distilled water, whether autoclaved or not, or adjusted to pH values ranging from 5.3 to 7.9, proved to be a poor environment for sporangial production. This is contradictory with the findings of Tweedy (158) who reported distilled water, both autoclaved and non-autoclaved, provided a better environment than several other solutions for sporangia production. Goode (62) found that production of sporangia was greatly reduced when pond water was sterilized by heating. In the present experiments there were no significant differences in numbers of sporangia produced on the ninth and tenth days after agar discs from fungus cultures had been immersed and incubated in autoclaved and non-autoclaved solutions.

Races of P. fragariae were found to differ greatly in their ability to produce .porangia. Of the four races tested race A-4

produced sporangia most abundantly, followed by race A-1. It has also been shown that different races of the fungus differ in their ability to utilize various natural and artificial substrates.

It is probable that ability to produce sporangia, to utilize various nutrient substrates and to tolerate toxins and other materials could be used to supplement the methods now used to distinguish races of P. fragariae. Until much more information is available, however, use of differential strawberry varieties will continue to be the most satisfactory means of race identification.

Vegetative growth of P. fragariae was inhibited in autoclaved potato-dextrose medium. Autoclaving for five minutes or longer evidently produces a fungitoxic substance, as has been suggested by McKeen (100). Vegetative growth of P. fragariae is influenced by size and shape of the culture vessels. Probably this is a response to O_2-CO_2 changes of the media brought about by depth and surface area of the media. Growth rate of P. fragariae was found to be correlated with the kind and amount of inoculum added to culture media. Unless all factors are carefully controlled growth studies based on short term experiments are likely to lead to mistaken conclusions.

The antibiotics pimaracin, penicillin and polymyxin added to agar media suppress contaminants and are therefore useful in isolating many Phytophthora species from diseased tissues (46).

Unfortunately P. fragariae was inhibited by these and other antibiotics tested. Isolation of P. fragariae from red-stele-diseased strawberry roots would be greatly facilitated if antibiotics could be found which are inhibitory to the other organisms found in strawberry roots but non-inhibitory to P. fragariae.

SUMMARY

Certain Douglas fir bark materials and their ammoniated forms incorporated into Phytophthora fragariae-infested soils at rates of 40 to 100 tons per acre were found to reduce the incidence and severity of red stele disease of strawberry plants grown in the treated soils. Some bark preparations afforded a high degree of red stele disease control in the first and second years following treatment, but by the fourth year red stele disease was severe in plants grown in all soils. Hot water-extracted bark and all ammoniated bark preparations afforded greater red stele disease suppression than other materials tested. Ammoniated barks were found to have a toxic effect on strawberry plants grown in soils amended with these materials. However, plants which survived in soil with ammoniated bark were larger, heavier, more vigorous, and had a higher concentration of nitrogen and phosphorus than plants grown in other soils.

Races of P. fragariae were found to differ in their ability to produce sporangia in culture. Race A-4 consistently produced more sporangia than other races. Race A-1 was intermediate in this ability.

The type of liquid in which cultures were immersed greatly

influenced sporangium production. Soil leachate, river and stream waters, and Hoagland's solution provided good environments for sporangium production while distilled water and dilute V-8 juice did not. Sporangia produced in Hoagland's solution did not open and release zoospores as readily as those formed in other liquids. Sporangium production occurred uniformly in heat-treated and non-heated liquids.

Pure cultures of P. fragariae were isolated from strawberry plants from several locations in the Willamette Valley. A method of identifying races of the fungus is suggested which may afford greater economy of plants than existing methods.

Heat treatment of certain media, which normally support good growth of P. fragariae, caused the production of a toxin or toxins inhibitory to this fungus. Races of P. fragariae were found to differ in their response to heat-treated media, however. High concentrations (10^{-5} and 10^{-4} M) of indoleacetic acid (IAA) inhibited growth of the fungus. Size and shape of culture containers markedly influenced growth rate.

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