OCCURRENCE, BEHAVIOR AND CONTROL OF *VERTICILLIUM ALBO-ATRUM* REINKE AND BERTH. IN SMALL FRUITS

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INTRODUCTION

Verticillium albo-atrum Reinke and Berth., the fungal pathogen responsible for the Verticillium wilt disease in small fruits, is of almost universal distribution, and is capable of infecting a large number of other crops. In many cases the disease has been confused with conditions resulting from attacks by other pathogens, especially in the decades following the original description of the causal agent in potatoes in Germany.

In Oregon, Verticillium wilt of potatoes and eggplants was reported shortly after plant pathological investigations were started on any scale. As early as 1904, black raspberries in the Northwest were known to be affected, serious losses being reported in some cases (79). The intensive planting of hops and potatoes in the Willamette Valley in the first 25 years of this century has undoubtedly played an important part in building up the disease severity. Native wild black raspberries have been found with Verticillium wilt, indicating that in all probability the pathogen has not been introduced, but is native to the local soils.

Verticillium albo-atrum is able to survive for long periods in the soil as the microsclerotial resting stage (70); the planting of any crop having a degree of susceptibility to the fungus is enough to ensure its continued viability in the soil.

Black raspberry growers in the Willamette Valley have been concerned about the Verticillium wilt problem for many years as it is undoubtedly the main cultural factor limiting production. In 1955 a grower-supported project was set up to investigate various aspects of the disease, especially possible control measures.

A survey of the better black raspberry plantings in the areas where this crop is grown most extensively--Washington, Multnomah, Marion, Yamhill, Linn and Clackamas counties--was conducted in 1955-56. Although Verticillium wilt was found to be the most important factor responsible for the decline of the plantings, it was by no means the only disease involved. Armillaria root-rot and root-rot complexes similar to those described in red raspberries (28 and 47) were also frequently encountered. The Verticillium wilt disease was not found to be causing much significant damage in red raspberry plantings.

Verticillium wilt has been recognized as an important disease of strawberries in British Columbia (40), California (59) and the Eastern United States (9) for several years. In Oregon, however, it has apparently been confused to some extent with the black root-rot complex. Weather conditions in the Northwest are such that symptom expression in the widely grown Marshall variety is poorly defined.

A survey of the better strawberry plantings in the areas of Oregon in which this crop is principally cultivated was made in the summers of 1955 and 1956. It was found the disease was quite prevalent in some plantings, making them uneconomical in a few cases.

Measures for the avoidance of Verticillium wilt have been well-established for small fruits and other crops, but the economical control of the disease once the soil has become infested has yet to be achieved. Investigations were conducted to determine whether control with chemicals could be obtained at rates feasible for the grower. Control measures applicable to Verticillium wilt of other crops can generally be adapted for use against the disease in small fruits. For small fruit plantings, which usually are maintained for five to ten years, a more complete eradication of the fungus from the infested soil is necessary than for an annual crop such as potatoes.

Biological control of Verticillium wilt by the addition of crop residues is showing considerable promise as a long-term control measure. Inoculation of the soil with micro-organisms antagonistic to *Verticillium albo-atrum*, perhaps in conjunction with addition of crop residues or chemical treatment, is another method which might be effective. Breeding for resistance to Verticillium wilt of black raspberries is a possibility which must not be overlooked, but it would be difficult to obtain this without changing the character of the species, as there seems to be little or no evidence of within-species resistance and the nearest outside source appears to be in some of the commercial derivatives of the wild Pacific coast trailing blackberry, *Rubus ursinus* Cham. and Schlecht (75).

Varieties of red raspberry and strawberry having a fair measure of tolerance to Verticillium wilt have been known for several years and are widely grown in Oregon.

The inhibition of microsclerotia formation is a possible means of reducing the pathogen to the status of a root inhabitant and thereby greatly simplifying its control (5). The importance of being able to detect the presence and amount of the Verticillium wilt fungus in the soil, both from the grower's and the investigator's points of view, led to studies on the possibility of obtaining more rapid and accurate methods than those available at the present time.

LITERATURE REVIEW

The disease

A. Symptoms in small fruits

In black raspberries the appearance of the disease above ground may vary according to the distribution of infection in the roots (81). When the whole root system is involved, the entire plant becomes dwarfed and pale yellowish areas appear between the main leaf veins. Usually these symptoms occur on the lower leaves early in the summer, spreading further up the plant later in the season. When only a few roots are infected on one side of the plant, the above-ground symptoms are usually confined to the same side. In extreme cases a bluish streak extends up the side of the cane. In primocanes the disease spreads very rapidly, inducing symptoms of vascular discoloration and wilting of the leaves.

When infection takes place through the small secondary roots produced directly from the main crown of the plant, the entire crown soon becomes involved and the plant may die by the end of the season. Lateral roots may often remain uninfected and produce small plants from adventitious buds. Similar descriptions have been given by Berkeley and Jackson (6), Hockey (23) and Lawrence (34). Lawrence reported, however, that defoliation and wilting take place from the top of the plant downward, but Rudolph (51) pointed out that this is erroneous.

In red raspberries the symptoms appear on the new canes late in the season, the first sign of infection being a yellowing of the lower leaves in the intervenal areas (20). Affected leaves often curl up at the margin and ultimately fall prematurely, the terminal tuft being the last to survive. A blue stripe on the stem, extending from the ground upwards, proved to be the most reliable diagnostic symptom in the field. During the winter the dead fruiting canes and shrivelled buds are the only outward indication of the disease. The dead buds occupy positions in a vertical line and the underlying tissue is discolored. As the leaves appear, some of the canes are noticeable for the dwarf character of the leaves and the poor development of the fruiting laterals. The canes may produce undersized fruit or die before the fruit is matured.

In strawberries under California conditions the outer leaves usually droop or wilt, older leaves turn brown, followed by the gradual death of the plant (59). Roots are usually unchanged in appearance but may be reduced in number. Runners may or may not die, depending on whether they were established before or after the mother plant became seriously infected. In the first case, the runners die owing to their failure to receive support from the mother plant. In the second case runner plants were often observed to wilt and die soon after becoming established, either through infection via the stolon or through the new roots.

It was observed by McKeen and Bosher in British Columbia (40) that wilting of strawberry plants becomes evident in the fruiting season and the plants often survive for a while and develop a few small leaves in the center of the crown and sometimes one or two spindly side crowns, but produce little or no marketable fruit.

Braun (9, 10) found that in New York runner production by diseased plants is somewhat reduced and that later in the season the diseased mother plants and their runners develop a characteristic reddening of the petioles and stolons. The older leaves show premature reddening and yellowing and the outermost leaves eventually turn brown and die. Early symptoms appear soon after the blossom stage when the affected plants show discoloration, poor growth, and wilt on sunny days. The wilted plants recover in the evening, but they gradually lose this ability as the season progresses.

Symptoms in youngberry, dewberry and other susceptible blackberries are not sufficiently different from those in red raspberry to justify individual description, although the blue stripe symptom is less common in some of these species (51).

B. Disease development

Dimond (12) pointed out that the rapidity of disease development depends on several factors, mainly environmental, in any particular host. Infection of the host occurs through the roots and the fungus penetrates fairly directly to vascular tissue. Thereafter further mycelial growth is almost always restricted to conductive elements of the xylem. Mycelium continues to grow through the root and stem and in many cases it eventually appears in petioles and the growing point. In late stages of the disease, especially in nonwoody hosts, the fungus causes disintegration of stem tissues generally and may fruit on the surface after the plant dies. By this time, extensive rotting of the roots may have occurred, probably as a result of infection by secondary pathogens.

It was shown by Thomas (59) that Verticillium is rarely isolated from strawberry roots, but the fungus is readily obtained from the crowns.

Bewley (8) observed that in tomatoes the fungus destroys the cortex at the point of entrance and enters the wood where it grows upward into the stem.

Braun (10) pointed out that strawberry plants weakened by Verticillium infection may be more susceptible to root-rotting organisms. Roots of badly affected plants are usually decayed but it has not been established that this is due directly to Verticillium.

Incidence of plant pathogenic nematodes in the soil does not contribute to the severity of disease development as far as could be told from the results of McClellan, Wilhelm and George (38).

Van der Meer (61) reported that mildly affected cherry trees may recover from Verticillium infection unless the roots are reinfected. Also Rudolph (51) made the observation that even very severely attacked fruit trees may exhibit a most extraordinary recovery in the following year. Roberts (49) found that tomato plants are less susceptible to Verticillium when young and after eight weeks they are infected from the soil much more consistently. These results indicate that there may be stages in the growing cycle of other plants in which little or no infection can take place.

C. Dissemination and spread

The studies by Isaac (26) indicated that *Verticillium albo-atrum* is spread by root growth and contact rather than by mycelium growing towards the root. The fungus must first kill the host, then the neighboring plant must send roots into the remains.

Roberts (49) demonstrated that killing of a central infected plant in a field hastened the spread of *Verticillium* to healthy surrounding plants. Rudolph and Harrison (52) observed that the spread of *Verticillium* in irrigation water was usually found to be against the direction of flow.

Wilhelm (69) reported that dissemination of *Verticillium* may occur by wind that carries pieces of tissue on which aerial microsclerotia have formed. Undoubtedly much spread of the fungus occurs through the distribution of infected plant propagation parts. Keyworth and Bennett (32) reported, however, that strawberry runners taken from severely affected plantations produce apparently healthy plants when grown in clean soil. Spread of the fungus by cultivation of crop remains into the soil is an important way by which the fungus can be spread in a field where infection was initially restricted to local areas. (51,pp. 254-256)

D. Cause of wilt and other symptoms

Dimond (12), in integrating the various theories on the mechanism of wilt induction, pointed out that no one toxin or enzyme will account for the syndrome of any one wilt disease. Epinasty and leaf yellowing appear to be caused by ethylene formation by the pathogen and possibly to some extent by the host. Vascular discoloration appears to result from melanin production in living cells, brought on by disorganization through the pectic enzymes produced by the pathogen.

Verticillium albo-atrum produces pectic enzymes which partially or completely hydrolyse pectins exposed in pits and vessels. The hydrolytic products can form calcium gels if partially hydrolysed, or gums if acted upon by other enzymes. In either case the vessels become plugged and water shortage develops in the leaves. This leads to wilting that becomes more acute with time. Many other workers have attempted to explain the wilting phenomenon (8,17,18,34,45,53).

Earlier workers were of the opinion that mycelial growth was sufficient to block the vessels and cause wilt (7,61), but this view has been disproved by subsequent investigations and the integrated theory, as put forward by Dimond (12) has gained general acceptance. It was shown by Van der Meer (61) that the gum formed in the wood of infected cherry trees is insoluble in water, 96% ethyl alcohol, ether, nitric and sulfuric acids and potassium hydroxide. Boiling in nitric acid was the only treatment which dissolved the gum. Transformation of cell wall pectin into a gum of this nature would be sufficient, therefore, to destroy the permeability of the cell walls to water or plant sap.

E. Taxonomy of the fungus

Recent work on the variation of isolates of *Verticillium* obtained from mint plants, in which they were responsible for wilt, has indicated that the name *Verticillium albo-atrum* Reinke and Berth. would be more accurately applied to all of the systemic strains of *Verticillium* (16).

It was shown by Fulton, working with a raspberry isolate (14), Gauger, working with a mint isolate (16), Nelson, working with another mint isolate (45) and Presley, working with a cotton isolate (48) that four distinct morphological variants of *Verticillium* could be obtained by single spore isolations from each source. Wide variations between these four types for pathogenicity, microsclerotia formation and potentiality for further variation in single spore isolates were demonstrated. Hence the attitude of Rudolph (50) that all isolates should be designated *Verticillium albo-atrum* Reinke and Berth. and that attempts to make further separations at the species level should not be made, would seem to be more logical.

A species of *Verticillium* capable of causing weak root infections of maple and other hosts was given the name *Verticillium intertextum* by Isaac and Davies (27). The fungus forms aerial hyphae aggregated into mycelial strands in culture and is incapable of systemic vascular growth.

F. Characteristics of the fungus

While Wilhelm (68) and Isaac (25) regard *Verticillium albo-atrum* as a root inhabitant, the opinion of Luck (35) that the fungus is a weak root-inhabitant effectively elevated to the status of soil inhabitant by the microsclerotia, would seem to be more plausible. Luck found that unsterilized muck soil inoculated with conidia and mycelium of Verticillium albo-atrum was no longer infective after five months. When the experiment was repeated with microsclerotia, there was no drop in inoculum potential.

Wilhelm (70) found that a proportion of the microsclerotia were still viable after thirteen years in culture and after fourteen years in field soil without any susceptible hosts being present. These results contrast with earlier experiments by McKay (39) and Zeller (81) in which they found that the fungus is only viable for one year in infected potato and black raspberry tissue. It is possible that they were working with one of the hyaline types of the fungus in which the formation of microsclerotia does not take place.

Wilhelm (65) found no apparent relation between soil type, climatic environment or crop history and the vertical distribution of Verticillium albo-atrum in the soil. Samples of twenty soils showed that the fungus is frequently present to a depth of thirty-six inches, although the amount at this level is usually one-third to a quarter as much as in the surface layers.

Recent results (5) have shown that the formation of microsclerotia by Verticillium albo-atrum is apparently linked with the production of the black pigment (melanin) which they contain. Regulation of melanin production produced a corresponding variation in the number of microsclerotia formed. The pattern of variation of this fungus in culture is difficult to explain. Gauger (16) found that variations occur in the isolates from successive spores on conidiophores of each of the four morphological variants he described. This is surprising in view of the fact that the majority of the conidia are uninucleate and the same conidiophore nucleus is responsible for providing the genetic material of all the conidia it produces. Although Gauger made hyphal-tip isolations, it was not possible to say with certainty whether heterocaryosis occurs.

G. Host Range

Rudolph (51) has tabulated the species of plants susceptible to Verticillium wilt and listed about 134 species in 18 orders that were known to be affected in 1931. Since that time the host range has been extended by Baker, Snyder and Hansen (4), Snyder, Hansen and Wilhelm (58) and Wilhelm, Raabe and Smalley (74).

Susceptible small fruit species are black and red raspberry, Crandall blackberry, dewberry, nectarberry (51), boysenberry and youngberry (75) and commercial varieties of strawberry (59).

Other crops commonly planted in Oregon, such as potatoes, beans, peas, cabbages, brussels sprouts, hops, cherries, mint, tomatoes, squash, watermelon and cucumber, possess varying degrees of susceptibility to Verticillium wilt (51). In soils where potatoes, hops or tomatoes are grown the fungus builds up to such proportions that planting with black raspberries or strawberries would be disastrous.

(79). The fungus is able to maintain itself in the soil with other crops, and possibly to build up its inoculum potential to varying degrees. Braun (10) pointed out that grains are the only group of plants that are entirely immune.

H. Host specificity

Ludbrook (36) is of the opinion that there is little or no biologic specialization among the systemic strains of *Verticillium*, but important differences in pathogenicity have been recorded for isolates from different sources. Nelson (44) points out that *Verticillium albo-atrum* is characterized by non-specific pathogenesis. He demonstrated that isolates from cotton, pepper, eggplant, snapdragon, okra, blackberry and other very susceptible plants have not infected peppermint under the most favorable conditions. Snyder, Hansen and Wilhelm (58) reported a marked difference in the host-specificity of isolates from stock, radish, nightshade, cabbage and brussels sprouts, which did not infect Bonny Best tomatoes, and isolates from clove and Crandall blackberry, which did.

It was shown by Horner (24) that 17 isolates of *Verticillium albo-atrum* from 11 hosts were infectious to peppermint. The discrepancy with Nelson's results (44) is apparently due to the fact that the latter interpreted lack of symptoms as evidence of lack of infection.

Rudolph (51) summarized the cross-inoculation data known in 1931 and considers that unsuccessful inoculation experiments were due mainly to the fact that the investigators used too few test plants or that environmental conditions were unfavorable for disease development.

A variety of inoculation methods have been used in treating pathogenicity and host specificity of vascular wilt fungi. Bewley (8) reported that symptoms are produced more rapidly when the mycelium of Verticillium albo-atrum is introduced into the hypocotyl or internode of tomato plants than when the fungus is placed directly in the soil. Rudolph (51) used a spore suspension to infest the soil but found that plants grown in the soil were not uniformly infected and considered the method to be unsatisfactory.

Wellman (64) compared several inoculation techniques and demonstrated that dipping the roots in a spore suspension gives the most satisfactory results. Naturally infested field soil produced uneven infection and sterilized soil inoculated heavily with cultures of the fungus also gave erratic results. Inoculations by pouring fungus suspensions over the soil or in holes at the base of the plants gave fair results but disease development was slow.

Subsequent to Wellman's report, the root-dip method has been used, with slight variations, in almost all of the investigations by Gallegly (15), Green (18), Keyworth (30), Scheffer and Walter (53), and Wilhelm and Thomas (74). The method has the advantage over that used by Bewley (8) of easier application and less probability of contamination.

I. Diseases producing symptoms similar to Verticillium wilt in small fruits.

Jones (28) found that _Armillaria mellea_ was responsible for unhealthy growth of raspberries in British Columbia. The observations of Childs and Zeller (11) showed that two strains of _Armillaria mellea_ may be distinguished. One strain from former Douglas fir plantings is apparently entirely saprophytic, as roots of orchard trees covered with the fungus were unaffected. The other strain, found on former oak tree sites, was pathogenic and on all areas from which oak trees had been removed, some of the orchard trees were dying.

In strawberries the black root rot condition is responsible for above-ground symptoms similar to those of Verticillium wilt.(9,10)

In studies on root rot conditiohs of red raspberries in northern Utah, Powelson (47) describes symptoms of dwarfing of the canes, bronzing and scorching of the leaves, and death of individual canes or the entire stool. The principal fungi found to be associated with the roots of affected plants were species of _Cylindrocarpon_, _Coniothyrium_, _Pythium_ and _Fusarium_. It was demonstrated that _Pratylenchus_ species and other nematodes were also involved in the complex.

Assay methods

A. Susceptible plants

Wilhelm (65) found that microsclerotia of _Verticillium albo-atrum_ are too small to be screened or washed from the soil, the method used for Sclerotium rolfsii and Phymatotrichum omnivorum. Similarly Luck (35) reported that the specific gravity of the microsclerotia fluctuates over a wide range and is not sufficiently different from that of muck soil to allow separation by flotation techniques.

The most successful method available at the present time involves the growing of susceptible plants in the soil to be tested. Wilhelm (65) using tomato seedlings transplanted in the cotyledon stage, related his infection index (the percentage of Bonny Best tomato plants which became infected with Verticillium after six weeks when planted in 8 inch pots, 10 plants per pot) to the inoculum potential. He found that, within broad limits, this infection index is directly proportional to inoculum potential and is at least a function of the inoculum potential, the inoculum distribution and the past crop history of the soil.

The results of Snyder, Hansen and Wilhelm (58) indicate that Bonny Best tomato may not be the best assay plant for Verticillium albo-atrum since isolates from stock, radish, cabbage and brussels sprouts did not infect it.

B. Plating

Paharia and Kommedahl (46) consider that the dilution technique does not give a true picture of soil microflora. Martin (37) found that it is possible to inhibit bacteria in soil dilution plates by using streptomycin and rose bengal. Recent work by Tolmsoff (60) shows that even without bacteria the heavily-sporing and fast-growing fungi such as species of Penicillium, Mucor, Trichoderma and Pythium make dilutions of the order of 1:1,000,000 necessary to obtain readable numbers of colonies. Tolmsoff found that when microsclerotia of Verticillium were added to soil at a rate of one million per cc. of soil, it was not possible to detect the fungus at the dilution of 1:10,000,000 necessary to make reading of the plates possible. Tests with the same inoculum showed that 50 microsclerotia per cc. of soil were sufficient to infect potato plants. Many soils appear to contain about ten million spores of the heavily-sporing fungi per cc., so that dilutions will be heavily biased in favor of these species unless a selective medium can be developed.

Control measures

A. Cultural

1. Crop rotation. While crop rotation is suggested by most writers as a possible means of eradicating Verticillium from infected soil, data from cross-inoculation and rotation experiments (51) indicate that in practice this method is restricted in application. It is an effective means of preventing further buildup of the fungus in the soil, but there is little confirmed evidence of disease control.

Earlier reports by Zeller (81) and McKay (39) state that almost complete control of the disease could be obtained by 3 to 5 year rotations with non-susceptible crops. Guba (19) however, demonstrated that no control was obtained by rotation, even over a long period, with non-susceptible crops, and this appears to be the opinion held by most workers at the present time. It was shown by Wilhelm (70) that the fungus persisted for 14 years in field soil without any susceptible host present. Similarly Nelson (45) was able to obtain almost 100% infection in a field which had been planted with non-susceptible crops or fallowed for 12 years. Zeller (79) gave a good example of what can happen if two susceptible crops are grown on the same ground in successive years. He described a case in which almost 100% infection of black raspberry plants had occurred in a field where potatoes had been grown the previous year. Fifty percent of the plants were dead or dying.

2. Nutrition. Roberts (49) found a marked decrease in infection of tomato plants starved of nitrogen. Gallegly (15) confirmed these results under controlled environmental conditions. Subsequent experiments by Roberts (50) demonstrated that when nitrogen and phosphorus are sufficient for good growth of tomato plants, addition of potassium had no effect on Verticillium infection.

Presley (48) showed that higher rates of nitrogen increased Verticillium wilt of cotton while heavier applications of potassium were effective in decreasing the incidence of disease.

No evidence was found by Nelson (45) that minor elements have any effect on incidence or severity of wilt.

3. Environment. Bewley (8) described a method of decreasing the severity of Verticillium wilt in tomatoes in the greenhouse. He held the infected plants for several days at a temperature of 25°C., lightly watered, in a greenhouse shaded by an application of whitewash.

Nelson (45), working with mint, found almost as severe symptom expression at 28°C. as at 24°C., which indicates that maximal temperatures for disease development vary with the host plant used.

Himelick (22) demonstrated that disease-free mint planting stock could be obtained by treating rhizomes at temperatures of 47°C. for 55 minutes, 48°C. for 35 minutes or 49°C. for 20 minutes.

It was shown by Wilhelm (66) that occurrence and severity of Verticillium wilt in California was not greatly affected by the pH of the soil within the range in which susceptible crops are commonly grown. Severe outbreaks of the disease may occur on soils well in the acid range.

Guba (19), in attempting to control Verticillium wilt of eggplants, treated infected soil with sulfur for four consecutive years which reduced the soil pH to about 4.2. Plants still showed almost 100% infection. Further pH reductions controlled the disease but the plants grew very poorly. Guba concluded that artificial acidification is impracticable as a soil treatment.

In the investigations of Van der Meer (61) it appeared that disease development is much more rapid in drought and that under very moist conditions the susceptible plants suffer but little from the disease. Nelson (45) by increasing the soil moisture from 70% to 85%, was able to considerably reduce the severity of Verticillium wilt of mint. The disease was much worse in saturated soil than with 85% moisture. Keyworth (29) found in experiments on hops that it is not possible to say that the disease symptoms are more evident in wet summers, but he nevertheless recommends improvement of soil drainage as a control measure.

4. Roguing, sanitation, weed control. Rudolph (51) summarized the situation very effectively in saying that all writers stress the great necessity of clearing the soil of all infective material. Dead or dying plants with as much of the root system as possible, and all plant debris and weeds, should be carefully removed and burned.

Host range studies (51) showed that all the major dicotyledonous weeds are susceptible to Verticillium wilt and may therefore be responsible for keeping fields infested for a long period of time if they are not controlled.

Several workers (39, 51) have shown that roguing of single plants may lead to a greater incidence of infection by the end of the season because roots of adjacent plants come into contact with the moribund remains on which the fungus is growing. The standard recommendation is the removal of two neighboring healthy plants in addition to the one showing signs of infection.

5. Use of clean planting stock. Keyworth (29) found that cuttings of hop plants should not be taken from infected fields for propagation purposes, or, if this is impossible, they should be taken from areas well away from where infection has occurred. Other writers are in general agreement that plant parts used for pro-

pagation should be taken only from healthy stock.

Keyworth and Bennett (32) observed that runners taken from severely diseased strawberry plantations may produce apparently healthy plants when set in clean soil.

B. Chemical control

1. Soil disinfestation. Schoevers (55) reported that one ounce of a mixture of $5\frac{1}{2}$ parts of ammonium carbonate to one part of copper sulfate in 2 gallons of water was somewhat effective in controlling Verticillium wilt when applied to the soil as a drench. Steam was shown to be successful but impracticable on a large scale. Two percent formalin was found to be effective in greenhouse applications at a rate of 2 liters per square meter, but treatment was rather costly. Keyworth (29) recommended the use of formalin for the treatment of areas from which infected hop plants have been removed. The method requires 8 gallons of 2% formalin to be poured down the sides of the hole from which the infected plant has been removed and replacement with uninfected soil.

It was found by Wilhelm (67) that fermate and dithane, at rates of 0.1% of the dry weight of the soil, caused a considerable reduction in the inoculum potential of Verticillium infested soil, while spergon at the same rate was ineffective.

Studies by Nelson (45) showed that Verticillium wilt of mint could be controlled by applications of chloropicrin at the rate of 500 pounds per acre. He pointed out that steam sterilization is effective but is too expensive. Formalin and nematocides were found to be practically ineffective. The investigations of Wilhelm and Ferguson (73) demonstrated that under field conditions chloropicrin at 35.3 gallons per acre (about 500 pounds) reduced infection of tomato plants from 100% to 9.1%, and chlorobromopropene (CBP-55) at 46 gallons per acre reduced infection from 100% to 76.4%. Allyl bromide was found to have an effective rate similar to that of chloropicrin in preliminary tests, but was not used in the field trials, presumably because of its obnoxious nature. Chloropicrin was found to diffuse in all directions, while allyl bromide and CBP-55 only diffused downwards.

Munnecke and Lindgren (42) and Wilhelm (72) found that methyl bromide at 4 pounds per 100 square feet was ineffective in controlling _Verticillium_ in the soil, and Wilhelm (72) further reported that ethylene dibromide at 100 gallons per acre, DD at 100 gallons per acre, and carbon disulfide at 600 gallons per acre were insufficient to control the fungus in the soil.

Anderson and Okimoto (1) found that a compound, 3,5-dimethyl-tetrahydro-1,3,5,2-thiadiazine-2-thione, now known as Mylone, was effective at comparitively low rates against species of _Pythium_, _Fusarium_, and _Phytophthora_ in the laboratory. This material completely controlled _Phytophthora cinnamomi_ of tomatoes at a rate of 50 pounds per acre in greenhouse tests.

Young and Tolmsoff (78) demonstrated that 190 pounds per acre of sodium N-methyl dithiocarbamate (Vapam) was extremely effective in controlling the early maturity disease of potatoes, the principle cause of which is Verticillium albo-atrum. In spite of heavy increases in yield, all potato plants in the treated areas were found to be infected with Verticillium. Residual effects in both weed and disease control were observed when potatoes were planted the following year.

2. Chemotherapy. Hilborn and Delp (21) found that several antibiotics were able to reduce the severity of disease in tomato and potato plants infected with Verticillium albo-atrum. Dimond and Davis (13) working with Fusarium wilt of tomato, pointed out that benzothiazoles are not active as systemic fungicides but alter the metabolism of the host to produce resistance.

Waggoner (63) reported that control of Verticillium wilt of potatoes by use of certified seed, less susceptible varieties and crop rotation had not been adequate in the northeastern United States. Chemotherapy by use of 2,4-D and chelated metals was attempted, but no well-marked control was obtained. 2,4-D reduced symptom expression of the disease when sprayed to run-off at a rate of 500 and 1000 parts per million. The plants in these treatments gave a lower yield than those in the control plots.

Recent work by Becker (5) has shown that the formation of microsclerotia in a mint isolate of Verticillium albo-atrum could be regulated. Microsclerotia formation was found to be linked with melanin production and inhibition of the polyphenol oxidase enzyme which is involved in the synthesis of this pigment, could be achieved. Polyphenol oxidase is known to require copper ions as activators, through which the flow of electrons to oxygen is mediated. By chelating the copper available to the organism with Versenates or dithiocarbamates, microsclerotia formation can be completely inhibited *in vitro*.

Zentmyer and Erspamer (82) found that treatment of avocado trees with therapeutic concentrations (50-100 parts per million) of N-methyldithiocarbamate (Vapam) applied through a sprinkler system was strikingly effective in reducing infection by *Phytophthora cinnamomi*.

C. Biological control

Millard and Taylor (41) were able to reduce potato scab by inoculating soil with another species of *Streptomyces*, which effect they considered to be due to competition rather than antagonism. Anwar (2) demonstrated that an isolate of *Bacillus subtilis* protected barley plants from infection by *Helminthosporium sativum* when both organisms were added to the soil under greenhouse and field conditions. No control of *Fusarium lini* in flax was obtained using other microorganisms which had looked promising in this respect in laboratory tests.

Ark and Oswald (3) reported that some strains of *Streptomyces scabies* exerted strong antibiotic action against *Fusarium oxysporum* and *Verticillium albo-atrum* when grown on potato dextrose agar at 28°C.

Recent observations by Wilson (77) demonstrate that certain soil fungi are able to produce a substance or substances in sterilized muck soils which are active against *Verticillium albo-atrum*. Greenhouse trials with the antagonistic fungi indicated the possibility of modifying the pathogenic effects of *Verticillium* in tomatoes. Species of *Streptomyces*, *Fimetaria*, *Podospora*, *Chaetomium* and *Stachybotris alternans* effected good control.

The investigations of Wilhelm (67) show that substantial reductions in the inoculum potential of *Verticillium albo-atrum* in soil could be obtained by the addition of blood meal, fish meal and cottonseed meal at approximately equivalent nitrogen levels. The suppression of the fungus was thought to be due to competition by the saprophytic microflora, favored by the presence of the amendments.

D. Resistance

1. Nature of resistance. Studies by Keyworth (31) indicate that the site of differential resistance of hops to *Verticillium albo-atrum* is in the roots. Grafting experiments showed that stems of resistant varieties were susceptible when grafted onto a non-resistant root-stock. The resistance of tomato plants, however, was found by Scheffer and Walter (54) to be located in the stem when cut stems

of resistant and susceptible varieties were inoculated with a spore suspension of Fusarium. The resistant varieties recovered as soon as new xylem tissue had been produced.

2. Resistant varieties. Sherbakoff (57) points out that in spite of the great number of plants affected and the widespread damage caused by _Verticillium albo-atrum_, there has been relatively little breeding for resistance against this pathogen. The reason is probably that there is little promise of success with a pathogen having such a wide host range.

Nelson (43) described a non-commercial variety of spearmint with excellent resistance to Verticillium wilt. He was able to incorporate this resistance into commercial peppermint varieties without much loss of oil quality. Cotton and tomato varieties which are tolerant or resistant to Verticillium wilt have also been developed.(57).

It was shown by Guba (19) that selection of plants showing Verticillium wilt tolerance over several years did not produce any resistant variety. The observations of Zeller (81) show that all black and purple varieties of raspberry are susceptible. Some varieties were able to live for several years after becoming infected, although the yield of berries was very poor. Thomas (59) observed that the Marshall variety of strawberry showed some resistance to wilt since only a few of the plants die when grown in infected soil. No evidence of resistance was found by Braun (10) in strawberry varieties commohly grown in the north-eastern states.

Recent investigations by Wilhelm (71) show that the strawberry varieties Sierra and Blakemore and some clones of the wild *Fragaria chiloensis* are resistant. The criterion for resistance used was the ability of the plants to withstand three successive inoculations with *Verticillium* without showing symptoms, although infection may have occurred. Progenies from crosses of highly resistant parents were usually less than 50% resistant.

In studies by Wilhelm and Thomas (75, 76) the following bramble varieties were found to be wilt resistant: clones of *Rubus ursinus* Cham. and Schlecht., the wild Pacific coast trailing blackberry, and the derived commercial varieties Logan, Mammoth, Himalaya, Oregon Evergreen, Cascade, Chehalem and Ollalie. F_1 progenies of crosses of Lloyd George raspberry (susceptible) x Logan (resistant) and Boysen (susceptible) x Himalaya (resistant) were all found to be resistant. The Logan variety appears to breed true for Verticillium wilt resistance, while the susceptible Young, Nectar and Boysen varieties, and the resistant Ollalie, appear to segregate for resistance and susceptibility.

METHODS AND MATERIALS

During the course of the investigation several materials and methods were used repeatedly. These are described in this section, while explanations of any special techniques will be given with the data from the individual experiments for which they were used.

Culture media

Potato dextrose agar was used for isolation and culture work throughout the course of the investigation. This medium was prepared according to the following formula:

Decoction from 200gm. of potatoes

Dextrose, 20gm.

Agar, 17gm.

Water to make 1 liter

Shortly after the investigation was started, it was found to be necessary to reduce bacterial growth in the medium used for isolation purposes. Streptomycin, at a concentration of 50 parts per million, was found to be effective for this purpose. The antibiotic was added to the medium prior to the final autoclaving as its activity was not significantly affected by 15 pounds of steam pressure for 20 minutes. When isolations were made for *Verticillium* alone, 2% water agar with 50 parts per million of streptomycin was found to be the most convenient culture medium.

Isolation

Plant material for isolation was surface sterilized by immersion for short periods (one to three minutes, depending on the nature of the material) in a one to five dilution of commercial sodium hypochlorite (Chlorox). When root material was involved, soil was removed by washing in running tap water prior to surface sterilization. After immersion in Chlorox the tissue was placed on a sterile surface (fresh paper towels were found to be satisfactory) and cut into sections of appropriate size with sterilized instruments. The pieces of tissue were then placed on the medium in petri plates, taking precautions to prevent contamination from spores in the air, and allowed to incubate for three to six days at room temperature.

Isolates of fungi obtained in this way were made by transferring a small quantity of spores or mycelium from an uncontaminated colony in the petri plate to a test tube agar slant. Further transfers to petri plates were sometimes necessary to free an isolate from contaminants before placing it in a slant.

Greenhouse procedures

A sandy loam:peat moss mixture, to which an appropriate amount of balanced fertilizer had been added, was used in all experiments unless otherwise designated. In the preliminary cross-inoculation experiments this soil was autoclaved for four hours at 15 pounds pressure. This treatment led to a lack of uniformity in the results and was not used in subsequent work. No evidence of contamination from the unsterilized soil was found in extensive trials.

Seed used in the cross-inoculation and assay experiments were treated with a commercial seed protectant at the recommended rate.

Inoculation

Czapek broth was used in the preparation of cultures for inoculation purposes. This medium was prepared according to the following formula:

Sucrose	30gm.
Sodium nitrate	3gm.
Dipotassium hydrogen phosphate	1gm.
Magnesium sulfate	0.5gm.
Potassium chloride	0.5gm.
Ferrous sulfate	0.01gm.
Water to make 1 liter	

One-hundred milliliters of the Czapek broth in 250ml. Erlenmeyer flasks were inoculated with mycelium and spores from cultures on potato dextrose agar and placed on a mechanical shaker for four to seven days. The contents were then treated for a short time in a Waring blendor and diluted with three volumes of water. Plants to be inoculated were removed from the soil and after washing were placed in the diluted inoculum. After a period of 15 to 20 minutes the plants were removed and replanted in potting soil.

THE OCCURRENCE OF VERTICILLIUM WILT OF SMALL FRUITS IN OREGON

Black raspberries

The main areas of the Willamette Valley in which black raspberries are grown, Clackamas, Linn, Marion, Multnomah, Washington and Yamhill counties, were visited during the summers of 1955 and 1956 to determine the seriousness of Verticillium wilt in this crop. Attention was given mainly to plantings which had received adequate care but were partially or wholly diseased.

Plants having a diseased appearance were examined and the symptoms recorded. Petioles, canes, crowns or entire plants were brought back to the laboratory for isolation. An attempt was made to select plants with representative symptoms in each planting, but in cases where there were plants with other symptoms, these were also examined. A total of 70 farms were visited in the survey, 24 of which were found to have plants infected with *Verticillium* (Table 1).

The disease was found to be most prevalent in Clackamas, Marion, Washington and Yamhill counties. In Linn and Multnomah counties there was little evidence of Verticillium wilt and disease problems were due to other causes. In Linn county, Armillaria root rot was apparently the main disease responsible for decline of the plantings.

Strawberries

Scattered strawberry plants in various locations throughout the state were found to be infected with Verticillium wilt in 1955. A survey was carried out in the summer of 1956 to determine the severity

Table 1. Frequency of isolation of Verticillium albo-atrum from diseased black raspberry plants in the Willamette Valley in 1955 and 1956.

County	Diseased plants examined	Plants with Verticillium	Farms Visited	Farms with Verticillium
Clackamas	92	27	16	7
Linn	32	-	11	-
Marion	44	9	10	6
Multnomah	19	2	8	1
Washington	43	16	14	6
Yamhill	31	13	9	4
TOTALS	259	67	70	24

of the disease in this crop. Only the better plantings were visited to eliminate complications which might result from poor cultural practices.

Three fields in the central Willamette valley in which there were plants with a diseased appearance were sampled at random. The fields were examined in early and late summer to determine whether there had been any increase in the incidence of the disease in the intervening period. Two outer petioles were taken from each plant and isolations were attempted to determine whether the plants were infected with Verticillium (Table 2).

In one planting of the Marshall variety all of the 25 plants from which isolations were made were found to be infected. In the other Marshall planting 28 out of 70 plants were infected while in

Table 2. Incidence of Verticillium wilt in strawberries in the Willamette valley in 1956.

Variety	Acreage	Time of isolation July 20-21	September 6
Marshall	2	25/25*	4/4
Marshall	3	28/70	9/30
Siletz	1	17/25	24/30

* Numerator - the number of plants from which Verticillium albo-atrum was isolated: denominator - the number of plants from which isolations were made

the Siletz field 17 out of 25 plants yielded Verticillium.

Eighteen plantings, totalling 156 acres, were visited in the Eastern Oregon strawberry plant growing areas (Umatilla, Malheur and Jefferson counties). Plants with an unhealthy appearance were brought back to the laboratory for plating (Table 3).

Of the plantings examined, 7 were found to have plants infected with Verticillium wilt. No significant increase or decrease in the incidence of infection between the first and second samplings could be detected. In general these strawberry plantings were not seriously affected by Verticillium wilt except for three of the smaller fields visited. No Verticillium was isolated from plants taken from three fields of the Washington variety but two fields of the Willamette variety and one field of the Canby variety contained a few plants which were found to be infected. A few blackberry plantings were visited, but none of them appeared to have wilt disease problems.

Table 3. Frequency of isolation of Verticillium albo-atrum from diseased strawberries in Eastern Oregon in 1956.

		Time of Isolation			
Variety	Acreage	July 6	July 18	July 27	September 13
Marshall	2	18/25*			7/8
Siletz	1	1/7			4/8
Marshall	44		1/3	0/19	
Marshall	18		0/1	0/9	
Marshall	1½		1/3	12/17	
Marshall	7		0/3	0/5	
Marshall	5		2/2	0/21	
Siletz	1		0/2	0/4	
Marshall	1		1/3		
Marshall	1		1/2		

* Numerator - the number of plants from which Verticillium albo-atrum was isolated: denominator - the number of plants from which isolations were made.

SYMPTOMS OF THE DISEASE IN OREGON

Black raspberries

During the survey of black raspberry plantings in the Willamette valley records were kept of the symptoms of all the plants examined. The frequency of isolation of Verticillium from these plants was compared with external appearance to determine which symptoms are consistently indicative of infection by the fungus (Table 4).

Although some symptoms are more frequently associated with the disease than others, none of them were completely diagnostic. Bluestem was the only single symptom which showed much correlation with infection. Plants with a combination of symptoms, for example bluestem, yellowing and wilting of the leaves of the primocanes were more consistently found to be infected (Figure 1).

Figure 1. Black raspberry plant from a field heavily infested with Verticillium. Symptoms are wilting and yellowing of the leaves, bluestem and splitting.

Table 4. Frequency of isolation of Verticillium albo-atrum from black raspberry plants showing various symptoms.

Appearance of plant	Frequency of isolation of Verticillium
Bluestem	4/12*
Bluestem and splitting	7/14
Bluestem and yellowing	10/23
Bluestem and primocane wilt	7/20
Bluestem and fruiting cane wilt	1/1
Bluestem, yellowing and primocane wilt	11/23
Bluestem, yellowing and fruiting cane wilt	1/9
Bluestem, primo- and fruiting cane wilt	1/5
Bluestem, hellowing, primo- and fruiting cane wilt	1/8
Bluestem, yellowing, splitting, primo- and fruiting cane wilt	9/12
Yellowing	0/9
Yellowing and primocane wilt	2/15
Yellowing and fruiting cane wilt	1/13
Yellowing, primo- and fruiting cane wilt	0/13
Primocane wilt	3/31
Primo- and fruiting cane wilt	1/14
Fruiting cane wilt	1/8
Stunted growth	2/15
No symptoms (from infected field)	5/14
TOTAL	67/259

* Numerator- the number of plants from which Verticillium albo-atrum was isolated: denominator- the number of plants from which isolations were made

Splitting of the stem was generally found in young plants which had been set out in heavily infested soil (Figure 2). All of the individual or combined symptoms observed could apparently be brought about by other diseases. It is significant that 5 out of 14 symptomless plants from an infested field were found to be infected with Verticillium.

Strawberries

In an attempt to correlate the presence of Verticillium wilt infection with external appearance, isolations were made from a large number of strawberry plants from two fields in which the disease was known to be prevalent (Table 5).

Table 5. Frequency of isolation of Verticillium albo-atrum from strawberry plants showing various symptoms

Appearance of plant	Variety Marshall	Variety Siletz
Vigorous growth	0/2	3/12
Healthy	0/17*	13/22
Slow growth	2/8	5/5
Foliar discoloration	7/9	9/10
Poor runner formation	-	7/7
Runners dying	-	2/2
Wilting of outer leaves	-	3/3
Small leaves	-	4/4

* Numerator- the number of plants from which Verticillium albo-atrum was isolated: denominator- the number of plants from which isolations were made

Figure 2. The stem of a black raspberry plant from a field heavily infested with Verticillium. Symptoms are splitting, bluestem, wilting and yellowing of the leaves.

Healthy vigorous plants were taken in addition to plants with symptoms of foliar discoloration (premature reddening and yellowing of the leaves), wilting of the outer leaves, poor runner formation, slow growth, and death of the runner plants (Figure 3).

Figure 3. Marshall strawberry plant heavily infected with Verticillium. Symptoms are poor growth, poor runner formation and foliar discoloration.

It is evident that the Siletz variety is more tolerant of Verticillium infection since 13 of the 22 apparently healthy plants examined were found to contain the fungus. None of the symptomless Marshall plants were infected.

In the Marshall variety foliar discoloration (reddening and yellowing of the leaves) was associated with Verticillium infection in the majority of cases (Table 5).

In the Siletz variety slow growth, foliar discoloration, poor runner formation, dying of the runners, wilting of the outer leaves and small leaf size could be consistently correlated with the presence of Verticillium infection.

DISEASES OF SMALL FRUITS WHICH MAY BE CONFUSED WITH VERTICILLIUM WILT

Black raspberry diseases

Armillaria root rot. Examination of the roots and crowns of plants showing yellowing and wilting of the leaves revealed a white mycelial mat, frequently with rhizomorphs (Figure 4), in some of the plantings which were visited in the survey. No sporophores were observed at any time so that identification of the fungus as *Armillaria mellea* (Fr.) Quel. could only be tentative. It is possible that the fungus is not a primary pathogen, or requires preliminary damage to the roots before it can become established. Evidence that the latter might be the case was found in plants which had been damaged by the larvae of the raspberry root borer, and which showed a more frequent incidence of the white mycelial fungus.

Raspberry root rot. One recently established planting of black raspberries was found to have a large area of declining plants showing symptoms of bluestem, wilt and yellowing of the leaves. Preliminary examination of the stem tissues by isolation failed to reveal the presence of *Verticillium*. When isolations were made from the roots and crowns of plants showing typical symptoms, a number of weakly pathogenic fungi were obtained. In order of frequency of isolation, these were *Cylindrocarpon* sp., *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp., *Collectotrichum* sp., and *Phomopsis* sp. This complex of fungi is similar to that found in red raspberries (47). Some of the above fungi were occasionally isolated from the crowns of plants infected with *Verticillium*.

Figure 4. The crown of a black raspberry plant attacked by Armillaria root rot and the larva of the strawberry crown moth. The appearance of the crown after it was taken from the field (left) and the appearance after incubation for 7 days in a moisture chamber (right).

Strawberry crown moth (Ramosia bibionipennis). The larva of this insect was found to be causing considerable damage in a few plantings of black raspberries. The borer may bring about the death of the affected plants by girdling the crowns (Figure 4). Plants may show wilting and yellowing of the leaves, lack of vigor and occasionally bluestem.

Water soaking. Black raspberries are very sensitive to water-logging of the soil. When plants have been subjected to a period of partial or total flooding they frequently show yellowing of the leaves and wilting (Figure 5).

Figure 5. Black raspberry plant in a field which had previously been flooded on two occasions.

Strawberry diseases

Plants which were showing poor growth, wilting and reddening and yellowing of the leaves were found in some cases to be infected by Cylindrocarpon sp., Fusarium sp., Pythium sp., and Rhizoctonia sp., fungi which are usually associated with the black root rot complex of strawberries. The roots of these plants were invariably blackened, but plants from which Verticillium was isolated also showed this symptom in some cases.

FACTORS INFLUENCING THE INCIDENCE OF THE DISEASE

Crop history

In the survey of black raspberry plantings the crop history was determined as far back as possible. In some cases the growers were unable to recall what crops had been planted in the ground beyond the previous five years, or more frequently the farm had changed hands within this period. These facts may account for some of the discrepancies found when crop history was related to the frequency of isolation of *Verticillium* from the plantings (Table 6).

When *Verticillium* was found to be present in the black raspberry plantings, and cereals were the only crops known to have been planted previously, it is very probable that potatoes or hops had been grown in the field at some earlier date. In most cases a recent history of potatoes, tomatoes, hops or black raspberries was associated with infection of the black raspberry plantings examined.
Rotation with two years of a cereal crop did not eliminate the fungus from ground in which black raspberries had been previously grown. Plantings in fields which previously had been exclusively in pasture were consistently free of the disease. A history of strawberries did not lead to infection of the black raspberry plantings examined. In one case infection was found in a field which had been previously planted with golden seal, a non-susceptible herb. The explanation for this probably lies in the fact that potatoes were grown in the ground some 20 years before. The heavy manuring applied to the herb, in conjunction with

Table 6. Influence of recent crop history on Verticillium wilt incidence in black raspberries.

Recent Crop History	Incidence of Verticillium wilt
Alfalfa, grain	0/1*
Black raspberries	3/5
Black raspberries, potatoes	0/1
Black raspberries, clover (2)**	1/1
Black raspberries, cereals (2)	2/3
Cereals	3/11
Cereals, vetch, clover	0/1
Hops, Cherries, beans (6), fallow (1)	1/1
Cleared from timber	1/8
Cleared from timber, potatoes (1)	0/1
Clover	1/2
Golden seal (*Hydrastus canadensis*)	1/1
Hops	2/2
Pasture	0/11
Peaches	1/1
Potatoes	4/4
Potatoes, tomatoes	1/1
Prunes	0/3
Strawberries	0/3
Strawberries (3), cereals (2)	0/2

*Numerator- the number of plantings from which *Verticillium albo-atrum* was isolated: denominator- the number of plantings from which isolations were made.

**The number of years the crop was planted is given in parenthesis where known. The time sequence is from left to right.

the presence of large numbers of susceptible weeds, was apparently sufficient to maintain the inoculum potential of the fungus in the soil.

From these observations it is evident that fields in which potatoes, hops or black raspberries have been grown should be tested for the presence of Verticillium before planting with black raspberries.

Nutrition

Field observations on the relation of nutritive factors to incidence of Verticillium wilt in black raspberry plantings were consistent with results reported by other workers (48). Where heavy applications of barnyard manure or fertilizer of high nitrogen content were known to have been made, it was invariably found that when the disease was present the plantings were more severely affected than when little nitrogen had been added.

Environment

A well-marked seasonal variation in the incidence of the disease appears to take place in both black raspberries and strawberries. During the winter momths isolations from plantings which were known to be heavily infected during the previous summer were largely unsuccessful. This may be due to the winter temperatures being unfavorable for the survival of the fungus in the host, or to the low amounts of nutrient materials present in the vessels of dormant plants.

When severely infected black raspberry and strawberry plants were brought into the greenhouse and maintained at 60°-70°F. under winter light conditions, there was a gradual decrease in the incidence of the disease in the plants. From this observation it seems likely that light is the important factor involved, the photosynthetic process being held at a level at which insufficient food material is available to the fungus.

DIAGNOSIS OF THE DISEASE IN THE LABORATORY AND FIELD

Since field diagnosis of Verticillium wilt of small fruits based on symptoms alone is unreliable, attempts were made to determine whether other information would give a more dependable indication of infection. The crop histories of the black raspberry plantings visited in the survey were ascertained as far as possible, but complete correlation with Verticillium wilt incidence was not found (Table 6). On ground in which potatoes or hops had been recently grown the black raspberry plantings were infected in the majority of cases. When a diseased planting has a recent crop history of potatoes or hops and the plants show symptoms which are most consistently associated with Verticillium wilt, it is very probably, but not conclusively, infected with the disease.

Laboratory diagnosis by isolation from tissues of the suspected plants is a more dependable method. It has been found, however, that isolations may be negative in the winter and early spring, even though the plants were known to be infected the previous summer.

Isolation from susceptible weeds was not consistently reliable as a means of determining whether the soil in a field is infested. In one field in which potatoes had been grown for a number of years, nightshade (Solanum nigrum L.), mallow (Malva rotundifolia L.), pigweed (Amaranthus retroflexus L.) and lambsquarters (Chenopodium album L.) were all found to be infected with Verticillium. In another field which had originally been a hop yard, nightshade, pigweed, lambsquarters and groundsel (Senecio vulgaris L.) were not infected with the disease. In the greenhouse susceptible plants became quite heavily infected when grown in soil samples from the latter field.

SPREAD OF THE DISEASE THROUGH INFECTED PROPAGATION STOCK

Black raspberries

Mother plants which had been found to be infected with Verticillium wilt were tipped into cans of sterilized soil and allowed to root during the winter months. In the spring, isolations from the tip plants were negative for *Verticillium*. Tip plants which had established themselves in badly infected plantings were also found to be free of *Verticillium* when isolations were made in the spring.

Strawberries

Plants showing typical symptoms of Verticillium wilt were selected from plantings in which the disease was known to be present. Isolations were made from the mother plants and from the first three runner plants of one stolon (Table 7).

Plants which had been taken from a field in which *Verticillium* infection was known to be present and set out by another grower in clean ground were found to be completely disease-free. This observation led to studies on the incidence of the disease in propagation stock at the time of digging. Isolations were made from crowns, roots and petioles of mother plants and runners growing in heavily infested soil.

Ten mother and runner plants of the Marshall variety were found to be completely free of *Verticillium*, although isolations from 25 plants in the same area of the field showed infection the previous summer. Mild infections were found in the crowns of 3 out of 15 mother plants and runner plants of the Siletz variety, at the time of digging, in a field

Table 7. Incidence of Verticillium albo-atrum in the runners of infected strawberry plants.

Distribution of infection	Clones examined Marshall	Siletz
Mother, 1st, 2nd and 3rd runner plants	0*	1
Mother, 1st and 2nd runner plants	2	1
Mother and first runner plant only	5	3
Mother plant only	1	2
Mother and 2nd runner plant	1	0
Total clones examined	10	8

* Infection determined by isolation from two petioles per plant.

in which 17 out of 25 of the mother plants were infected the previous summer.

To determine whether infection of the runner plants takes place from the soil or from the mother plant, isolations were made from the runner plants and the connecting stolons of clones growing in heavily infested soil. In clones of the Marshall variety the mother and runner plants, but not always the connecting stolons were infected. In 7 clones of the Siletz variety all of the stolons between the infected runner plants and the mother plant yielded Verticillium on isolation. In another Siletz clone an infected runner plant was attached to a healthy mother plant. One Marshall clone had the mother and second runner plants infected while the first runner plant was healthy. From these observations it appears that in the summer runner plants of the two varieties examined may become infected via the stolons or directly from the soil once the roots are established.

THE PATHOGENICITY AND HOST RANGE OF VERTICILLIUM ISOLATES

To determine the host specificity of *Verticillium* isolates from various sources, a wide range of susceptible plant species was inoculated and the presence and degree of infection ascertained by isolation after a period of 8 weeks.

The cross-inoculation studies were conducted in part to determine whether any of the plant species tested would be suitable for bioassay of the fungus in the soil.

Plants to be inoculated were grown from seed in sterilized soil, or obtained from disease-free propagation stock. In the latter case preliminary isolations were made to ensure that the plants were not already infected with *Verticillium*. Inoculations were made by immersing the roots in a spore suspension for about 15 minutes. A minimum of three plants of each species were inoculated with each isolate. In the cases where *Verticillium* could not be isolated from at least one of the inoculated plants, the inoculations were repeated with fresh plant material (Table 8).

Plant species inoculated

Black raspberry, Munger var.	*Rubus occidentalis* L.
Eggplant, Black Beauty var.	*Solanum melongena* L.
Balsam*	*Impatiens* sp.
Tomato, Bonny Best var.	*Lycopersicum esculentum* L.
Potato, Netted Gem var.	*Solanum tuberosum* L.

*Several commercial varieties of balsam were used in the course of the investigation but no differences in susceptibility to *Verticillium* could be detected between them. Further references will be simply to balsam.

Plant species inoculated (continued)

Mint, Mitcham var.	Mentha piperita L.
Red raspberry, Washington var.	Rubus idaeus L.
Groundsel	Senecio vulgaris L.
Dandelion	Teraxacum officinale Weber
Lambsquarters	Chenopodium album L.
Pigweed	Amaranthus retroflexus L.
Strawberry, Marshall var.	Fragaria sp.
Strawberry, Siletz var.	Fragaria sp.

Isolates used for inoculum

Source of isolate	Index number*
Verticillium albo-atrum	
Black raspberry (Rubus occidentalis L.)	B-3-O-, B-7-W, B-5-Mu, B-6-W
Hop (Humulus lupulus L.)	H-4-C, H-4-L
Mint (Mentha piperita L.)	Mn-5-S, Mn-51-H, Mn-51-N
Potato (Solanum tuberosum L.)	Po-5-S, Po-6-C, Po-9-H
Strawberry (Fragaria sp.)	St-9-O, St-1-D
Maple (Acer macrophyllum Persh.)	Mp-11-O
Peony (Paeonia officinalis L.)	Py-40-H
Snowberry (Symphoricarpus albus L.)	Sn-43-H
Mallow (Malva rotundifolia L.)	Mw-45-H
Red raspberry (Rubus idaeus L.)	R-50-F
Tomato (Lycopersicum esculentum Mill.)	T-32-C
Verticillium intertextum	
Lotus corniculatus L.	L-1-G

*See Appendix A for details

Table 8. Host specificity of Verticillium isolates.

Plants inoculated	Area of isolation	Isolates used for inoculum B-3-O	B-3-N	B-6-W	B-5-Mu
Black rasp-	stem	-	x	x	-
berry	root	x	x	x	x
Eggplant	stem	x	x	x	x
	root	x	x	x	x
Balsam	stem	x	x	x	x
	root	x	x	x	x
Tomato	stem	x	x	-	x
	root	x	x	x	x
Potato	stem	x	x	x	-
	root	-	-	-	x
Mint	stem	-	-	-	x
	root	-	x	-	x
Red rasp-	stem	-	-	-	-
berry	root	-	-	-	x
Groundsel	stem	x	x	x	x
	root	-	-	x	-
Dandelion	crown	x	-	-	x
	root	-	x	-	-
Lambs-	stem	x	x	x	x
quarters	root	x	x	x	x
Pigweed	stem	x	x	x	x
	root	x	-	x	x
Marshall	crown	x	-	x	x
strawberry	root	-	-	-	-
Siletz	crown	-	-	x	-
strawberry	root	-	-	-	-

x Plant parts found to be infected on isolation.
- Plant parts found to be uninfected on isolation.

Table 8 (continued). Host specificity of Verticillium isolates.

Plants inoculated	Area of isolation	Isolates used for inoculum H-4-O	H-4-N	Mw-45-H	Mp-11-O
Black rasp-	stem	x	x	x	-
berry	root	x	x	x	x
Eggplant	stem	x	x	x	x
	root	x	x	x	x
Balsam	stem	-	x	x	x
	root	-	x	x	x
Tomato	stem	x	x	x	x
	root	-	x	x	x
Potato	stem	x	x	x	x
	root	-	-	-	-
Mint	stem	-	x	-	x
	root	-	x	x	-
Red rasp-	stem	-	-	-	-
berry	root	x	-	-	-
Groundsel	stem	x	x	x	x
	root	-	x	-	x
Dandelion	crown	-	x	-	-
	root	-	x	-	-
Lambs-	stem	-	x	x	x
quarters	root	-	x	x	x
Pigweed	stem	-	x	-	x
	root	x	-	x	x
Marshall	crown	x	x	x	x
strawberry	root	-	-	-	-
Siletz	crown	x	x	-	-
strawberry	root	-	-	-	-

X Plant parts found to be infected on isolation.
- Plant parts found to be uninfected on isolation.

Table 8 (continued). Host specificity of Verticillium isolates.

Plants inoculated	Area of isolation	Isolates used for inoculum Mn-5-S	Mn-51-H	Mn-51-N	Py-40-H
Black rasp-	stem	x	x	x	-
berry	root	x	x	x	x
Eggplant	stem	x	x	x	x
	root	x	x	x	x
Balsam	stem	x	x	x	x
	root	x	x	x	x
Tomato	stem	x	-	x	x
	root	x	x	-	x
Potato	stem	x	-	-	-
	root	x	-	-	-
Mint	stem	-	x	x	-
	root	-	x	-	-
Red rasp-	stem	-	-	-	-
berry	root	-	-	-	-
Groundsel	stem	x	-	x	x
	root	-	-	x	x
Dandelion	crown	x	-	-	-
	root	-	-	-	x
Lambs-	stem	x	-	x	x
quarters	root	x	-	x	x
Pigweed	stem	-	-	x	x
	root	-[1]	-	x	x
Marshall	crown	-	-	-	x
strawberry	root	-	-	-	-
Siletz	crown	-	-	-	-
strawberry	root	-	-	-	-

x Plant parts found to be infected on isolation.
\- Plant parts found to be uninfected on isolation.

Table 8 (continued). Host specificity of Verticillium isolates.

Plants inoculated	Area of isolation	Isolates used for inoculum Po-5-S	Po-6-C	Po-9-H	R-10-F
Black rasp-	stem	x	x	x	x
berry	root	x	x	x	x
Eggplant	stem	x	x	x	x
	root	x	x	x	x
Balsam	stem	x	x	x	x
	root	-	x	x	x
Tomato	stem	x	-	x	x
	root	x	x	x	x
Potato	stem	x	x	x	x
	root	x	-	-	-
Mint	stem	x	x	x	-
	root	x	-	x	x
Red rasp-	stem	-	-	x	-
berry	root	-	-	-	-
Groundsel	stem	-	x	x	x
	root	-	x	x	-
Dandelion	crown	-	x	-	-
	root	-	-	-	-
Lambs-	stem	-	x	x	x
quarters	root	-	x	x	x
Pigweed	stem	-	-	x	x
	root	-	x	x	x
Marshall	crown	-	x	x	x
strawberry	root	-	-	-	x
Siletz	crown			-	x
strawberry	root			-	-

x Plant parts found to be infected on isolation.
- Plant parts found to be uninfected on isolation.

Table 8 (continued). Host specificity of Verticillium isolates.

Plants inoculated	Area of isolation	Isolates used for inoculum Sn-43-H	St-9-0	St-1-D	T-32-C	L-1-G
Black raspberry	stem	x	–	–	x	–
	root	x	x	x	x	x
Eggplant	stem	x	x	x	x	–
	root	x	x	x	x	x
Balsam	stem	x	x	x	x	–
	root	x	x	x	x	x
Tomato	stem	x	x	x	x	–
	root	–	x	x	x	x
Potato	stem	–	x	x	x	–
	root	x	–	–	–	x
Mint	stem	–	–	x	x	–
	root	–	–	–	–	–
Red raspberry	stem	–	–	x	–	–
	root	–	–	–	x	x
Groundsel	stem	–	x	–	x	–
	root	–	x	–	x	x
Dandelion	crown	–	–	x	–	–
	root	–	x	–	–	x
Lambs-quarters	stem	–	x	x	x	–
	root	–	x	x	x	x
Pigweed	stem	x	x	x	–	–
	root	x	x	x	x	x
Marshall strawberry	crown	x	–	x	x	
	root	–	–	–	–	
Siletz strawberry	crown	x	x	x	–	
	root	–	–	–	–	

x Plant parts found to be infected on isolation.
– Plant parts found to be uninfected on isolation.

A few of the plant species inoculated were susceptible to all of the isolates, but in general there was evidence of a small degree of host specificity. Eggplant, tomato, black raspberry and balsam were readily infected by almost all of the isolates tested. In some cases the fungus did not invade the stem tissue of these plants during the course of the experiment. Some of the isolates, for example B-5-Mu and H-4-N, were infective to a wider range of species than others. The isolate Mn-5-S was the only one which induced symptoms when inoculated into mint. This culture of the fungus was originally obtained from a mint plant with symptoms, and was infective to fewer plant species than the symptomless mint isolate, Mn-51-H.

Negative cross-inoculation results are not very dependable as there are several factors which may interfere with disease development. It is possible that in some cases the environmental conditions of the experiment, or the stage of growth of the inoculated plants, were unfavorable for infection. Apparent differences in the pathogenicity or host range of the isolates may be a direct result of different rates of sporulation in the liquid culture medium. The growth of some of the cultures was largely mycelial; others produced a yeast-like growth and one or two grew mainly in the sclerotial form.

The four weed species, groundsel, dandelion, lambsquarters and pigweed, were infected by most of the isolates, as were potato, mint and Marshall strawberry. Washington red raspberry and Siletz strawberry were infected by a minority of the isolates. The significance of the results in red raspberry is doubtful, however, as the plants were not in a vigorous condition at the time of inoculation.

Although successful isolations were made from the stems and crowns of most of the strawberry and potato plants inoculated with *Verticillium albo-atrum*, the fungus rarely grew from the root tissue.

The isolate from *Lotus corniculatus*, which closely resembles the *Verticillium intertextum* described by Isaac (27), was re-isolated from all of the plants inoculated with the exception of mint and the two strawberry varieties. The isolate produced hyaline, ropy mycelium in culture, with typical verticilliate conidiophores. It is apparently incapable of vascular infection and may be regarded as a root rotting organism.

From the above data it is apparent that eggplant, tomato, balsam and black raspberry could all be used for bioassay purposes. In practice black raspberry would be inferior in this capacity because of the length of time required for germination of the seed.

METHODS FOR THE DETERMINATION OF THE PRESENCE AND AMOUNT OF *VERTICILLIUM ALBO-ATRUM* IN THE SOIL

During the course of the investigation methods of assaying for the presence and amount of *Verticillium* in the soil were compared. A rapid, quantitative method would be very desirable for both the grower and the research worker and would be of special importance to the grower of certified planting stocks.

Plating

1. Direct plating. Small amounts of soil which were known to be heavily infested with *Verticillium* were sprinkled over the surface of plates of 2% water agar to which 50 ppm. of streptomycin nitrate had been added. After five days of incubation at room temperature *Verticillium* could be detected in about half of the plates by careful searching with the microscope. This method is useful for the detection of *Verticillium* in the soil but gives only a rough estimate of the amount of the fungus present. Detection of the fungus in slightly infested soil might require large numbers of plates. Another drawback of the method is the difficulty sometimes experienced in distinguishing *Verticillium albo-atrum* from similar forms such as *Verticillium intertextum* and *Gliocladium roseum*. A chemical which would favor *Verticillium* but would inhibit the growth of other rapidly spreading fungi, such as species of *Mucor*, *Penicillium* and *Trichoderma*, would make the direct plating method a very practical one. No such materials are known at the present time.

2. Dilution plating. The dilution of small amounts of soil in sterile water, followed by mixing with an agar medium and pouring into petri plates, is a method which may readily be applied to the estimation of the soil fungi which sporulate profusely. The dilutions necessary to obtain readable plates usually make it impossible to detect *Verticillium* in any but very heavily infested soils. To make a quantitative assay of the *Verticillium* present in a 10 acre field at least a thousand dilution plates would be necessary.

Soil from 3 check plots and 5 plots which had received a chemical treatment 2 months previously was diluted with sterile water and plated out in potato dextrose agar to which 50 ppm. of streptomycin nitrate had been added (Table 9).

No colonies of *Verticillium albo-atrum* were observed on any of the dilution plates. When soils from the same check plots were tested by the direct plating method, it was possible to detect a few verticilliate conidiophores. In all probability these were of *Gliocladium* as colonies of this fungus appeared on dilution plates of the same soil. Susceptible plants grown in this soil under greenhouse conditions became heavily infected with *Verticillium albo-atrum* after eight weeks of growth.

Susceptible plants

From the cross-inoculation data it was evident that Bonny Best tomato, Black beauty eggplant and balsam could possibly be used in bioassay studies. To determine which of these plants was most suitable for the detection of *Verticillium* in the soil, a heavily infested

Table 9. Fungi recovered by the dilution method from soil taken from plots in which the untreated soil was known to be heavily infested with Verticillium albo-atrum.

Treatment	Soil dilutions 1/100,000	1/1,000,000	1/10,000,000
Check I	9P, 1R, 3T* 4U	1P, 1R	-
Check II	17P, 5F, 3A, 3U	2P, 1A, 1F, 1G, 1R	-
Check III	13P, 1A, 1G, 1F, 6U	6P, 1R	1P
Allyl bromide	2P, 1T, 1U	-	-
CBP-55	8P, 1T	2P	-
Chloropicrin	3T, 3U	2U	-
Mylone	-	-	-
Vapam	1P	-	-

* The total number of colonies on three replicated plates of each dilution is given with the following abbreviations: A- Aspergillus sp., F- Fusarium sp., G- Gliocladium sp., P- Penicillium sp., R- Rhizopus sp., T- Trichoderma sp., U- unidentified species.

sandy loam was thoroughly mixed and the plants grown in it from seed, transplants and cuttings. Seeds were treated with a commercial protectant, transplants were made using seedlings in the cotyledon stage, and cuttings were treated with a rooting hormone before planting. The plants were maintained for 8 weeks in the greenhouse without supplemental lighting and with temperatures of 60-70°F. After the 8-week period the plants were removed from the soil and isolations were attempted to determine the Verticillium infection index (Table 10).

Eggplant gave the highest infection index with all three propagation methods under the conditions of the experiment. Growing the plants directly from seed appeared to be most favorable for infection. The infection indices for tomato and balsam were comparable for the three methods of propagation, and were generally less than half those obtained with eggplant. It is concluded that under winter light conditions eggplant is the most effective bioassay plant of the three species tested and that growth from seed is the best method of propagation.

Table 10. A comparison of various methods of bioassay for _Verticillium albo-atrum_ in naturally infested soil under winter conditions using susceptible plants.

	Method of propagation		
Plant used	Seed	Transplant	Cutting
Balsam	33*	18	20
Eggplant	80	73	60
Tomato	33	20	20

* Infection index- the number of plants from which _Verticillium albo-atrum_ was isolated, 15 soil samples, 5 plants per sample, calculated as a percentage.

To determine the shortest time necessary for the infection of susceptible plants grown in infested soil, balsam and eggplant were grown from seed in soil samples taken from the check plots of the field disinfestation trials. The plants were maintained in the greenhouse under summer light conditions. Isolations were made from the seedlings 2,4,6 and 8 weeks after sowing (Table 11).

Table 11. Rapidity of infection of seedlings of susceptible plants by Verticillium albo-atrum from naturally infested soil under summer conditions.

Time after sowing	Infection of plants Balsam	Eggplant
2 weeks	0*	0
4 weeks	17	17
6 weeks	50	64
8 weeks	94	100

* Infection index- the percentage of plants from which Verticillium albo-atrum was isolated, 9 soil samples, 5 plants per sample.

A period of 8 weeks is necessary for balsam and eggplant to become almost completely infected by Verticillium when the plants are grown from seed in the summer. After 4 weeks of growth a few of the plants had become infected, after 6 weeks this had risen to over half of the plants from which isolations were made. Under summer conditions there is no significant difference between the rapidity and amount of infection of balsam and eggplant when grown from seed.

A similar experiment was conducted to compare the rate of infection of transplants of balsam and eggplant. Seedlings in the cotyledon stage were transferred to infested soil which had been maintained in an air-dried condition for 5 months. Isolations were made from the plants after 5 and 6 weeks of growth in the greenhouse under spring light conditions (Table 12).

Table 12. Rapidity of infection of transplants of susceptible plants by Verticillium albo-atrum from naturally infested soil under spring conditions.

Time after transplanting	Infection of plants Balsam	Eggplant
5 weeks	33*	65
6 weeks	28	60

* Infection index- the percentage of plants from which Verticillium albo-atrum was isolated, 4 soil samples, 10 plants per sample.

The transplants of eggplant gave a higher infection index than those of balsam under these conditions. This may be due to the fact that soil which had been kept for 5 months in an air-dried condition would contain only the microsclerotial form of the fungus, and these would be present at a lower concentration than the conidial and mycelial forms. Eggplant roots are fibrous and permeate the soil rapidly while those of balsam grow mainly on the surface and are not extensively branched. Roots of eggplant would therefore have a better chance of coming into contact with the microsclerotia. The slight decrease in the infection index after six weeks is not significant and it was concluded that maximum infection had taken place after 5 weeks of growth.

CONTROL STUDIES

Short term crop rotations or fallowing are of little use in eliminating *Verticillium* from the soil. In the course of the investigation trials were conducted to determine which chemicals were effective as soil disinfestants, and the minimum rates of these chemicals which would give adequate control.

Soil disinfestation trials in the field

Five chemicals were applied to the soil in three fields in which infestation with *Verticillium* had been demonstrated by bioassay with susceptible plants and by the fact that a large number of the black raspberry plants in these fields were infected with the fungus. The chemicals were applied at rates which should completely eliminate the fungus from the infested soil. The plots were 6 ft. x 15 ft. and at each farm the five chemical treatments and a check plot were replicated three times.

The soil in the plots was cultivated to a fine tilth to a depth of at least 9 in. prior to the application of the chemicals. The volatile materials were injected into the soil with a hand fumigation gun to a depth of 6 in. at 12 in. staggered intervals. The solid material (Mylone) was evenly distributed over the surface of the soil and thoroughly mixed with it by two successive cultivations. A water seal was then applied by hand or with a sprinkler system. The temperature of the soil was approximately 65°F. at the time of application. Soil samples were taken from the three sets of plots two months after treatment and brought to the greenhouse for bioassay with seedlings of susceptible plants (Table 13, Fig. 6)

Figure 6. The effects of five fungicides on the Verticillium infection index of balsam and eggplant grown in the treated soil. Soil samples taken two months after treatment. Representative plants shown after eight weeks growth.
Check, I - allyl bromide, II - CBP-55, III - Mylone, IV - chloropicrin, V - Vapam.

Table 13. The effects of five fungicides on the Verticillium infection index of balsam and eggplant grown in the treated soil.

Treatment	Rate (lb/acre)	Time after treatment 8 weeks	16 weeks**
Allyl bromide	375	0*	0
CBP-55	350	19	16
Chloropicrin	440	0	0
Mylone	400	0	0
Vapam	440	0	0
Check	-	97	34

* Infection index- the percentage of plants from which Verticillium albo-atrum could be isolated after 8 weeks growth, 6 soil samples, 5 balsam plants and 5 eggplants per sample.
** Plants grown in the winter months.

Complete control of the fungus in the soil was obtained with allyl bromide (375 lb./acre), chloropicrin (440 lb./acre), Mylone (400 lb./acre) and Vapam (440 lb./acre). CBP-55 (350 lb./acre) reduced the incidence of the fungus but did not eliminate it from the soil. Allyl bromide, Mylone and Vapam at these rates completely eliminated weeds in the plots. Chloropicrin was partially effective and CBP-55 did not have any effect on weed growth. *

The apparent decrease in the infection index of the soil taken from the plots 16 weeks after treatment may be attributed to the fact that the growing conditions were less favorable for the infection of the assay plants at this time.

* See Appendix B for details of proprietary materials.

Greenhouse control trials

From the field trials it was evident that CBP-55 was ineffective. To determine the minimum rates of the four remaining chemicals which would control the fungus in the soil, a greenhouse trial was set up, making the conditions as near ideal as possible.

Soil from a heavily infested field was air-dried for 5 months so that the microsclerotia would be the only form of the fungus present. The soil was thoroughly mixed and put through a sieve to remove lumps. The portion of this soil to which the volatile materials were to be added was placed in 5 gallon containers and watered to near saturation. Fumigants were introduced into holes in the soil with pipettes. The holes were then immediately covered and a water seal applied. The solid material was mixed with the dry soil, placed in #10 cans and thoroughly watered. The containers were opened after 5 days at 70-75°F. and the chemicals allowed to diffuse out of the soil. When the majority of the fumes had dissipated the soil was placed in #10 cans and planted with eggplant seedlings in the cotyledon stage. The soil to which the solid material had been added was planted at the same time. The plants were lightly watered and maintained at a temperature of 60-65°F. in the greenhouse. After 6 weeks of growth the eggplants were removed and isolations made (Table 14).

Under these conditions, allyl bromide, Mylone and chloropicrin at a rate of 200 lb./acre eliminated the fungus from the soil, while Vapam was effective at 100 lb./acre. Plants growing in the 500 and 750 lb./acre treatments of allyl bromide, Mylone and Vapam, and the 750 lb./acre treatment of chloropicrin, showed varying degrees of phytotoxicity. Allyl bromide and chloropicrin at the 100 lb./acre rate, and Mylone at the 200 lb./acre rate were effective in completely inhibiting weed growth. Although it would be very difficult to obtain such ideal

Table 14. The effects of four fungicides, applied to the soil at six rates, on the *Verticillium albo-atrum* infection index of eggplants grown in the treated soil.

	Chemical applied			
Rate (lb./acre)	Allyl bromide	Chloropicrin	Mylone	Vapam
50	17*	11	30	10
100	4	2	2	0
200	0	0	0	0
400	0	0	0	0
500	0	0	0	0
750	0	0	0	0
Check	57		46	

* Infection index- the percentage of plants from which *Verticillium albo-atrum* was isolated after 6 weeks growth, 4 replicates per treatment, 10 plants per replicate.

conditions for treatment in the field, it should be possible to use lower rates than those applied in the field trials. Soil temperatures of the range used in the greenhouse would occur only in the summer in Oregon, so it is unlikely that treatments with lower rates of material would be effective if applied in the spring prior to planting.

RESISTANCE STUDIES

The development of small fruit varieties resistant or tolerant to attack by *Verticillium albo-atrum* will very probably provide the ultimate solution to the control problem.

Black raspberries

Five-hundred black raspberry seedlings of the Munger variety were inoculated three times at intervals of about a year with *Verticillium* isolates which had been obtained from infected black raspberry plants. One hundred and eight of the plants survived the three inoculations and 9 of these were found to be infected on isolation. The remaining plants could possibly be used for propagation stock as they appeared to be resistant to the disease. The plants were growing under greenhouse conditions during the course of the experiment, with temperatures of 60-70°F., and this has been shown to bring about a decline in the incidence of the disease during the winter months. Also the plants were somewhat pot-bound and were not in a vigorous state of growth at the time of the third inoculation. Further inoculations will be necessary before the plants can be regarded as resistant to Verticillium wilt.

Strawberries

Plants of several strawberry varieties were examined for the presence of *Verticillium albo-atrum* by isolation. The plants were growing in randomised plots in soil which was known to be infested with the fungus. In most cases there were 14 plots of each variety and 12 plants per plot. Five plants in each plot were sampled by plating two of the outer petioles (Table 15).

Table 15. The susceptibility of several strawberry varieties to infection by Verticillium albo-atrum.

Strawberry variety	Plants infected
Marshall	28/70*
Siletz	4/70
Northwest	4/70
Donner	3/10
Lassen	15/45
Shasta	18/70
Sierra	1/50
2127	21/70
2235	24/70

*Numerator- the number of plants from which Verticillium albo-atrum was isolated: denominator- the number of plants from which isolations were made.

The strawberry varieties Marshall, Lassen, Donner, Shasta, 2127 and 2235 were more or less susceptible to the disease, although the Marshall plants were not declining as seriously as were plants of the other five varieties.

The varieties Siletz, Northwest and Sierra were lightly infected with the disease and might be regarded as fairly resistant under the conditions of the experiment. From the preceding data on Siletz plants grown in a heavily infested soil (Table 2), it is evident that the resistance of this variety is not maintained under conditions of high inoculum potential, although the plants still showed good disease tolerance.

DISCUSSION AND CONCLUSIONS

The fact that Verticillium wilt is an important disease of black raspberries and strawberries in Oregon is undoubtedly due to the extensive cultivation of highly susceptible crops in the affected areas in the first three decades of the century. Potatoes, hops and eggplants may build up the inoculum potential of originally lightly infested soil in a very short time, especially if the plant remains are not removed from the field. The long life of the microsclerotial resting stage of *Verticillium*, in conjunction with the presence of susceptible weeds and the planting of crops mildly susceptible to the disease, has apparently been sufficient to maintain the infectivity of many of the fields in which small fruits are now grown.

Since other diseases may produce symptoms similar to those generally attributed to infection by *Verticillium albo-atrum*, reliable diagnosis of diseased plants in the field is not possible. Certain syndromes, such as bluestem, splitting of the stem, yellowing and wilting of the primocane leaves, in connection with other data such as crop history, make reasonably accurate predictions possible in some cases. In the winter and early spring months the fungus is not generally present in the tissues of Marshall strawberry and Munger black raspberry plants growing in heavily infested soil. This makes diagnosis by isolation unreliable at this time of the year and indicates that the spread of the fungus through infected propagation stock is not an important factor in these crops. Strawberry runners taken from severely affected plantings have been observed to produce apparently healthy plants when grown in uninfested ground (32). Prune and cherry trees also have been reported to recover from infection by the following season (51,61). The reason for the decline of the disease in the winter is not clear.

The symptoms of splitting of the stem, found occasionally in black raspberries growing in ground heavily infested with Verticillium, has not been described previously. The epidermal and cortical regions of the lower stem are split vertically, a condition reported by Van der Meer (61) on maple, red and black currant and gooseberry. The diseased stems became edematous, followed by splitting and exudation of fluid. On microscopic examination mycelium was observed in the xylem vessels on the side where the splitting occurred.

In the host-specificity studies the majority of the isolates tested were infective to the plant species inoculated. Mint, when inoculated, developed symptoms of Verticillium wilt with only one of the isolates. This isolate had originally been obtained from mint and was infective to 5 of the 14 other plant species. Another mint isolate, although infective to mint plants, did not cause symptom development, but was able to infect 8 out of 14 of the other plant species. Thus specialized strains of Verticillium albo-atrum exist, an observation which is in agreement with the findings of Horner (24). Nelson (44) concluded that isolates which did not cause symptoms to develop in mint were not infective, but this was found not to be the case. No evidence of specialization was encountered in the isolates from strawberries and red and black raspberries.

Estimation of the fungus in the soil presents several difficulties. The use of plating methods for quantitative determinations requires the development of a selective medium. These methods have some application to the qualitative detection of the fungus in the soil at the present time. Bioassay by growing susceptible plants in the soil is a method which has been in use for several years (65). This technique has the disadvantage of requiring a longer time and more space than plating. Bonny Best tomato has been used as a test plant but was found to be very unreliable under winter growing conditions in the greenhouse. From the host specificity trials eggplant and balsam were found to be suitable on the basis of susceptibility to all of the isolates tested. In a comparative bioassay experiment, eggplants became infected with *Verticillium* from naturally infested soil more rapidly and completely than Bonny Best tomatoes or balsam plants under winter conditions. It seems that the susceptibility of eggplant is not affected by the unfavorable growing conditions, and there does not appear to be such a long period of resistance in the seedling stage of eggplant as with Bonny Best tomato.

Short term crop rotations have been recommended as a means of eliminating the fungus from the soil (39, 81). In the present study the observations on the crop histories of black raspberry plantings affected with *Verticillium* indicated that rotations with non-susceptible crops did not control the disease, which is in agreement with the conclusions of several other workers (19, 45, 65).

Chloropicrin at a rate of 500 lb. per acre has been reported to eliminate *Verticillium* from the soil (73). In the course of this investigation chloropicrin, Mylone, allyl bromide and Vapam were all found to be effective at rates of about 400 lb. per acre. The cost of applying the chemicals at this rate would be prohibitive to most growers. Greenhouse trials using the same materials under controlled conditions showed that complete control could be obtained with lower dosages. The chemicals were applied to moist, sieved soil which was maintained for several days at a relatively high temperature. These conditions are almost impossible to obtain in Oregon fields in the early spring months. To have even moderately good soil conditions it is necessary for the grower to postpone the date of planting by several weeks. In small fruit plantings the chemicals should be applied in the late summer when soil conditions are best for chemical disinfestation. The diseased plants would have to be removed immediately after harvest and the soil treated while conditions for the application of volatile materials were still favorable. Efficiency of the more volatile fumigants such as chloropicrin might be improved by covering the treated ground with tarpaulins after applying the water seal.

From isolation data and field observations it is apparent that the severity of infection of a crop is related to the amount of Verticillium in the soil. This is also in agreement with the conclusions of Keyworth, who found that severity of infection is related to the inoculum potential in the roots of hop plants, which would be proportional to the amount of fungus in the surrounding soil (31). Apparently healthy black raspberry plants were found to be infected with the fungus in several cases where the soil was lightly infested. In moderately infested soils mild symptoms were usually apparent, but growth was not greatly impaired. In heavily infested soil the plants usually showed characteristic symptoms and poor growth. These observations indicate that it may be possible to reduce, but not necessarily eliminate, the fungus from the soil and still obtain a satisfactory commercial control. This would require smaller amounts of chemicals than are necessary for complete control. Total elimination of the fungus from the soil, although desirable, is not economical with the materials available at the present time, and does not appear to be essential. Soil disinfestation by the application of chemicals would be more valuable if carried out in conjunction with deep plowing, as the fungus commonly occurs to a depth of 2-3 feet (65).

Soil disinfestation is not a permanent control measure and the development of resistant varieties in the future is to be hoped for. Resistant strawberry varieties are known but are poorly adapted to growing conditions in the Northwest. If a suitable variety could be produced, several years would be required for it to displace the well-established Marshall variety on the market unless the fruit characteristics were similar.

Incorporation of the resistance of some clones of the wild Pacific Coast trailing blackberry into black raspberry will probably prove to be the most profitable approach in developing a variety resistant to Verticillium wilt, although this may be a difficult task if the character of the fruit is to be maintained.

SUMMARY

1. Verticillium wilt was found to be an important disease of black raspberry and strawberries in Oregon. Red raspberries and the other commonly grown trailing berries were not severely affected by the disease.

2. Although certain symptoms are usually associated with the presence of the disease, it was not possible to make a reliable diagnosis in the field. Root rots, water soaking and insect damage produced symptoms similar to those usually attributed to Verticillium infection.

3. A crop history of potatoes, black raspberries or hops may build up the inoculum potential of the fungus in the soil. Other susceptible crops and weeds appeared to be responsible for the maintenance and the possible introduction of the fungus. Short term rotations with a non-susceptible crop did not eliminate the fungus from the soil.

4. Studies on the incidence of infection in propagation stock indicated that spread of the disease through this means may not be a serious threat in Marshall strawberries and Munger black raspberries. The fungus could not be isolated from plants in heavily infested soils at the time of propagation.

5. The inoculation of a range of susceptible plants with isolates of Verticillium from different sources demonstrated a low degree of host specificity of the fungus.

6. The presence of Verticillium in the soil was determined most reliably and rapidly by growing transplants of eggplant in soil samples

in the greenhouse. Isolations could be made after five weeks of growth under conditions of at least 12 hours of daylight and 60°F.

7. Vapam, Mylone, chloropicrin and allyl bromide at rates of about 400 lb. per acre were found to control the fungus in the soil in field trials. Greenhouse tests indicated that lower rates might be effective in the field if conditions at the time of application were sufficiently favorable for the fungicidal action of these chemicals.

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Key to index numbers of isolates used in cross-inoculation studies.

Index number	Plant source	Location	Remarks
B-3-O*	Black raspberry	Corvallis	Non-sclerotial[x]
B-5-Mu	Black raspberry	Multnomah Co.	
B-6-W	Black raspberry	Washington Co.	
B-7-W	Black raspberry	Washington Co.	
H-4-C*	Hop	British Columbia	
H-4-L*	Hop	Polk Co.	
Mn-5-S*	Mint	Marion Co.	Symptoms in mint
Mn-51-H*	Mint	Marion Co.	No symptoms in mint
Mn-51-N*	Mint	Marion Co.	
Po-5-S**	Potato	Klamath Co.	
Po-6-C*	Potato	Deschutes Co.	
Po-9-H**	Potato	Klamath Co.	
St-9-O*	Strawberry	Marion Co.	Non-sclerotial
St-1-D	Strawberry	Yamhill Co.	
Mp-11-O	Maple	Corvallis	Non-sclerotial
Py-40-H*	Peony	Multnomah Co.	
Sn-43-H*	Snowberry	Marion Co.	
Mw-45-H*	Mallow	Marion Co.	Non-sclerotial
R-50-F*	Red raspberry	Corvallis	Non-sclerotial
T-32-C	Tomato	Central Oregon	
L-1-G***	_Lotus corniculatus_	Douglas Co.	_Verticillium intertextum_ Isaac.

* Supplied by C.E. Horner, Oregon State College.
** Supplied by W.J. Tolmsoff, Oregon State College.
*** Supplied by C.M. Leach, Oregon State College.
x Sclerotia not formed when isolate grown on potato dextrose agar.

Technical names, activities and sources of proprietary materials referred to in the text and tables

Trade name of chemical	Technical name of chemical	Activity	Source
Allyl bromide	3-bromo-1-propene	100%	The Dow Chemical Co.
CBP-55	chlorobromopropene	55%	Shell Chemical Corp.
Chloropicrin	trichloronitromethane	100%	Larvicide Products Inc.
Mylone-85W	3,5-dimethyltetrahydro-1,3,5,2-thiadiazine-2-thione	85%	Carbide and Carbon Chemicals Co.
Vapam-4S	sodium N-methyl dithiocarbamate	4 lb./gallon	Stauffer Chemical Co.