THE INFLUENCE OF QUANTITY OF INOCULUM ON THE SEVERITY OF
VERTICILLIUM WILT

by

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I am grateful for the data recorded by many workers which have substantiated our interpretations of Verticillium wilt. I hope that none will take offense to the reinterpretations offered, and I hold myself responsible for any reinterpretations which in the future may prove erroneous. It is sincerely hoped that these reinterpretations lead to further progress in understanding and controlling this disease and are not merely wasted effort serving to further confuse our understanding of the pathological problem.

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INTRODUCTION

The acreage of land planted to potatoes in Oregon has not changed substantially during the past 58 years; approximately 30,000 to 40,000 acres have been planted annually from 1900 to 1958. The major variety of potatoes presently grown in Oregon is the Russet Burbank (Netted Gem) variety.

During the early part of the present century potatoes were grown primarily in the Willamette Valley in western Oregon where irrigation was not required for their cultivation. With the availability of irrigation, three new potato-producing areas developed and potatoes are now grown in the Ontario area in eastern Oregon, in central Oregon and in the Klamath basin in southern Oregon.

One of the limiting factors in potato production in the Willamette Valley during the first two decades of the present century was Verticillium wilt (70, 71, 72), a disease caused by the fungus Verticillium albo-atrum Reinke and Berth. The destructiveness of this disease was temporarily left behind when potato production moved to new areas, but Verticillium wilt is now a limiting factor in the production of potatoes in central Oregon and the Klamath basin (150, 151, 152).

Approximately 10,000 to 12,000 acres of potatoes are grown annually in the Klamath area of southern Oregon. Verticillium wilt was estimated to have reduced yields by at least 500,000 sacks

(100-pound sacks) of potatoes in the Klamath area during 1957 (152). This was considered to be a conservative estimate of yield losses.

The Verticillium wilt disease did not appear suddenly in the Klamath area and all evidence indicates that many years of continuous potato culture were required for the disease to become severe. Many fields which 30 years ago produced between 300 and 500 sacks of potatoes per acre now produce less than 200 sacks per acre in spite of improved cultural practices. Fields which have been in potato production fewer years produce correspondingly larger yields. The severity of symptoms in the plants is closely correlated with the number of crops of potatoes raised on the land. This correlation between disease severity and cropping history has been reported in other potato-producing areas (10, 84).

The relationship between wilt severity and cropping history and the fact that effective control of Verticillium wilt was obtained by soil fumigation, although plants on treated soil were infected (150, 151, 152), led to the investigations reported in this thesis. These experiments were designed to determine the relation between inoculum potential of Verticillium in the soil and the severity of Verticillium wilt in potatoes.

LITERATURE REVIEW

<u>HISTORICAL</u>

Reinke and Berthold (91), in 1879, described a disease of potatoes in Germany and the fungus associated with the symptoms they observed. The fungus was described as a new species of the genus <u>Verticillium</u> and named <u>Verticillium</u> <u>albo-atrum</u> Reinke and Berthold. They showed conclusively by inoculation experiments that at least part of the symptoms, which they assumed to be associated with a single disease, were due to the invasion of potato plants by their newly described fungus.

Reinke and Berthold (91) believed that there were at least three types of disease on potatoes incited by <u>Verticillium</u> <u>albo-atrum</u> which they designated types A, B and C. Form A first appeared in the plants in July and was characterized by a yellowing and withering of the older leaves with a progressive upward development of these symptoms. Rolling of the leaves and formation of brown foliage spots frequently but not always occurred. The vascular system was yellow and filled with mycelium that remained confined to the vascular elements until the infected part died, whereupon the fungus invaded the dead tissues.

Form B, like form A, was said to appear during the middle of July and the above-ground symptoms were essentially the same in both forms. The main distinction between the two was that form B caused the underground parts of the stem and larger roots to develop large brown spots and fissures in the bark tissue. Form B was further

characterized by the presence of _Verticillium_ in the roots and its complete absence from the stems. Forms A and B were said to occur separately or in combination on the same plant.

Form C of the disease was believed to arise when tubers produced by plants affected by forms A or B were planted. Form C was characterized by the sudden wilting and death of young shoots, resulting in elimination of the disease since new tubers were not produced by these plants and since it was believed that the pathogen overwintered in infected tubers. Form C was further characterized by glassy appearing stems that were fragile and broke easily when bent. These symptoms described for form C did not always develop when tubers were planted from hills affected with forms A and B. Frequently healthy plants grew from these tubers, or other forms of the disease reappeared.

At the time that Reinke and Berthold were working on Verticillium wilt, plant pathology was still in its infancy. With the exception of late blight, caused by _Phytophthora infestans_, none of the economically important diseases of potato had been recognized or described. There had been a condition of potatoes in Europe since the earlier part of the nineteenth century which had commonly been called Krauselkrankheit in Germany. Rudolph (95) pointed out that this term had been used collectively to include potato diseases in general and that it included several distinct potato diseases. Reinke and Berthold believed that Krauselkrankheit was a single potato disease with various manifestations. It is not surprising, therefore, to find in their description of Verticillium wilt the symptoms that

properly belong to several distinct diseases which were described and characterized as separate diseases 30 to 40 years later.

Reinke and Berthold encountered difficulty in reproducing disease symptoms of the various forms of what they believed to be Verticillium wilt. Inoculation experiments with V. albo-atrum frequently failed to produce any symptoms in potato plants. They interpreted their results as indicating that the fungus had to spend a given number of generations passing from parent plant to the tubers before certain forms of the disease appeared. When tubers were taken from diseased hills and planted in the field, some of the symptoms which they were unable to produce by inoculation with Verticillium appeared in the new plants. They believed their observations substantiated their interpretation and did not consider the possibility of plants contracting Verticillium wilt or other diseases from natural sources in the field. They believed that tuber transmission was the primary mode of perpetuation of Verticillium wilt.

Reinke and Berthold observed that Verticillium gained entrance to the tubers through the stolons, but they were unable to find the fungus in the tubers at depths greater than 15 mm. from the point of attachment to the stolon. It was believed that this was the location at which the fungus overwintered within the tuber. In attempting to explain the mode of disease transmission from the tuber to the new plant it is significant that Reinke and Berthold did not believe that Verticillium grew directly throught the tuber and into the new shoots. Instead, they claimed that it grew back out of its stem-end location

within the tuber by the path through which it entered. It was believed to then penetrate only the cork layer of the tuber and grow along the cork layer until reaching a shoot which it penetrated, invaded and killed. Dark mycelium was found on the surface of tubers, and it was believed that this observation substantiated their interpretation of tuber transmission.

Among the many manifestations of the disease as originally described on potatoes was a rapid collapse and wilt of young plants. This has proven very misleading to later workers who have not realized that in all probability this symptom was associated with some disease other than Verticillium wilt. Those who have looked for this symptom in Verticillium wilt have been unsuccessful in finding it. This symptom has occasionally been attributed to the disease without sufficient evidence that there was a relationship between infection of young potato plants by Verticillium and rapid wilt and collapse of the host. Pethybridge (86) and Rudolph (95) realized that the slowly progressive type of wilt and death of plants as described by Reinke and Berthold was probably the type associated with Verticillium wilt. Their own experiences with the disease led them to suggest that the term "Verticillium wilt" be abandoned in preference for "Verticillium hadromycosis" since the wilting phenomenon is not always the most obvious symptom of the disease under natural conditions.

There were two major errors made by Reinke and Berthold (91) in their original description of the Verticillium wilt disease. One of

these errors was the assumption that most of the disease symptoms they observed were induced by V. albo-atrum. In view of the fact that Verticillium wilt can occur in potatoes in combination with virus-induced diseases, blackleg, Rhizoctonia, and other diseases, it is apparent how Reinke and Berthold could have interpreted these various diseases as different manifestations of Verticillium wilt, particularly since these other diseases of potato had not yet been described.

The other major error made by Reinke and Berthold was the assumption that since potato tubers were infected by Verticillium, tuber transmission of the disease would necessarily be one of the primary modes of disease perpetuation. These two errors have had a strong influence on the concepts of the Verticillium wilt disease and on the subsequent areas of investigation.

A number of observations recorded by Reinke and Berthold serve to support the concepts of the Verticillium wilt disease as they are to be presented in this thesis. They are: (a) the first appearance of symptoms typical of Verticillium wilt occurred near the middle of the growing season; (b) symptoms appeared on the older, lower leaves first; (c) typical disease symptoms could be present without appreciable, if any, amounts of fungus mycelium in stems; (d) in the tubers, the fungus was localized in the stem-end; (e) not all tubers from diseased hills contained Verticillium; (f) the fungus did not penetrate through the tuber in gaining entrance to

new shoots from infected tubers; and (g) difficulty was encountered in obtaining disease symptoms upon reinoculation with Verticillium.

TIME OF INFECTION

Little effort has been made to determine the time of infection of host plants by Verticillium under natural conditions. The general opinion on the time of infection is well illustrated by Rudolph's (95, p. 268) statement that, "Infection may take place at any time during the growing season,--". This statement was made without supporting data. Referring to the disease in potatoes, Waggoner and Taylor (123, p. 168) followed different lines of reasoning and stated that, "Verticillium wilt symptoms first appear on the plants in the field during late July or early August. Therefore, inoculation must occur during June." Pethybridge (86) had conflicting ideas on the time of infection, and although he was unable to find Verticillium in potato plants until after several weeks of growth had been made---even when infected tubers were planted---, he stated that infection could take place in potatoes during any stage of growth.

All of the published data suggest that natural infection occurs at specific stages of plant development, or that the host is far more susceptible to infection during certain stages of development than at others. This would appear to be true for many diverse plant species grown in widely separated areas.

Reinke and Berthold (91) reported that typical Verticillium wilt on potatoes first appeared during the middle of the growing season. Pethybridge (86) was unable to find mycelium in potato stems until several weeks after tubers had been planted. He was unable to detect invasion of plants for an extended period of time even when they were grown from tubers that were known to be infected. McKay (71) was not able to isolate Verticillium from roots or stems of potato plants with any degree of consistency until several weeks after planting. Even when viable inoculum was in direct contact with young plants, infection had not taken place. Ludbrook (68) felt that there was some unexplained reason for the delay of wilt symptoms on potatoes grown in infested soil in the greenhouse. He believed that this delay in symptom expression could not be explained on the basis of soil or air temperatures or soil moisture, and his data substantiated this view. McLean et al. (75) reported that various varieties of potatoes grown in the field did not show symptoms of wilt until later stages of plant development. Young (150) reported that symptoms first appeared several weeks after planting tubers.

Occasionally, abnormal plants that grow much taller and appear exceptionally vigorous develop in plantings of Netted Gem potatoes. Nielsen (84) reported that these variants made good growth and showed no symptoms of Verticillium wilt until late in the season, while adjacent non-variants were severely wilted. The variants blossomed later in the season and required a longer growth period to develop yields comparable to those produced by the normal, severely wilted

plants. These findings could indicate that tuber formation and infection by Verticillium were delayed in the variants, even though environmental conditions were favorable for infection and disease development earlier in the season.

In greenhouse experiments, Tolmsoff and Young (117) found that potatoes grown in artificially infested soil were not infected until tuber formation was initiated.

Dana (16) reported that "sunburn" of tomato fruit was a chief cause of yield losses in plants infected with Verticillium. As disease developed, foliage no longer shaded the fruit and, apparently, disease developed after fruit had been set.

Guba (30) reported that eggplants grown in the field did not show symptoms of Verticillium wilt until after fruit had been set. In an extensive breeding program in which resistance or tolerance to Verticillium wilt was the objective, symptoms developed later in the season on late flowering varieties than on early flowering varieties. Sterile varieties showed no wilt at all or only mild symptoms late in the season.

Snyder and Rudolph (105) infested soil with V. albo-atrum and observed that pepper plants grown in the soil did not show symptoms of wilt until approximately ten weeks after planting. Kendrick and Schroeder (50) obtained wilt of muskmelon grown in infested soil six weeks after planting. Isaac (44) found that brussels sprouts did not show symptoms of wilt in the field until three to four months after planting.

Tompkins and Ark (119) reported that strawflower did not show symptoms of Verticillium wilt until after flowers had been set. This was about five months after plants had been transplanted to the field, and symptoms developed uniformly over fields after flowers were set.

Cotton grown in California is reported to make normal growth and show no symptoms of wilt until early summer (37). Available data (64, 102) on soil and air temperatures in New Mexico and California during the growing season show that the temperatures during the early part of the growing season would be expected to produce more severe disease than occurs later in the season---providing that the plants were infected earlier in the season. Presley (89) stated that Verticillium could attack cotton in any stage of growth, but his illustrations showing susceptible and tolerant varieties growing side by side in the field reveal that the susceptible varieties made almost normal growth prior to being defoliated by Verticillium wilt. Arndt (4) was unable to isolate Verticillium from cotton seedlings grown in artificially infested soils for 21 days with temperatures of 18 to 24° C, which should have provided optimum temperatures for disease development.

Strawberry plants are reported to show the first symptoms of wilt after blossoms have been set and usually during the fruiting season. This is reported from areas as widely separated as British Columbia (74), Oregon (87) and California (116).

Raspberries grown in Canada (6), Washington (62) and Oregon (87, 153) first showed symptoms of wilt during the flowering and fruiting season.

The presence of mummified fruit on apricot trees affected by Verticillium was reported by Goheen (23), which would indicate that disease developed during the fruiting season.

Harris (34) reported that Verticillium wilt of hops occasionally appeared while the cones were half-grown, but normally appeared while the fully developed cones were ripening.

Van der Meer (121) was the first to suggest that certain perennial hosts had to be reinfected through the roots annually for disease symptoms to reappear. He observed that entire cherry trees or branches could recover the year following a severe case of wilt, and the discolored xylem was confined to the growth made during the year of the severe attack. A severe case of wilt in cherries could be perpetuated or increased in severity by planting tomatoes between the trees, but interplanting with non-susceptible crops resulted in decline of wilt symptoms in subsequent years.

Rudolph (95) reported a similar situation with prune and peach trees in which symptom expression was associated with the growing of tomato plants between trees, but trees could recover entirely in following years and discolored xylem was confined to growth made during the year of the severe attack. Rudolph also believed that annual reinfection may be necessary for reappearance of wilt symptoms.

Zentmeyer (154, 155) reported the recovery of avocado trees following a year of symptom expression, and here again wilting appeared to be correlated with the interplanting of trees with tomato plants. Liming (65) observed that elm trees frequently recovered from Verticillium wilt after a year of symptom expression.

Hops have perennial roots that develop new stems annually. Harris (35) found that hops frequently showed wilt one year but not the following. Keyworth (54, 56) isolated from many hop stems for several consecutive years and found that hills infected one year were frequently uninfected the next.

Raabe and Wilhelm (90) inoculated rose plants resistant to Verticillium wilt by the root-dip method and obtained invasion and mild symptom expression during the first year, but the same plants were free of Verticillium the second year after inoculation.

Wilhelm (142) inoculated strawberry plants by dipping the roots in a spore suspension. Plants were grown in the greenhouse for a period of time and it was shown by isolations from petioles that the plants were infected. These plants were then set out in the field and the following season isolations from the same plants showed that infection had declined by 78 per cent. Pratt (87) recovered Verticillium readily from strawberry petioles during the summer, but had difficulty in recovering it from petioles of the same plants during the winter.

Pratt (87, p. 46-47) transferred raspberry plants severely affected by Verticillium wilt to the greenhouse. Under temperature conditions favorable to wilt development, incidence of disease

declined during the winter.

Two phenomena appear to be consistently associated with
Verticillium host-parasite relationships under field conditions:
(1) the delay in symptom expression on many distantly related host
species until the growing season has progressed considerably, and
usually until the time of flowering or fruiting; and (2) the apparent
recovery of perennial hosts in years following severe attacks. There
is a complete lack of data in the literature suggesting that under
natural conditions hosts are infected during early stages of develop-
ment. There are no reports in which Verticillium was known to be the
cause of wilting and dying of seedlings in the field. Yet, under
conditions of artificial inoculation in which active fungus mycelium
was brought into direct contact with host tissue, wilting has fre-
quently occurred within several days.

Bewley (8, 9) and Rudolph (95) obtained wilting of tomato
seedlings nine days after inoculation of the roots with a spore and
mycelium suspension. Virgin and Maloit (122) and Scheffer et al.
(101) obtained wilt of tomato seedlings eight days after inoculation
by the root-dip technique. Schaible et al. (100) reported initial
wilt symptoms on tomato seedlings one week after root-dip inocula-
tion, and pronounced stunting of plants occurred within three weeks
after inoculation. Using the root-dip inoculation method on cotton
seedlings, Wiles (130) observed wilting in seven days, Presley (88)
within 14 days, and Robinson et al. (94) in 10 days. Using this
same method of inoculating eggplant and cucumber, Robinson also
obtained wilting of these hosts in ten days. Zentmeyer (154, 155)

reported wilting of avocado seedlings within two weeks after inoculation by the root-dip method. Symptoms of wilt appeared in two weeks over a soil temperature range of 15 to 30° C. Webb and Schultz (125) obtained wilting of potato seedlings in 12 to 30 days using root-dip inoculation.

There is no reason to believe that the host is resistant to infection during early stages of development in the field while being susceptible during similar stages under experimental conditions. Nor is there reason to believe that environmental conditions are unfavorable for infection or disease development in the field while the plants are young. Temperature records (64) reveal that temperatures were more favorable for disease development early in the growing season of cotton, but disease developed in the older plants under less favorable environmental conditions.

The fact that infection is readily obtained by artificial inoculation of seedlings, by the root-dip method, but apparently does not occur or is unimportant in seedling infection under field conditions suggests that some factor is present in artificial inoculation and absent in the field. With artificial inoculation by the root-dip method, mycelium and conidia of Verticillium are brought into direct contact with host tissue. There is no experimental evidence showing that viable mycelium or conidia of Verticillium are present in soil in the field. On the contrary, Wilhelm (137, 140, 141, 143) has stressed the importance of the resistant microsclerotia as the surviving infective units in the field. Wilhelm (137, 143) reported

that <u>Verticillium</u> <u>albo-atrum</u> is incapable of making saprophytic growth in unamended soil, and mycelium and conidia were found to be very transient under natural conditions. Luck (67) confirmed this and reported that mycelium was non-infective after relatively short incubation periods in soil. Some of Isaac's (41) and McKeen's (73) data can be interpreted as showing the transiency of conidia and mycelium in the soil.

Therefore, it is likely that the host-parasite relationships are different in natural infection and in artificial inoculation. Without the presence of viable mycelium and conidia in field soil, infection might result from root excretions stimulating germination of the dormant, resistant microsclerotia and resting mycelium. It is conceivable that stimulation of germination might occur at certain stages of host development, and this could explain the reason for delayed invasion of hosts under natural conditions.

The area immediately adjacent to plant roots, known as the rhizosphere, profoundly influences the development of soil organisms (13, 15, 66, 113, 114, 126). Both qualitative and quantitative differences are present in the rhizosphere microflora as compared to soil further from the roots and the predominant types of organisms present in the rhizosphere change with age of the plant (13, 15). Kerr (52) and Kerr and Flentje (53) have recently shown that chemical stimuli are very likely to be involved in host infection by <u>Pellicularia</u> <u>filamentosa</u>. Sequira (104) found that spores of the banana-wilt <u>Fusarium</u> were induced to germinate by materials diffusing

from roots. Buxton (13) recently showed that the rhizospheres of different pea varieties had a striking selective action on physiologic races of Fusarium oxysporum f. pisi, and the selective rhizosphere effects were associated with susceptibility of the pea varieties to wilt.

Nematologists are becoming aware of the intricate relationships between plant-parasitic nematodes and the hosts that the nematodes feed on. With several nematode diseases the host plant stimulates the hatching of dormant eggs and attracts the larvae (24, 127, 128, 129, 149). Widdowson and Wiltshire (127) recently showed that potato roots excrete water and acetone soluble materials believed to be organic acids. The root exudate caused maximum stimulation of hatching of the potato-eelworm cysts with concentrations as low as 0.1 mg. active material per liter of water (.1 ppm). The stimulatory effect upon hatching changed with age of the plants. Different hosts stimulating the same nematodes are believed to produce different stimulants in some cases (24).

Wilhelm et al. (147) reported that seeds of an obligate parasite, Orobanche ramosa L., (the broomrape plant) were stimulated to germinate in the presence of tomato roots. Apparently, the stimulatory effect was very localized in the soil, because planting three successive crops of tomatoes into infested soil and preventing reproduction of the parasite did not result in a successive decrease in numbers of infections with succeeding crops.

The phenomenon of a host plant stimulating its own invasion by a parasite is not new and there is reason to believe that this condition may exist with the Verticillium wilt disease. Otherwise, it is difficult to explain the prolonged survival of microsclerotia in field soil in the absence of susceptible hosts (141, 131), the delayed invasion of plants grown in the field under conditions favorable for disease development, the abundance of infections occurring with a small volume of naturally infested soil (132, 136), and to relate these facts with the transiency of conidia and mycelium in soil (67, 137, 140, 141, 143). If infections occurred at random from microsclerotia germinating at random, the inoculum potential of Verticillium would be expected to be very unstable and should not accumulate appreciably; however, this has not been the case (10, 30, 37, 64, 84, 87, 102, 132, 152).

DEGREE OF INVASION

There are different degrees of host invasion and symptom expression with Verticillium wilt. This has remained one of the most characteristic features of the disease since it was first described by Reinke and Berthold (91). Rudolph (95) was aware of this peculiarity of the disease and stated that, (95, p. 212) "The disease is peculiar in that it may attack any suscept in all parts, or it may be confined sharply to one side or even to only one shoot or limb of the affected plant," and (95, p. 220) "In the leaves, the disease picture is analagous to that seen in the stems. The infection may

be sharply localized or it may involve the entire organ. In some instances, only a lobe of a leaf or only one leaflet of a compound leaf may become diseased."

This peculiarity of Verticillium wilt has been observed by nearly all workers, and attempting to explain the phenomenon has been a center of much speculation and investigation. The phenomenon has not yet been adequately explained. Many workers have controlled the degree of wilt in experiments, but have not appreciated the significance of their results as related to the disease as it develops in the field.

Zeller (153) reported that unilateral symptom expression by raspberry plants could be traced to infection of a limited number of roots on the same side of the plant to which symptoms were confined. When infected roots were distributed around the plant, symptoms were more general over the entire host rather than being confined unilaterally.

Keyworth (60) found that the severity of wilt in hops was correlated with the extent of root infection, and that the extent of root infection determined the extent of stem invasion. The degree of root invasion did not influence the upward growth of the fungus in the stems, but it did influence the quantity of the vascular system invaded.

From the reports of Zeller (153) and Keyworth (60) alone, it can be seen that the severity of wilt is influenced by the quantity of the root system invaded or by the number of roots infected. It might

then be assumed that by controlling the number or quantity of roots infected, infection could result in a range of disease severity from symptomless infection to a very severe wilt. Much of the data in the literature support this view when reinterpreted.

Harris (35) isolated from many hop plants taken from infested fields, but isolations were made from symptomless plants as well as those showing symptoms. He concluded that most of the hop plants in fields were infected whether they showed symptoms or not. He believed that nearly all hops were infected, but that some special soil conditions were required for symptom development. In the absence of these special conditions infected plants failed to develop symptoms. Keyworth (56) confirmed the findings of Harris, and also concluded that the number of infected hop plants was not represented by the number showing symptoms. He ruled out the possibility that late infection was responsible for the high percentage of symptomless infected plants, and, in other experiments, Isaac and Keyworth (47) showed that a mild infection could remain mild throughout the growing season. Keyworth (54) also believed that some soil factor determined whether infected hop plants showed symptoms, but he showed that the soil factor was not soil moisture as Harris (35) had believed.

Keyworth conducted a number of other experiments showing the influence of root invasion on disease severity. In grafting experiments (60, 61) a susceptible and a tolerant variety of hops were used. The tolerant variety became infected at the same time as the susceptible variety and the fungus progressed up the stem in both

varieties at the same rate, but the tolerant variety failed to show
symptoms of wilt or showed only mild symptoms. Susceptible and
tolerant varieties were each grafted on their own rootstock, and,
in addition, tolerant tops were grafted on susceptible rootstock
and susceptible tops were grafted on tolerant rootstock. Soil inocu-
lations were made with a virulent isolate of Verticillium and the
illustrations (60) show that the results obtained were consistent.
The susceptible variety showed severe wilt when on its own root-
stock but it showed mild wilt when it was grafted on tolerant
rootstock. The tolerant variety showed mild wilt when grafted on its
own rootstock but it showed severe wilt when grafted on susceptible
rootstock. Keyworth also found that the root system of the tolerant
variety of hops was not as extensively invaded as the root system of
the susceptible variety.

Keyworth (60, 61) believed that the stems of both tolerant and
sensitive varieties were highly resistant to invasion by
Verticillium. He suggested that extensive stem invasion and acute
symptom development on 'sensitive' root systems might result from:
a lowering of stem resistance by root impairment or by diffusion of
fungal toxins; an increase in the invasive power of the pathogen
through the establishment of a 'food base'; or the production of a
'fungal accelerator'.

Talboys and Wilson (108), in an extensive series of graft
experiments, confirmed Keyworth's findings that disease severity
was influenced by the degree of root invasion. They were unable to

demonstrate translocation of specific 'resistance' or 'sensitizing'
factors to explain severe wilting arising from 'sensitive' root
systems and mild symptoms resulting from 'tolerant' root systems.
They suggested that a study of the initial phases of root infection
might give valuable information helping to explain differences in
degree of host invasion. In a series of reports by Talboys
(109, 110, 111), data were presented to show that wilt severity in
hops is determined during the infective phase of disease develop-
ment. Thus, the reports by Harris (35) and Keyworth (54, 56) that
disease development is influenced by some soil factor are sub-
stantiated since infection arises from the soil.

Isaac and Keyworth (47) and Keyworth (60) concluded that there
are two strains of Verticillium that attack hops. The mild strain
is reported to show moderate to mild symptoms of wilt on suscep-
tible hops and no symptoms on tolerant hops. The more virulent
strain caused severe wilt on susceptible hops and mild symptoms
on tolerant varieties. Using various methods of inoculation
it was shown that the severity of wilt was influenced by the method
of inoculation and not by the strain of the pathogen. Mild symptoms
were obtained on susceptible hops with both strains when inoculum
was injected into the vascular system of the stem. Severe symptoms
were obtained on the susceptible variety of hops with both strains
when they were injected into the pith of stems. Two isolates of
each strain were used and when the experiment was repeated, results
were the same.

In selecting varieties of hops for resistance to wilt,
Keyworth (55) tested resistance in soil that was artificially
infested with diseased hop stems. All varieties were found to be
infected, but the degree of symptom expression differed markedly
between varieties with some showing no external symptoms. These
varieties were then tested for resistance in fields that had pre-
viously produced hops with different degrees of wilt. In soil that
previously had the most severe wilt, all varieties showed symptoms,
while in soil that previously had a mild case of wilt results were
similar to those obtained in the artificially infested soil.
Keyworth interpreted these results as possibly showing the presence
of different strains of Verticillium in different fields.

Some of Keyworth's conclusions and beliefs conflict or are not
complimentary to each other. Thus, he believed that the severity of
wilt is associated with the extent of root invasion (60, 61), but
also stated that Verticillium grows in the soil (58). He believed
that a single infection is sufficient to cause severe wilt (60), but
found that plants could become invaded by a virulent strain of the
pathogen and show very mild symptoms (60). He believed that some
soil factor influenced disease development (54, 55), but showed that
the method of inoculation was important in determining wilt severity
(60). It was stated that the stemsof hop plants are highly resistant
to invasion by Verticillium (60, 61), but the pathogen was shown to
develop upward rapidly in inoculated stems (60).

As will be pointed out later, Keyworth's results and observations can largely be explained on the basis of the influence of inoculum potential which, apparently, was not obvious to him because he believed that Verticillium grows in the soil. However, Keyworth's experiments were well designed and much valuable information has come from them. His finding that the extent of root invasion is associated with disease severity is important because many of the reports in the literature on Verticillium wilt can be interpreted as showing the influence of extent of root invasion in disease development.

Other workers have tried fewer methods of inoculation which has resulted in a more limited range of symptom expression. Haenseler (31) and Ludbrook (68) were unable to obtain wilt of pepper with inoculations of many Verticillium isolates. Bewley (8) obtained mild symptoms of wilt with soil inoculations, while Snyder and Rudolph (105) obtained severe wilt of pepper with heavy soil inoculations.

Wilhelm (142) showed that susceptible varieties of strawberry would exhibit symptoms of wilt after a single root-dip inoculation, while moderately resistant varieties showed wilt after plants were inoculated a second time, and resistant plants did not show wilt after the third inoculation. Resistant plants were frequently infected after the third inoculation but did not show symptoms of wilt in the greenhouse. When planted in field soil heavily infested with Verticillium, resistant plants occasionally showed mild to moderate wilting.

Van Adrichem and Orchard (120) reported that three successive root-dip inoculations of strawberry crosses at eight-week intervals resulted in wilt of susceptible lines after one inoculation. Plants with moderate resistance showed wilt after the second root-dip inoculation. Plants showing only mild symptoms after the third inoculation were classified as being tolerant to Verticillium wilt and those showing no wilt after the third inoculation were classified as being resistant.

Rudolph (95) made over 250 stem inoculations on apricot trees with Verticillium from apricot, tomato, peach and raspberry. Some of the isolates produced a definite streaking of the wood and could be recovered above the point of inoculation but not more than five of these inoculations were successful in producing even mild symptoms. The isolate originally from diseased apricot did not produce symptoms. In another experiment, 300 inoculations were made in the canes of healthy raspberry plants with the same isolates but none of the inoculations produced symptoms.

Bewley (8) inoculated tomato plants by hypocotyl wound-inoculations, internode wound-inoculations and by planting tomatoes into artificially infested soil. He observed that (8, p. 119), "The hypocotyl and internode inoculations were the first to show typical disease symptoms but the soil inoculations, although longer in producing first symptoms, produced a complete wilt as soon as the hypocotyl inoculations. Internode inoculations---the fungus travelled up one side of the plant first and produced a wilt on this

side only." In another experiment, using hypocotyl and soil inocu-
lations, only the data obtained from the soil inoculations were
presented because (8, p. 120), "----these being the more comparable
with those of plants naturally infected in nurseries."

Caroselli (14) was able to influence the severity of wilt on
maple by controlling the quantity of inoculum injected into trunks
of trees. The greater the quantity injected, the more severe the
wilting symptoms were. Inoculations made lower on the trunks were
more effective in producing wilt than inoculations higher up.
Inoculations of petioles and leaf-blades failed to produce symptoms.

Gravatt (27) encountered difficulty in obtaining consistent
wilt symptoms when maple was inoculated with various isolates of
Verticillium. He believed that there was a multitude of strains
of the pathogen, each possessing a different capacity to produce
symptoms. Haenseler (32) observed a similar situation in inocu-
lation of peach trees with isolates of Verticillium obtained from
wilted peach trees. It was difficult to obtain wilt symptoms and
Haenseler concluded that very special conditions were required for
infection. Winter injury to plants was suggested as being a pre-
requisite for infection. Winter injury is not required for
development of peach wilt in California (95). Haenseler con-
sidered infection and symptom expression to be synonymous as so
many workers have done.

Nelson (82, 83) considered infection and symptom expression
to be synonymous. This led him to the conclusion that the mint

strain of <u>V. albo-atrum</u> was specific in its pathogenicity to mint and that other strains of <u>Verticillium</u> were incapable of infecting mint. Horner (38, 39) has shown that this is not true and that the mint strain is capable of infecting and existing in other hosts without symptom expression, and that other strains are capable of infecting mint with no symptom expression. Thomas and Webb (115) reported an isolate of <u>Verticillium</u> from potatoes capable of inducing wilt in peppermint, although symptoms were milder and somewhat different from those induced by the peppermint strain.

Snyder et al. (106) reported that certain weeds could be symptomless hosts of <u>V. albo-atrum</u>, and this has been observed by other workers. Wilhelm (144) pointed out that symptomless invasion of nightshade (<u>Solanum sarachoides</u>) was associated with the invasion of very few roots. Wilhelm and Thomas (138) traced high levels of inoculum in strawberry fields to a previous build-up on symptomless, native nightshade weeds.

Rudolph (96) showed that flax is a host of <u>Verticillium</u>, but symptoms of disease were very subtle with stunting the most obvious symptom. Discoloration of vascular tissue did not occur.

Workers (1, 26, 76, 86, 101) have reported wilt in terms of vascular discoloration without sufficient proof that it is closely correlated to incidence or severity of wilt. Rudolph (96), Snyder et al. (106), Baker et al. (5), Wilhelm (144), and other workers have mentioned several hosts in which vascular discoloration was not a symptom of disease or infection. McKay (71, 72) pointed out that

this symptom is not reliable for determining either the presence or absence of Verticillium in tubers of potatoes, but vascular discoloration of tubers has been used in reporting incidence and severity of wilt in potatoes (1, 26, 86). Vascular discoloration has frequently been used in recording data when other symptoms were not consistent or obvious.

Luck (67) found that mint plants could be infected by the mint strain of Verticillium without exhibiting any of the symptoms that are very characteristic for Verticillium wilt in mint. Symptomless invasion was believed to be due to factors other than environmental because some infected plants showed symptoms and others did not when they were grown adjacent to each other. Attempts to determine the nature of this apparent "resistance factor" were negative.

Hawkins and Higgins (36), in the first report of Verticillium wilt on cotton grown in Georgia, reported that symptoms were very mild, and yields were not reduced on infected plants. All plants cultured from had the pathogen.

Pratt (87) found that more than 30 per cent of the symptomless raspberry plants in a field were infected with Verticillium.

Folsom (20) was unable to get wilting of tomatoes with inoculation of Verticillium. Roberts (92, 93) and Wilhelm (132) reported that the absence of wilt symptoms in tomatoes was not a reliable indication of the absence of V. albo-atrum from such plants. Kadow (48) isolated Verticillium from the vascular tissue of tomato fruits grown on symptomless plants. Pegg (85) found that hyaline

"strains" of Verticillium could be present in the vessels of hosts
without causing external symptoms. Isaac (42, 43, 46) used dif-
ferences in pathogenicity between isolates as further reason for
ranking the isolates as individual species. He observed that certain
"species" could be present in the vessels of hosts without causing
external symptoms.

Nelson (83) found that the severity of symptoms on mint at the
time of isolation of Verticillium was not an indication of the
severity of symptoms which that isolate would produce upon reinocu-
lation into mint. Isolates obtained from mint showing very mild
symptoms were often among the most virulent when reinoculated, while
isolates obtained from severely wilted plants were frequently among
those that produced mild symptoms when reinoculated.

Felix (19) observed wilt of spinach in New York and isolated
V. albo-atrum from wilted plants, but several inoculation attempts
failed to produce wilt in spinach. Apparently, isolations were not
made to determine if infection had occurred without symptoms.

Varieties of tomatoes have been developed which, under condi-
tions of artificial inoculation by root-dipping, were resistant
or tolerant to Verticillium wilt (100). Other resistant varieties
of tomatoes have been developed in which the resistance broke down
(17, p. 410) "----under conditions very favorable to the develop-
ment of Verticillium wilt." This situation bears a striking
resemblance to that on hops already described by Keyworth (55).

Kendrick and Middleton (51) developed a disease index for Verticillium wilt of pepper in which the length of growth made between nodes was associated with the degree of plant resistance. Robinson et al. (94) found that varying the quantity of inoculum while root-dipping potato plants had a marked effect on the degree of stunting and time of appearance of wilt symptoms. Since all plants were inoculated at the same time, infection would have been expected to occur at the same time with the various levels of inoculum.

Reinke and Berthold (91) found that inoculations of potato plants with V. albo-atrum produced erratic results and frequently the inoculated plants failed to show symptoms. Apparently, it was not determined if the symptomless plants were infected. Robinson et al. (94) reported that in many cases isolations from potato plants yielded Verticillium when the plants showed no symptoms.

McKay (70, 71, 72) investigated Verticillium wilt of potatoes extensively. In his many field trials in which plants were artificially inoculated by placing infested plant material over the seed-piece, the results of wilt were invariably mild and in some seasons Verticillium wilt could not be distinguished from normal maturity. Most of his experiments (71) were repeated two and three times, not for confirmation but just to obtain wilting of plants. Even after two or three years of trials on separate experiments, results were disappointing.

McKay (71) was inclined to attribute the mildness or absence of symptoms in infected plants to "poor years for wilt", but he failed to describe a "good year for wilt". In some such "poor years for wilt", he obtained severe wilting of plants where inoculum was well distributed around the plants within a radius of 15 in., while inoculum placed in contact with seed-pieces produced mild wilt (71). McKay felt that these results were anomalous and repeated a similar experiment in another "poor year for wilt" and again obtained the anomalous results in which wilt was mild where inoculum was well situated for early infection, but poorly distributed for multiple root infections. Wilt was the most severe where inoculum was distributed in such a manner as to allow a greater infection area in the roots. These results are in good agreement with those described by Keyworth (60) who found that the severity of wilt was associated with the extent of root invasion.

McKay (70, 71, 72) changed from the use of stem isolations to tuber isolations because too many symptomless plants were infected in the field. Thus, frequency of stem invasion was not correlated with symptom expression. Apparently, this was confusing to McKay, and he lost faith in the use of symptom expression for recording disease severity. He states that (71, p. 466), "The percentage of tubers infected rather than the appearance of the plants during the growing season is used as the index for judging the extent of infection from the soil because it is the more reliable method." Thus, McKay's (70, 71, 72) data on tuber

transmission, field spread, and control through crop rotation are based on tuber isolations and do not represent disease severity since McKay pointed out that the association between appearance of plants and the results from tuber isolations was not constant. McKay was aware that Verticillium could be present in potato plants without inducing symptom expression but believed that this resulted from infection occurring late in the season, (71). Rudolph (95) also believed this, but neither worker presented data to support this view.

Ludbrook (68) carried out extensive field and greenhouse experiments in which numerous hosts and many isolates of Verticillium were employed. He obtained 46 isolates of Verticillium of the microsclerotial and "dauermycelium" types from several foreign countries and various areas within the United States. Using 11 isolates he inoculated 20 to 40 plants of potato, tomato, pepper, cucumber, eggplant and several woody hosts in the field during two consecutive years. Sand-cornmeal inoculum was placed near the seed or transplant. Inoculum was favorably located for early infection and infection occurred, as determined by isolations, but symptoms failed to develop with potato, tomato, pepper and cucumber while moderate to very mild symptoms appeared on eggplants. The results were the same during both years in which the trials were conducted. When these same isolates were used in greenhouse inoculations with inoculum mixed in the soil, eggplant and tomato wilted

severely while potato showed moderate wilt. Studies of optimum and maximum soil and air temperatures and optimum soil moisture for disease development in the greenhouse showed that severe wilt would have been expected in all of the hosts under the field conditions that prevailed if these environmental factors had played a major role in disease development following infection. Ludbrook (68, p.153) concluded that, "The difficulty experienced in obtaining the disease in the field at temperatures below the experimentally derived maximum leads to the belief that some factor or factors other than temperature must have lessened the susceptibility of the field-grown plants to the disease." There is no reason to believe that the susceptibility of the field-grown plants in Ludbrook's experiments was reduced. The results of Ludbrook (68), McKay (71) and many others can be explained on the basis of the quantity of the root system invaded by Verticillium.

Since the time that Verticillium wilt was described by Reinke and Berthold (91), there has remained a baseless assumption that still prevails. There have been no data to support the assumption, it has seldom been stated, but it appears to have been accepted by all who have worked with the disease. This assumption implies that a single infection of a host plant by Verticillium is as effective in causing disease symptoms as multiple infections. This assumption has led many workers to use stem inoculations which have frequently failed to produce wilt symptoms or have produced only mild symptoms. Keyworth (60) obtained severe wilting when Verticillium was

inoculated into the pith of hop stems, but with naturally infected plants it is doubtful if the pith becomes invaded until the plant is dead.

The assumption that a single infection is as effective in causing disease as multiple infections appears unreasonable when one considers that Verticillium grows predominantly longitudinally in the vessels (64, 95). Multiple root infections would then be expected to result in a greater quantity of the vascular system infected or a greater quantity of the fungus within the host. Since there are definitely different degrees of disease, these could be the result of having different quantities of the pathogen within the host. There are no data to support the belief that a single infection will continue to develop until the host is as thoroughly invaded as would result from multiple infections. Keyworth (60) pointed out that a mild infection in hops remained mild throughout the growing season.

Although data were not presented to substantiate the statement, Schneider (102, p. 141) concluded from his work on Verticillium wilt of guayule that, "There are also some indications that multiple infection are more serious than a single infection."

Talboys (110) recently reported the results of a histological study of hop roots during early stages of infection by Verticillium. The study included two varieties of hops, one highly susceptible and the other highly resistant, and two strains of Verticillium, one being mildly pathogenic and the other a virulent strain. Cortical

tissues appeared to offer some resistance to penetration of either pathogen in either host, but the mild pathogen was resisted more than the virulent one and the more tolerant host offered more resistance than the susceptible one.

Apparently, the resistance offered by cortical tissues was of a temporary nature or was a feable resistance because Talboys believed that the primary site of resistance was in the endodermis. The more resistant variety had a greater capacity to form suberized endodermal cell-walls in response to the presence of the pathogen than the susceptible variety. The suberized endodermal walls were found to delay invasion of the vessels and, frequently, prevent it. The virulent pathogen was capable of overcoming the suberized barrier more frequently than the mild strain. When the endodermal barrier was overcome the vessels were invaded.

The results of Keyworth (60), in which the mild and virulent strains caused equal wilting when inoculated into hop stems in equal quantities, are supported by the results of Talboys (110). Thus, the primary reason for differences in wilt severity between the varieties of hops studied is that fewer vessels become infected by Verticillium in the more resistant variety because of fewer successful root invasions.

It is possible that with a high level of inoculum in the soil successful penetrations to the vessels would be more frequent even in the resistant variety, and severe disease might result. This would effectively explain the differences in disease severity among

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highly resistant varieties of hops when planted in fields with past
histories of various degrees of disease severity. Keyworth (55)
found that many varieties of hops that were highly tolerant to
Verticillium infection when grown in artificially infested soil
showed wilt when planted in fields with previous histories of
severe wilt.

Although a rapid wilting of leaves is not a typical symptom of
Verticillium wilt on 'sensitive' varieties in the field, Talboys
(109) reported that this occurred without the preliminary chlorosis
when hops were grown in soil that contained a high concentration of
infested plant debris. Varieties of hops tolerant to Verticillium
wilt also developed acute symptoms under conditions of very heavy
soil inoculations (108).

CURRENT SEASON SPREAD OF VERTICILLIUM

Reinke and Berthold (91) suggested that Verticillium might
spread from hill to hill in potatoes during the growing season, and
in this manner one infected plant could serve to infect a number of
surrounding plants. This would imply that Verticillium had some of
the properties of a soil saprophyte although they did not believe
that it was capable of surviving in the soil for very long. This
is well illustrated by the significance they placed on tuber trans-
mission as a means for perpetuation and spread of the disease.

McKay (71) probably realized that his data did not support the
popular belief that Verticillium wilt is commonly tuber transmitted.
In some of his experiments the number of infected hills grown from

non-infected tubers was not significantly different from the number
of infected hills grown from infected tubers. McKay explained that
these data indicated that Verticillium had spread from infected
hills grown from infected tubers to healthy hills grown from
non-infected tubers. When experiments were designed to show spread,
a number of "poor years" for wilt were encountered and "spread" was
negligible. It should be pointed out that McKay's (71, p. 450;
72, p. 10) illustration of current season spread is definitely an
exception, as shown by his data. The figure shows a single inocu-
lated potato plant surrounded by four non-inoculated diseased
plants. McKay believed that Verticillium had spread from the
inoculated plant to the four adjacent non-inoculated plants during
the growing season. This was the only such case involving five
adjacent wilted plants during two years of experiments in which
more than 600 inoculated and uninoculated plants were grown side by
side.

Furthermore, McKay's (71) data show that in all probability
the occurrence of five adjacent wilted plants was the result of
natural field infestation by Verticillium. Some of McKay's experi-
ments were conducted in fields that had been planted to potatoes
only four to five years previously, and it was believed that this
was "clean soil" because McKay believed that Verticillium was
capable of surviving in the soil for only one or two years. However,
it should also be pointed out that McKay's methods did not allow the
detection of Verticillium in the soil for more than one year.

Without realizing the limitations of his techniques, McKay concluded that the fungus could survive for only one or two years in field soil.

McKay (71) believed that _Verticillium_ was capable of spreading in the field to a distance of 45 inches from the point of inoculation during the growing season. His data show that if it is capable of spreading this distance, it is also capable of spreading 75 inches because there was no significant difference in the number of infected hills located these distances from known sources of inoculum. From his data, it would appear that he arrived at this figure on distance of spread arbitrarily. Contrary to McKay's claims, his data substantiate the view that _Verticillium_ does not spread from plant to plant appreciably, if at all, during the growing season.

Under more carefully controlled conditions Roberts (92), Isaac (42) and Luck (67) have shown that _Verticillium_ does not spread to neighboring plants until after the infected plant dies. Then spread is believed to result from the growth of roots from neighboring plants into the area formerly occupied by the infected plant. This concept is important for it implies that _Verticillium_ does not grow through the soil as mycelium.

Wilhelm (143) stated that (p. 696), "Under the conditions of the experiments performed, independent saprophytic growth of _Verticillium albo-atrum_ could not be detected in three natural field soils during two months of winter." Isaac (42) confirmed this,

finding that neither the microsclerotial nor "dauermycelium" types of Verticillium grew through soil.

Garrett (22) believed that "apparent spread" of wilt diseases during the growing season was more apparent than real. He attributed "apparent spread" to a diminishing concentration of inoculum from a central point, but interpreted this as indicating later infection progressively further from the central point.

Some workers (71, 91) believed that current season spread resulted from the contact of roots between plants. Available data (42, 67, 92) show that this does not occur until after the infected plant is dead. But, when dead, the infected plant could serve as inoculum to infect neighboring plants without growth of the fungus through the soil.

Apparently, Keyworth believed that Verticillium is a soil inhabitant for he stated that (58, p. 537), "The fungus grows in the soil----." There are no data to support this view and, undoubtedly, Garrett's (22) classification of the fungus as a soil invader--- capable of invading the soil through the hosts which it attacks, but incapable of inhabiting the soil as a saprophyte----places the fungus in the proper group of soil-borne organisms.

SEED AND TUBER TRANSMISSION AND DISSEMINATION OF VERTICILLIUM

Rudolph (98) obtained fruit from 164 tomato plants that were severely wilted by V. albo-atrum. More than 26,768 seeds were taken from these fruits for isolations following surface sterilizing. A

total of two seeds were found to be infected by <u>Verticillium</u>. It was not shown that the infected seeds were capable of producing diseased plants, but Rudolph believed that seed transmission of <u>V. albo-atrum</u> by tomatoes was unimportant if it occurred at all. He did not state whether the seeds were removed from the fruit as they normally would be by commercial seed producers.

Kadow (48) allowed naturally infected fruit of tomato and eggplant to ferment prior to isolating from the seeds. He found that a very high percentage of both tomato and eggplant seeds were infected following this process. It was not determined if the infected seeds were viable or if they would produce infected plants providing that they were viable.

Guba (30) reported that Verticillium wilt was not seed-transmitted by eggplant but he did not present the data that might have led him to this conclusion.

Burton and deZeeuw (12) attempted to settle the question of whether <u>Verticillium</u> is seed-transmitted by eggplant. They tested over 12,000 seeds of six different varieties from six major seed-producing areas in the United States. They failed to detect <u>Verticillium</u> in any of this seed. They found that naturally infected eggplants yielded infected fruit but the seeds in these fruit were not normally infected. In one case, seeds from a decaying fruit were found to be infected but greenhouse and field tests failed to produce seed transmission of the disease from infected seed.

Tompkins and Ark (119) reported that Verticillium wilt was not seed-transmitted by strawflower. Isaac (44, 46) found that Verticillium was not seed-borne on brussels sprouts and other vegetables but particles of plant refuse mixed with the seed contained considerable contamination by the pathogen.

Rudolph and Harrison (99) isolated from various parts of 3,371 cotton bolls and found that 4.44 per cent of the receptacles were infected but none of the seeds were infected. Miles (77, 78) reported that Verticillium was not seed-transmitted by cotton. Seed grown on the Atlantic coast and planted in that area did not produce diseased plants, while seed from the same source produced diseased plants in widely separated areas in Greece. Presley (89) reported that Verticillium was not seed-transmitted by cotton.

Taubenhaus (112) isolated the fungus from 199 cotton seeds out of 1,441 seeds that were tested. By artificial inoculation, Allen (3) showed that infected cotton seeds remained viable but it was not determined if the infected seeds would produce diseased plants.

The source of propagation stock with vegetatively propagated plants such as raspberries (62, 87), strawberries (59, 87) and mint (67) has been shown to have relatively little influence on disease development. Even when such plants were taken from diseased fields a considerable lapse in time---generally several years---was required before wilt became obvious or severe. This would be about the same length of time required for the disease to appear in crops that are grown from seed where it is believed that

the disease is not seed-transmitted. Keyworth and Bennett
(59, p. 309), working with strawberries, concluded that, "It is thus
clear that the performance of the runners was unrelated to the dis-
ease status of their parent plants. It was obviously greatly
influenced, however, by the disease status of the ground on which
they were planted." Pratt observed with strawberries that
(87, p. 50), "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."
It was not made clear whether this was determined on the basis of
isolations or symptom expression. However, Pratt found that runner
plants could become infected from either the mother plant or the
soil by the time that they formed new roots. Pratt also found that
tip plants of raspberry produced by infected mother plants were free
of infection, as determined by isolations.

Despite all of the work that has been done on seed transmis-
sion of Verticillium wilt and transmission through propagation
stock, little can be said about the influence of these plant struc-
tures on disease transmission and dissemination. The available data
indicate that seed transmission of Verticillium probably plays only
a minor role in dissemination of the pathogen, particularly with the
current use of seed-protectant fungicides. The available data also
indicate that under certain conditions infected fruit can produce
infected seed, but it still remains to be determined, under carefully
controlled conditions, whether infected seed will produce infected

plants. Since infection and symptom expression have so frequently been considered synonymous----and it was pointed out that this is not true----absence of symptoms have frequently been interpreted as indicating absence of infection. For this reason, much of the seed transmission work is of doubtful value because it only shows that symptoms of disease were absent but it does not show whether Verticillium was absent. It is likely that the amount of inoculum disseminated on seed would not suffice to produce symptoms even if it was enough to cause infection.

One of the most widely accepted beliefs concerning transmission of Verticillium wilt is that the disease is tuber transmitted by potatoes. Reinke and Berthold (91) based much of their work and their description of the disease on this assumption. They found the fungus in tubers to a depth of not greater than 15 mm. from the point of attachment to the stolon. In attempting to explain how the fungus moved from this location to the new shoots, they said that it grew back out of the tuber through the same path by which it entered. It was believed to then penetrate the cork layer on the outside of the tuber and grow along the cork layer until reaching a new shoot. Reinke and Berthold knew that Verticillium was not capable of growing through parenchyma tissue, and the fact that they were able to find the fungus only in the stem-end of tubers must have led them to believe that the fungus passed from the tuber to new shoots by the route they described. They said that dark mycelium was found on the surface of planted tubers, and they believed that this substantiated

their interpretation without appreciating that the dark mycelium could have belonged to any of a number of soil fungi.

Other workers (86, 95) have expressed the improbability of tuber transmission occurring as Reinke and Berthold described it. The idea of tuber transmission was not dropped, however. Only the concepts on how the fungus moved from an infected tuber to the new shoots have been modified, and most workers appear to have accepted the conclusions of Pethybridge (86).

Pethybridge (86) was not satisfied that Verticillium was localized in the stem-end of tubers for he observed that when tubers were cut in half, into eye-ends and stem-ends, and planted in the field, both ends of the tubers produced equal numbers of diseased plants. Without considering that infection could have come from the soil, Pethybridge concluded that the fungus must be distributed throughout the tuber in the vascular system from the stem-end to the eye-end. He believed that he had demonstrated this through three observations. One was the fact that either end of the tuber was capable of producing diseased plants when grown in the field. A second observation was that when an infected tuber was cut near the point of stolon attachment, Verticillium grew out of the vascular tissue of the tuber but this did not demonstrate the depth to which the fungus had penetrated. The third observation was that when tubers were sliced lengthwise and observed under the microscope, "mycelium" was found distributed the entire length of tubers. In the first place, observing a sliced tuber the entire

length with the optics available at that time and discerning
mycelium from other structures that resemble mycelium within a
tuber would have been questionable. Secondly, it is a well-known
fact (70, 71, 72) that many fungi inhabit the interior of tubers
and Pethybridge did not make isolations from the interior of tubers
to identify the mycelium, which he assumed to be Verticillium.

Another weakness of the tuber transmission theory as proposed
by Pethybridge (86) is that for the fungus to move from the tuber
to the new shoots, the tuber must remain intact. He found that
potato plants in the field grown from infected tubers did not
become infected until after several weeks growth, but he did not
determine if the tubers had decayed by this time.

It is not known whether the small quantity of Verticillium
present in an infected tuber is capable of surviving through the
decomposition process of the tuber in the soil. Rudolph (95)
pointed out that the pathogen plays no role in this decomposition
process and it is highly improbable that the fungus increases
during decomposition because of its poor competitive nature in the
presence of other soil organisms as pointed out by Wilhelm (137,
143). The preceding section on current season spread also empha-
sizes that Verticillium is a poor soil saprophyte. During
decomposition of certain types of organic matter in the soil,
(118, 134, 136) even relatively small quantities of organic matter
have been found to greatly reduce the population of microsclerotia.
It is therefore doubtful whether the small quantity of Verticillium

in an infected tuber would survive the concentrated decomposition occurring in a decomposing potato tuber and this inoculum may never be available to infect roots after it is released from the tuber.

McKay (70, 71, 72) advocated the tuber transmission theory of Verticillium wilt of potatoes. His influence on the subject can be seen by the many references citing his conclusions regarding the importance of tuber transmission. McKay accepted the conclusions of Pethybridge and believed that Verticillium was distributed throughout the tuber. It is interesting that McKay isolated from over 12,000 tubers to determine if they were infected with Verticillium but invariably isolations were made from the stem-end where the presence of many other fungi frequently interfered with detection of the pathogen.

McKay (70, p. 822) set up an experiment that should have shown the influence of tuber transmission on Verticillium wilt. Tubers were obtained from healthy and wilted potato fields from several locations in Oregon and from fields in Michigan, Wisconsin and Colorado. Tubers from these various locations were planted on newly cleared land after it was determined by isolations that they were infected or non-infected with Verticillium. Data were taken and isolations were made from tubers but none of these data were published showing the influence of tuber transmission.

In another experiment McKay (71, p. 440) states that, "---, in the 1917 tests plants grown on clean soil from Verticillium-infected seed gave 29.7 per cent of Verticillium infection in the yields; the

seed potatoes from which only 'miscellaneous fungi' were isolated gave 13.3 per cent, and those from which no organisms were isolated gave 18.9 per cent." By "clean soil" McKay meant fields that had not grown potatoes for at least four to five years. He considered the fungus to survive for only about one year in the field, and he stated that some of his experiments were conducted in fields that had grown potatoes as recently as four to five years previously. His results and some of the field diagrams (71, p. 443 and 446) on pattern of recovery strongly suggest that there was natural infestation of the soil which was believed to be free of <u>Verticillium</u>.

McKay (70, 71) and Pethybridge (86) found that not all tubers infected with <u>Verticillium</u> produced infected plants, but neither adequately explained this. Pethybridge suggested that lightly invaded tubers might produce healthy plants, while extremely invaded tubers would produce diseased plants.

McKay's (70, 71) results agree with those of Pethybridge (86) in that the stem-end of tubers planted in the field produced about the same amount of infected hills as eye-ends. McKay found that stem-end halves produced hills in which 22.7 per cent of the yield was infected and 24.6 per cent of the yield from eye-end halves was infected. It remains, however, to be shown that <u>Verticillium</u> is distributed throughout the tuber. Reinke and Berthold (91), Spieckermann (107) and others have been able to find the fungus in only the stem-end of tubers near the point of attachment to the

stolon. There are no data to show that the pathogen is normally
distributed through the tuber, and the results obtained by
Pethybridge and McKay in which either end of the tuber produced the
same number of diseased hills could be explained on the basis of
infection occurring from the soil. The data of both of these
workers show conclusively that the soil in which tubers were
planted had a greater influence on disease development than did the
source or part of the tuber planted.

Robinson et al. (94) attempted to determine the relative
importance of infected tubers in transmitting the disease as com-
pared to tubers with surface contamination. They concluded from
one experiment that (94, p. 32), "---surface inoculation caused
most of the wilt present and that vascular infection of seed pieces
was of little importance." A second experiment led them to the
conclusion that (94, p. 33), "This is further evidence that, at
least under some conditions, surface-borne inoculum of Verticil-
lium gives rise to far more wilt than does mycelium within the
vascular ring." Their data show that the amount of disease
resulting from planting internally infected tubers was negligible
and could easily be attributed to infection from the soil rather
than from the tubers. No mention was made of the severity of wilt
resulting from surface contamination of tubers. Dilutions were
made from the soil and plant debris taken from a sack used in
harvesting a field of wilted potatoes. More than 10 million viable

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particles of _Verticillium_ were present on the surface of a bushel of potatoes. These workers also claimed that _Verticillium_ grew from internally infected tubers into new shoots, but their data do not substantiate this claim and no conclusive evidence was presented to support it other than the claim.

Lefebvre (63) reported that potatoes grown on new land in Alaska were infected with _Verticillium_. Certified seed tubers from the States had been used for the planting.

Gratz (26) observed that the planting of tubers in Florida taken from severely wilted fields in Maine did not result in diseased plants in Florida. Temperature records over a period of 10 years showed that during the first two-thirds of the growing season for both areas average air temperatures were almost identical. Gratz concluded that if soil or air temperatures had influence on disease development between the two areas, and if the disease was tuber transmitted, Florida-grown potatoes should also have severe wilt.

Studies on tuber transmission of Verticillium wilt have invariably been carried out in the field where it has been assumed that natural infestation of the soil was absent. As a direct result of this assumption, the belief that internally infected tubers would necessarily produce infected plants has persisted without supporting evidence since 1879 when Reinke and Berthold (91) described the disease.

One aspect of tuber infection proves interesting, although no attempts have been made to explain it. McKay's (71) data show that tuber infection is largely independent of disease symptoms on the rest of the plant. Some plants showing severe wilt produced a normal number of tubers of which none were infected. The reverse was also true in that symptomless plants frequently produced a similar number of tubers of which nearly all, or all, were infected. This would imply that the general assumption that the fungus moves from infected stems to the tubers may not be true, and that infection of the stolons may occur independently of the rest of the plant or that some other unrecognized factor influences tuber infection. McKay (71) found that stolons were among the last part of a potato plant to become infected.

There has been very little direct evidence linking transmission of Verticillium wilt with infected seed or propagative plant parts such as runner-plants, stolons or tubers. Still, when the disease appears on a crop, it generally appears over a wide area that has had a similar cropping history. A few examples are: raspberries grown in the state of Washington (62), Oregon (87, 153) and in Canada (6); cotton in California (37), Arizona (89), Mississippi (76) and Greece (77); eggplants in Massachusetts (30); strawberries in New York (11), California (116, 146), Oregon (87), and British Columbia (74); tomatoes in California (95); potatoes in Oregon (152), Idaho (84) and Massachusetts (10); hops in England (54, 56); strawflower in California (119); cherries grown in association with

tomatoes or potatoes in Europe (121); and many others. Studies
of transmission have not adequately explained the appearance of the
disease over wide areas at about the same time. If the disease is
seed-transmitted it would be a very slow process as shown by
transmission studies, and it would reasonably be expected to occur
in localized areas once the organism was introduced to the soil.
However, this has not been the case.

Presley (89) expressed the opinion that Verticillium was
indigenous to the soils of Arizona. Lawrence (62) believed the same
to be true for soils in the state of Washington. Wilhelm (132, 140)
believed that infected plant debris and inoculum at the soil surface
could be blown long distances by wind. Isaac (46) found Verticil-
lium to be air-borne during the winter near fields that had produced
diseased crops. Isaac (44) also found that plant debris associated
with seed carried infective particles, and Robinson (94) found that
soil adhering to potato tubers taken from diseased fields carried
the pathogen in large quantity. Bewley and Buddin (7) found
Virticillium in water supplies. It would appear that all of these
factors could play an important role in the dissemination of
Verticillium. However, once the fungus was disseminated over an
area----by whatever means----its longevity could in effect make it
indigenous to an area. The development of disease over wide areas
as related to common cropping histories of fields could be related
to the build-up in concentration of the pathogen in the soil. This
would effectively explain why there is generally a delay period

before disease becomes severe in an area where a highly susceptible crop has been grown for only a few years. Verticillium wilt in its milder forms on many hosts could easily go undetected in an area until the pathogen become concentrated enough to cause economical losses and attract attention.

This interpretation would also explain the fact that with perennial woody hosts such as Avocados (154, 155), peaches, prunes (95), olive (106), cherry (121) and others, the disease severity appears to be related to the previous planting of annuals such as tomato or potato. These annual crops return considerable quantities of inoculum to the soil and continued growth of these between and under the woody hosts has resulted in more severe diseases on the woody hosts. However, when interplanting the annuals between the woody hosts was discontinued, disease severity declined.

Possibly, woody plants do not offer a suitable substrate for build-up of the pathogen, inoculum from such plants may not reach the proper zone in the soil to cause root infections, or the type of decomposition occurring with woody tissues returned to the soil might result in a decrease of the Verticillium inoculum present in and on them. With very few exceptions, tree hosts normally do not build up the disease potential (95, 106, 121, 154, 155), but instead the disease severity decreases in subsequent years even though the trees become infected. With semi-woody hosts such as hops (54, 56) and guayule (102), disease severity appears to increase or decrease slowly or remain about constant by continuous

cropping. Herbaceous crops such as tomatoes (141), potatoes (10), eggplant (30), mint (67), and many others tend to increase the disease potential of soils relatively rapidly under favorable environmental conditions.

Thus, severity of Verticillium wilt is almost invariably associated with the previous planting of annual herbaceous crops. Until it is demonstrated that seed transmission of Verticillium occurs commonly, the majority of data support the conclusion that the pathogen is widely distributed in nature and that its distribution is not related to agricultural areas, although its concentration is. The correlation between wilt severity and cropping histories of annual crops has been pointed out by many workers (10, 30, 54, 84, 87, 96, 106, 121, 141, 155).

DISEASE SEVERITY AS INFLUENCED BY QUANTITY OF INOCULUM

A complete lack of available estimates on the quantity of inoculum normally present in soils differing in disease potential has been the major obstacle to appreciating the influence that quantity and distribution of inoculum have on disease severity. No attempts have been made to determine the number of separate infections occurring on diseased plants in the field, and it has been generally accepted that a single infection is sufficient to induce severe disease.

Although Wilhelm (132) interpreted his data as showing the influence of inoculum potential on the number of infected plants,

they can be interpreted as showing the number of separate infections that might occur on a single plant grown in a small quantity of naturally infested soil. A biological assay for determining the relative inoculum potential of soils was devised. Each of many soils was assayed by placing soil in four 8-inch pots and planting ten tomato seedlings per pot. After six to eight weeks, isolations were made from the stems of all plants. A number of soils produced greater than 90 per cent infection in the 40 test plants and some produced approximately 100 per cent infection.

Assuming that each infection was represented by an invaded stem, a minimum of 9 to 10 infections occurred in each 8-inch pot of soil where 90 to 100 per cent of the test plants were infected. The soil in four 8-inch pots would hardly be the quantity to which the roots of a single vigorous plant would be exposed in the field. With 36 to 40 test plants becoming infected in this amount of soil, and with many of the plants undoubtedly having multiple infections, the amount of inoculum and the number of separate infections occurring per plant in the field when disease is severe might be much greater than previously anticipated.

In other experiments, Wilhelm (136) grew 100 test plants per 1.5 cu. ft. of soil. In unamended, naturally infested soil Verticillium was recovered from about 88 per cent of the plants. When the naturally infested soil was diluted at a ratio of 1:25 with disinfested soil 22 plants became infected, which would indicate that as many as 550 separate infections might occur from

1.5 cu. ft. of the undiluted, naturally infested soil. Similarly, when naturally infested soil was diluted at a ratio of 1:500 with disinfested soil, Verticillium was recovered from one plant which would indicate that the 1.5 cu. ft. of undiluted soil might cause 500 separate infections.

Wilhelm's (136) data show that the association between inoculum potential and frequency of recovery of Verticillium from the stems of young tomato plants is not a linear relationship. The relationship appears to become logarithmetic with increasing level of inoculum but, possibly, this relationship would change with age of the plants. Wilhelm's data show that naturally infested soils are capable of causing numerous infections per plant when the inoculum potential is relatively high.

Bewley (8) obtained 50 single-spore isolates of V. albo-atrum from diseased tomatoes in England and classified them into six groups on the basis of the quantity of microsclerotia and dark mycelium produced. Group I produced the least microsclerotia and dark mycelium, Group II produced more than Group I, Group III produced more than Group II, etc., and Group VI produced the most. When the "pathogenicity" of these isolates was tested by planting potato, eggplant, snapdragon, cucumber, sycamore, cotton, pepper and elm into soil infested by these isolates, disease severity was found to be related to the quantity of microsclerotia or dark mycelium produced. Bewley reported that (8, p. 125), "---there is some indication that the virulence of the strains is related to the

ability to produce carbonised hyphae and microsclerotia. Group I
was uniformly slow in producing the characteristic wilt, while
Group VI was most rapid----." The average number of days to wilt
symptoms was 31.3 days with Group I and 16 days with Group VI.
The average number of days until death of plants was 65 days with
Group I and 40 days with Group VI. The other groups produced wilt-
ing and death of plants at time intervals between the two extremes.
With some hosts, symptoms of wilt were absent but all plants were
infected, as shown by isolations.

Nelson (83, p. 83) stated:

"It has been observed that the most aggressive iso-
lates of the mint wilt pathogen produce sclerotia abun-
dantly. Conversely, those that are weak producers of
microsclerotia are likely to be less strongly pathogenic.
They usually induce disease symptoms after a more prolonged
incubation time, the symptoms are milder and the plants are
less severely injured.

(p. 82) "Of the many isolates of Verticillium cultured
from wilting peppermint and spearmint plants, comparatively
few have been non-pathogenic when tested for infectivity.
The pathogenic isolates have, however, displayed consider-
able variation in virulence. Some are only weakly para-
sitic, while others are much more aggressive and kill the
host plant in a comparatively short time. These variations
are not correlated with the conditions of the diseased
plant from which they were cultured. Some of the weakly
parasitic forms were cultured from plants that displayed
the most severe symptoms of wilt.

(p. 82) "Long continued culture of the most patho-
genic mint isolates on laboratory media has sometimes been
followed by a partial impairment in virulence or even com-
plete loss of infectivity. A similar behavior has been
noted with Verticillium isolates from other hosts,
including tomato, snapdragon, chrysanthemum, raspberry,
eggplant and cotton. ---all of them initially formed masses
of microsclerotia on culture media. (p. 83) In older cul-
tures decreased virulence is often associated with decreased

production of sclerotia. Complete disappearance of
sclerotia may signify loss of pathogenicity.
(p. 84) Restoration of sclerotial production has
sometimes increased the virulence of isolates that
had shown a degradation of pathogenicity following
a decrease in sclerotial production."

Both Nelson (83) and Bewley (8) interpreted their results as
indicating that microsclerotial production, or quantity of micro-
sclerotia, was associated with, but incidental to, pathogenicity.
Both workers used inoculation by soil infestation to arrive at
their conclusions.

Luck (67) observed a correlation between quantity of micro-
sclerotia produced and severity of wilt induced by isolates of the
mint strain of Verticillium. Horner (40) found that root-dipping
mint cuttings with the mint strain and planting the cuttings in
the field resulted in a rapid death of plants when inoculum was
concentrated, while a dilute suspension of the same microsclero-
tial and conidial inoculum produced mild wilt.

Presley (88) reported a relationship between the quantity of
microsclerotia produced by saltant strains of V. albo-atrum and
the severity of symptoms on cotton inoculated by root-dip. Kamal
and Wood (49) reported similar results using root-dip and soil
inoculation with cotton.

Tolmsoff and Young (117) used a single isolate of Verticillium
for soil inoculations on potato and found that a range of symptom
expression from symptomless invasion to severe and early wilt could

be produced by controlling the quantity of microsclerotia intro-
duced into the soil. Tuber yields were a reflection of the
differences in severity of symptoms and yields became progressively
smaller with increasing concentrations of microsclerotia.

Robinson et al. inoculated Irish Cobbler potato plants by
dipping roots in various concentrations of conidia ranging from
35 spores to eight million spores per ml. of inoculum. They found
that (94, p. 12), "Stunting, as based on 10 plants per treatment,
became steadily more pronounced with increasing inoculum load, and
was accompanied by earlier appearance of wilt symptoms. However,
in 27 days severe wilting occurred in plants inoculated with sus-
pensions containing only 7,000 spores/ml., and slight wilting
developed even with the most dilute spore suspensions. After 40
days, all inoculated plants showed severe and almost equal wilting
even though there were differences in growth dependent on the
initial size of inoculum." No interpretation of these results was
given, but they can be interpreted as showing the influence of
quantity of inoculum on the degree of root invasion and symptom
expression.

Although there is no reason to believe that conidia cause
infection of roots in the field, under conditions of root-dip
inoculation they do definitely serve as infecting units. It
would be reasonable to expect that inoculum consisting of eight
million conidia per ml. would cause countless numbers of infections
on the roots exposed to inoculum. Theoretically, there might have

been 228,571 infections per plant at the highest rate of inoculum

for each infection occurring at the lowest rate and, apparently,

all plants were infected at the lowest rate. It would also be

reasonable to expect that as the inoculum density decreased, the

root infections would be spaced further apart. With high concentra-

tions of inoculum the entire inoculated root system would soon be

thoroughly invaded, while lower concentrations of conidia with

infections spaced farther apart would require a longer time for as

much of the root system to be invaded. Even with the lowest con-

centration of inoculum used there might have been multiple infections

in the roots. Then it might reasonably be expected that eventually

the lower concentrations of inoculum would produce almost as severe

wilt as resulted with higher concentrations, but after a longer

period of time. The results obtained by Robinson et al. agree with

the findings of Keyworth (60) and McKay (71). Keyworth found that

the extent of symptom expression on hops was correlated with the

extent of root invasion, and McKay found that the distribution of

inoculum around and beneath potato plants influenced disease

severity.

Isaac (45) attempted to determine whether nutritional status

of the host or nutrition of the pathogen prior to infection had

more influence on disease development. He found that supplying

$NaNO_3$ to the host had far less influence on disease development than

when it was supplied to Verticillium in culture prior to inoculating

the host by infesting soil. Isaac interpreted this as indicating

that the $NaNO_3$ had made <u>Verticillium</u> a more virulent pathogen, but it might also be interpreted as indicating that more inoculum was present from greater growth with supplemented $NaNO_3$.

The majority of work reviewed on degree of invasion can be interpreted as showing the influence of quantity of inoculum on disease severity. Many of the results from field experiments on the control of Verticillium wilt can be interpreted similarly.

It is known that chloropicrin is effective in reducing the inoculum potential of <u>Verticillium</u> in the soil (139,145). Nelson (83) found that rates of chloropicrin up to 400 pounds per acre did not reduce the incidence of infection in mint stems but symptoms of wilt were delayed and less severe. These effects were proportional to the quantity of chloropicrin applied to the soil.

Young (150) and Young and Tolmsoff (151, 152) reported that Vapam was effective in reducing Verticillium wilt of potatoes as measured by the severity of foliage symptoms and increased yields. The degree of control was proportional to the quantity of Vapam applied to the soil. The incidence of infection in stems was not reduced even by rates of Vapam that nearly doubled yields, nor was the time of infection delayed as compared to untreated controls. Carry-over effects from one season to the next were present even though all of the infected plants were worked back into the ground.

Some cultural practices have been reported to control Verticillium wilt in certain areas. Guba (30) found that crop rotation of heavily infested soil with sod greatly reduced the severity of wilt on eggplant in spite of the prevalence of disease among the plants. An almost normal crop was harvested after sod, while disease remained severe in controls continuously planted to eggplant.

Leyendecker (64) reported that summer-fallow reduced the severity of wilt in cotton with a dry fallow being more effective than wet fallow, the latter giving no control. The reduction in disease severity and increased yields could not be explained on the basis of a reduction in the number of infected plants. Leyendecker concluded that a change in some soil factor or factors was responsible for the reduction in disease severity and suggested fertility, soil structure and microbial activity as possibly playing a role in the increase of yields. However, this does not agree with the finding that the dry fallow was more effective than wet fallow. These results can be interpreted as showing a decrease in the inoculum potential of Verticillium with dry fallow. This interpretation would not conflict with Wilhelm's (141) finding that Verticillium can survive in the field in the absence of susceptible hosts for 14 years because Wilhelm did not determine the percentage of microsclerotia surviving.

McKeen (73) found that when Verticillium was added to dry and moist soils that were kept dry and moist, respectively, for two months prior to planting, symptoms of wilt appeared later in plants

grown in soil that had been kept dry during the pre-planting incubation period. This can be interpreted as showing the influence of environment on survival of Verticillium, which in turn can be interpreted as showing the influence of inoculum potential on disease development.

McKay (71) reported that a three-to four-year rotation of infested land with grains and clover was sufficient to eliminate Verticillium from the soil. Zeller (153) reported that a three-year rotation with non-susceptible crops was sufficient to prevent infection of raspberries. Working with the "dauermycelium" form of Verticillium, Robinson et al. (94) concluded that a three-year rotation with clover and grain was effective in eliminating the pathogen from the soil, while a two-year rotation was effective in significantly reducing the incidence of disease in potato plants. Folsom (20) reported that a single year of rotation with clover was effective in eliminating wilt of potatoes, but this was based on a grower's observations.

Wilhelm (132, 141, 146) reported that crop rotation with non-susceptible crops for eight years or more was not effective in eliminating Verticillium from the soil. Thus, the report by Wilhelm appears to conflict with other reports, and workers have drawn hasty conclusions from these apparent discrepencies. However, it is necessary to consider the experiments on which the reports are based to understand that the reports do not necessarily conflict. They

can be interpreted as being complimentary when the influence of inoculum potential on disease development is considered.

A field suitable for experiments and naturally infested with Verticillium was not available for the rotation studies in McKay's experiments and he stated that (71, p. 465), "In order to have the diseased soil plots heavily contaiminated with the wilt organism a crop of potatoes was planted and inoculated at planting time ---." It is doubtful that the growing of a single crop of potatoes inoculated with Verticillium was sufficient to heavily infest the soil. Similarly, Robinson et al. (94) tested the effectiveness of crop rotation on a non-infested field, and the soil was artificially infested by planting a crop of tubers from a field of wilted potatoes. Zeller (153) infested his crop-rotation plots by planting and inoculating a crop of raspberries prior to imposing the rotations on the soil. Wilhelm's reports (132, 141, 146) on the ineffectiveness of crop rotation for eliminating Verticillium are based on studies of naturally infested soil in which, generally, many susceptible crops were grown prior to the rotations. Thus, Wilhelm's reports on the ineffectiveness of crop rotation are based on heavily infested soil. Wilhelm's data show that a substantial reduction in the inoculum potential resulted from either crop rotation or summer-fallow, but infection was not eliminated.

Both McKay (71) and Zeller (153) believed that Verticillium survives for only one or two years in the soil. When infection occurred in test plants after three years of rotation with

non-susceptible crops, they interpreted such infections as arising from foreign contamination or error. Thus infection had not been eliminated, although a reduction in the inoculum potential undoubtedly occurred. In this respect, their results agree well with those of Wilhelm (132, 141, 146) in that crop rotation reduced the level of inoculum without eliminating infection by Verticillium from the soil.

It is significant that the severity of wilt occurring in the rotation studies was not mentioned by McKay (71), Zeller (153), Robinson et al. (94) and Wilhelm (132, 141, 146). Apparently, it was considered that an infected plant was a badly diseased plant. McKay's (71) results are based on the frequency of recovery of Verticillium from potato tubers, and his results showed that under the conditions of his experiments tuber infection was not related to symptom expression in the plants. If crop rotation in his plots resulted in a decrease in disease severity, no mention was made of it, and the results have not proven satisfactory for field control in Western Oregon where the experiments were conducted. Nor have Zeller's (153) results on the control of wilt in raspberries in Western Oregon proven satisfactory for field control in that same area (87). If Verticillium wilt is as easily controlled by crop rotation as reported by Robinson et al. (94), this disease should not be a problem in that area. Under the conditions of their experiments, incidence of disease declined from one year to the next with continuous cropping of potatoes on the artificially

infested soil. Thus, their conclusion that a three-year rotation eliminated _Verticillium_ is questionable because of the low incidence of disease in the plots planted to potatoes for two consecutive years, and the low incidence of disease in all of the plots. Furthermore, no mention was made of whether control plots were included in the experiment.

Nelson (83) reported that a six-year rotation with potatoes, sugar beets, red beets, spinach and cabbage on land infested with the mint strain of _Verticillium_ was beneficial in that mint made unusually good growth when replanted following the rotations. However, he believed that rotation was not practical since many of the mint plants were infected. In another experiment, Nelson (83) found that crop rotation and fallow of heavily infested land for 13 years did not eliminate _Verticillium_ from the soil.

From the reports of many workers, it can safely be concluded that the inoculum potential of _Verticillium_ in the soil is the primary factor determining the severity of Verticillium wilt in the field. It can also be concluded that summer-fallow and crop rotation with non-susceptible hosts are effective in reducing the inoculum potential of _Verticillium_, but these measures of control are ineffective in eliminating the pathogen from the soil even when continued for periods of up to 14 years. The reports also indicate that the microsclerotial form of _Verticillium_ may be more difficult to control through cultural practices than the "dauermycelium" form.

ENVIRONMENTAL INFLUENCES

The nutrition of host plants has been shown to influence the
severity of Verticillium wilt but none of the alterations of host
nutrition have eliminated infection. Of the major elements required
by plants, available nitrogen has been shown to be the most influ-
ential on disease severity (21, 45, 57, 89, 93, 124). Less
obvious symptoms of wilt have resulted when plants were in a state
of nitrogen deficiency. Nelson (83) believed that nitrogen defi-
ciency predisposed mint plants to more severe wilt, but no experi-
ments were conducted to substantiate this belief.

Keyworth and Hewitt (57) reported that an excess or deficiency
of phosphorus, potassium, calcium, magnesium or manganese had no
affect on hop wilt. Gallegly (21) found that the available level of
phosphorus and potassium did not influence tomato wilt. Walker
et al. (124) obtained an increase in wilt severity on tomatoes with
an increase in nitrogen supplied to the host. In field experiments,
Presley (89) reported an increase in wilt severity of cotton for each
increase in the amount of nitrogen applied to soil and a decrease in
wilt severity for each increase in the amount of potassium added.
Roberts (93) found that the addition of potassium had no influence
on tomato wilt when nitrogen and phosphorus were readily available.
Nelson (83) reported that the addition of minor elements to soil had
no affect on severity of wilt in mint.

Soil factors other than availability of nutrients have been
suggested as influencing disease severity. Verticillium wilt has

been reported to be severe on sandy soils low in organic matter
(84, 95, 107, 119, 150, 151), on heavy, clay soils (95) and on soils
with a high organic-matter content (29, 83, 86, 119, 133).

In California (133) the disease was reported to be severe on
soils ranging in pH from 4.5 to 6.7. Van der Meer (121) found
Verticillium wilt in soils ranging in pH from 4.4 to 6.6. Wilhelm
concluded that (133, p. 777), "----the occurrence and severity of
Verticillium wilt is not greatly affected by soil reaction within
the range in which susceptible crops are commonly grown." Guba (30)
found that acidification of soil by applications of sulfur had very
little influence on wilt of eggplant within the pH range at which
eggplant would produce a commercial crop. Haensler (33) and
Martin (69) reported that acidification of soil by addition of sul-
fur decreased wilt while addition of lime increased disease, but
their results were not striking.

Nelson (83) made a thorough review of the influence that soil
moisture has on disease severity and pointed out, as did Rudolph
(95), that opinions on the influence of soil moisture vary. Some
workers (64, 68, 95) believe that moisture levels within the range
at which hosts are grown are relatively unimportant, while others
(8, 9, 73) believe that the disease is most severe with high
moisture levels, and still others (83, 102, 121) believe that a
soil with low moisture content induces more severe wilt.

A number of factors may have contributed to the disagreements
on moisture influence: (a) some workers have drawn conclusions from

general observations without experimental data to support their belief on the influence of soil moisture; (b) different hosts have been employed in moisture studies, and possibly the disease reaction of different hosts varies under different moisture levels; (c) as suggested by Nelson (83), soil temperatures may alter the influence of soil moisture; (d) not all experiments have been designed to separate moisture influence on the uninfected host from moisture influence on the diseased host; (e) various methods of evaluating moisture influence have been used, including incidence of infection, incidence of wilt, plant height, plant weight and general appearance of plants; (f) at least some hosts are not readily infected until later stages of plant development (117) which means that where infested soil is used, inoculum would remain in the soil until the plant became susceptible. Soil moisture could possibly influence survival of _Verticillium_ and differences in disease severity with various levels of moisture might be due to different levels of inoculum. Thus, it is necessary to separate moisture influence on the healthy and diseased host, and it is necessary to separate both of these from the influence of moisture on survival of _Verticillium_.

Soil and air temperatures have repeatedly been shown to have a strong influence on disease severity. Bewley (8) reported that tomato plants severely affected with wilt recovered when air temperatures were raised to 25° C. or higher. If the recovered plants were then grown at a temperature of about 20°, severe disease reappeared if the duration of time at which they were at 25°

or higher was short. If they were kept at the high temperature for 30 days and then moved to a temperature of 20°, disease reappeared but it remained mild. The temperatures reported by Bewley are subject to error since they were determined by averaging maximum and minimum daily temperatures, but the phenomenon of recovery by diseased tomato plants at high temperatures is a significant finding.

Schoevers (103) reported that 25° C. was the maximum for wilt development in tomatoes and Alexander (2) reported that severe wilt in tomatoes occurred in temperature tanks with temperatures ranging from 12° C. to 24° C. At 28° C., mild symptoms were present and plants made normal growth.

Under more carefully controlled greenhouse conditions, Ludbrook (68) studied the influence of soil and air temperatures using many "dauermycelium" and microsclerotial isolates and several hosts. The isolates of Verticillium were obtained from many areas within the United States and several other countries. Ludbrook concluded from his studies that (68, p. 151), "The most important environal factor influencing the disease appears to be temperature." He found that all isolates of Verticillium tested produced most severe disease on the various hosts at soil temperatures of 18° to 22°. Wilt severity declined rapidly at soil temperatures above 24°, and most isolates produced no symptoms or only mild symptoms at 28° to 30°.

Ludbrook (68) found that there was a definite difference in the ability of "dauermycelium" and microsclerotial isolates to produce wilt symptoms at soil temperatures of 26° to 30°. The "dauermycelium" isolates did not produce symptoms on tomatoes, eggplant or potatoes at a soil temperature of 28°, while microsclerotial isolates did induce symptoms at this temperature. Microsclerotial isolates usually produced mild symptoms at 30° except on tomatoes, which did not show symptoms at this temperature. This difference in the ability of "dauermycelium" and microsclerotial isolates to induce symptoms at higher temperatures has recently been confirmed by Edgington and Walker (18) and Robinson et al. (94). Other workers have reported this difference between the two forms and it appears to be correlated with the ability of the pathogen to grow at higher temperatures. The microsclerotial isolates grow at higher temperatures than "dauermycelium" isolates (18, 68, 94).

Ludbrook reported a difference in susceptibility between hosts at higher temperatures and stated that (68, p. 143), "----the maximum soil temperature for the occurrence of Verticillium hadromycosis in tomatoes inoculated with either a mixture of both species or Verticillium albo-atrum alone appears to be about 26°, as compared with a maximum of 30° for eggplants inoculated with V. dahliae or 28° for eggplants inoculated with V. albo-atrum, the air temperature in each case averaging 20°." The results of Edgington and Walker (18) show that tomatoes were not severely

affected with a soil temperature of 28°, regardless of air temperature, using "dauermycelium" isolates but their results disagree with those of Ludbrook in that they obtained severe disease at soil and air temperatures of 28° with the single microsclerotial isolate used. Since temperatures were not as accurately controlled in Ludbrook's experiments, possibly the reported temperatures were lower than actually existed, or there would appear to be a difference between the microsclerotial isolates used by Ludbrook and that used by Edgington and Walker.

Ludbrook (68) and Edgington and Walker (18) showed that soil temperatures influenced disease development more than air temperatures. Edgington and Walker (18, p. 597) stated that, "In the upper part of the stem the temperature was closer to air temperature than soil temperature for the majority of time, yet soil temperature was more effective than air temperature in influencing disease severity. It would seem, therefore, that the center of greatest activity of the fungus must have been in the roots and lower stem under the conditions provided in these experiments." Soil temperature was found to have more influence on the internal temperature of stems during the day than at night. At night, air temperature had more influence on internal stem temperature. The influence of soil temperature on internal stem temperature faded with increasing distance from the soil level.

The statement by Edgington and Walker in the preceding paragraph regarding the activity of _Verticillium_ in tomato roots, and the finding of Keyworth (60) that the area of root infection in hops determined the severity of wilt points to another possible misconception regarding the disease. It has generally been assumed that the roots merely serve as a means for the pathogen to gain entrance to the stem and that the stem, petiole and leaves are the areas of greatest fungus activity. It should also be pointed out that Reinke and Berthold (91) described a form of the disease in potatoes which, for the most part, accurately fits the description of the disease as it is now known to occur, but they claimed that symptoms of wilt could be present without any evidence of the fungus in stems of plants. Pethybridge (86) was unable to find mycelium in stems of potatoes until after symptoms had already appeared in the tops. Roberts (92, 93) did not find mycelium in tomato stems until after symptoms had appeared in the tops, and Keyworth and Bennett (59) occasionally failed to isolate _Verticillium_ from the upper parts of strawberry plants showing symptoms. All of these findings could indicate that _Verticillium_ is capable of doing major damage to the tops of plants without being present there, but through its activity in the roots. Green (28) did not believe that this was possible with tomatoes since symptom expressing parts of plants were found to contain the pathogen under the conditions of his experiment.

It is generally believed (18, 68, 94) that the small differ-
ence in maximum temperatures at which the "dauermycelium" and
microsclerotial isolates of Verticillium produce disease under
greenhouse conditions would adequately explain the geographic
distribution of these forms. Usually, the "dauermycelium" form
is found distributed in the cooler areas (68) of northern United
States, Canada, England and Europe, and possibly other areas.
On the other hand, the microsclerotial form is generally found
in southern areas as well as the cooler, northern areas. Tempera-
ture records (26, 64, 102) fail to show that the southern areas
are too warm for the development of the "dauermycelium" form and,
as pointed out by Gratz (26), the absence of Verticillium wilt
from Florida potato-growing areas cannot be explained on the
basis of temperature. The possibility that the resistant struc-
tures of the two forms differ in their resistance to adverse
environmental conditions has been entirely overlooked. If there
is a difference in their resistance to adverse environment, it
would be reasonable to expect that the "dauermycelium" form would
be more confined to the cooler areas, whereas the microsclerotial
form would extend further southward.

<center>MATERIALS AND METHODS</center>

Cultures

An isolate of <u>Verticillium</u> obtained from a wilting potato plant near Klamath Falls, Oregon, was used in the inoculation experiments. This isolate, designated KF-V14, produced microsclerotia in moderate abundance on potato dextrose agar in comparison with other isolates obtained throughout the state. If one considers the microsclerotial and "dauermycelium" <u>Verticillium</u> isolates as separate species, this isolate would be identified as <u>V. dahliae</u>. The "dauermycelium" type was rarely recovered from potatoes grown in Oregon. In this thesis, all isolates are regarded as <u>V. albo-atrum</u>.

Media

Potato dextrose agar containing 100 ppm streptomycin nitrate was used for isolations from plant tissues, for making dilutions of the inoculum, and for growing inoculum in the laboratory. The medium was prepared by the following formula:

Potatoes	200 gm.
Dextrose	20 gm.
Agar	20 gm.
Streptomycin nitrate (20 per cent active)	0.5 ml.
Tap water	1,000 ml.

Potatoes were peeled, sliced into quarters, placed in half of the volume of water and autoclaved for 20 minutes at 15 pounds pressure. Agar and dextrose were mixed with the remaining volume of water

in a separate container and were autoclaved simultaneously. After
autoclaving, the potato decoction was strained through cheesecloth
and enough water was added to restore the original volume. The
potato decoction was mixed with the autoclaved, melted agar and
dissolved dextrose, .5 ml of 20 per cent streptomycin nitrate was
added, and the mixture was dispensed into containers and autoclaved
for 20 minutes at 15 pounds pressure.

Isolations

Plant tissues were surface-sterilized by immersion in a 20
per cent solution of commercial Clorox (5.25 per cent sodium
hypochlorite) for two to three minutes. After surface-sterilization,
plant tissues were placed on clean paper towels where small sections
were removed to be plated. The paper towels served as blotters and
absorbed excess Clorox solution.

Five cross sections of the entire stem were usually taken at
each of several intervals from the ground level upward. That part
of the root system that could be removed from the soil with the
plant was entirely surface-sterilized. Roots were chopped up on
paper towels with a flamed razor blade and part of the root frag-
ments were scattered over the surface of the medium. Observations
were made on the apparent quantity of root fragments infected by
Verticillium.

Preparation of Inoculum

Two methods were used for the preparation of inoculum. In one method, similar to that used by Morton and Stroube (80), inoculum was grown on a sand-cornmeal mixture consisting of 1,000 gm. sand, 200 gm. cornmeal and 250 ml. water. Large quantities of inoculum were prepared by autoclaving the mixture, in proper proportions, at 10 pounds pressure for five hours. Following autoclaving, the mixture was inoculated with a spore suspension of Verticillium and incubated for two weeks at approximately 65° F. The inoculum was then dried to kill conidia and mycelium, and dilution plates were made to determine the approximate quantity of viable microsclerotia present. Counts obtained by dilution plates were underestimates of the quantity of microsclerotia present because many microsclerotia adhered to, and were embedded in, the sand and cornmeal particles.

The second method of preparing microsclerotial inoculum was similar to that used by Luck (67), except that the technique was modified to produce larger quantities of inoculum. Verticillium was grown on sheets of cellophane in 10" x 12" plastic containers. The containers were sterilized by rinsing with 95 per cent ethanol. Approximately 300 ml. of potato-dextrose-agar containing 100 ppm streptomycin nitrate was poured into each container. Sheets of cellophane were cut to fit inside the containers on the surface of the solidified medium. Cellophane sheets were autoclaved at 15 pounds pressure for 20 minutes, placed in a container of sterile water, individually to allow expansion, and then stretched over the

surface of the medium. A spore suspension of <u>Verticillium</u> was atomized onto the cellophane surface and the containers were incubated for 10 to 14 days at room temperature.

After the incubation period, the slimy layer of <u>Verticillium</u> growth on the cellophane was removed from several containers and macerated with approximately an equal volume of water in a Waring Blendor for three minutes. The macerated inoculum was poured over 10 to 15 pounds of finely screened, dry soil and was thoroughly mixed with the soil several times while drying and again after the mixture was dry. The dried inoculum was rescreened and dilution plates were made to determine the approximate quantity of microsclerotial units per volume of soil.

With either method of preparing inoculum, colonies that appeared in dulution plates made after the inoculum had been air-dried for one to seven days arose from microsclerotial particles. Dilution plates made while the soil was still moist produced many colonies that originated from conidia. The viable conidial population disappeared rapidly during drying, but the microsclerotial population remained constant for at least two to six weeks when the inoculum was dry. The conidial population decreased rapidly in moist soil when a small amount of the soil inoculum was incubated at temperatures of 10, 15, 20 and 25° C. After 10-day incubation in moist soil, 99.99 percent of the conidia were dead. The microsclerotial population remained constant under similar conditions.

Potato Culture

Potatoes of the variety Russet Burbank (Netted Gem) were used in the inoculum studies. Certified seed was used, and in both greenhouse experiments single-eye seed pieces were planted in the infested soil. In the inoculum experiment using sand-cornmeal inoculum, single-eye seed pieces were planted directly into soil without pre-sprouting. In the inoculum experiment using cellophane-grown inoculum, single-eye seed pieces were pre-sprouted in sand seven days before planting in soil.

At two- to four-week intervals plants were fumigated with TEPP (tetraethyl pyrophosphate) to kill aphids, and sprayed with chloro-benzilate (10 gm. per three gallons water) to kill mites resistant to TEPP.

Soils

Soil was obtained from two different sources for the two experiments. For the first experiment, in which inoculum grown on sand-cornmeal mixture was used, a fine, sandy loam soil was obtained from a field near Klamath Falls with severe Verticillium wilt. This soil was placed in No. 10 cans and autoclaved for three hours at 11 pounds pressure. Dilution plates made from the autoclaved soil showed that organisms capable of growing on potato dextrose agar had been eliminated from the soil. This soil was allowed to become reinvaded by air-born contaminants before it was used in the experiment.

The soil used in the second experiment in which inoculum was grown on cellophane was obtained from a semi-desert area east of Klamath Falls. This soil had never produced a cultivated crop and had a vegetative cover consisting primarily of sagebrush and Juniper. Twenty potato plants were grown in this soil before setting up the inoculum experiment, and the 20 plants failed to produce Verticillium when stem isolations were made. Therefore, this soil was not sterilized because it was considered to be free, or relatively free, of Verticillium.

THE INFLUENCE OF QUANTITY OF INOCULUM ON SEVERITY OF
VERTICILLIUM WILT OF POTATOES

Three observations led to an investigation of the importance
of inoculum potential in the development of Verticillium wilt.
Young (150) found that soil fumigation with Vapam (sodium
N-methyldithiocarbamate dihydrate) greatly reduced the severity of
Verticillium wilt of potatoes. Tuber yields resulting after Vapam
treatment were almost comparable to yields that would be expected in
the absence of this disease. However, in unreported data obtained
by making isolations from the plants in the Vapam experiments, 100
per cent of the stems were found to be infected in both treated and
untreated soil. Fertilizer experiments conducted with the Vapam
trials showed that disease severity was not reduced by addition of
fertilizers beyond a level optimum for growth. Therefore, the
reduction of disease by Vapam was probably not the result of
increased availability of nutrients to the plants.

The second reason for setting up the inoculum potential
experiments was because of the results obtained from root and stem
isolations from potato plants grown in naturally infested soil to
which various plant residues were added (118). Excellent control
of Verticillium wilt was obtained by the addition of oat and
barley residues to soil in the greenhouse, but the incidence of
stem invasion was not reduced. It was apparent that in effective
treatments the percentage of infected roots was reduced.

The third reason for setting up the inoculum potential experiments was because of the observation that the severity of Verticillium wilt in the field appeared to be closely correlated with the number of susceptible crops grown in the soil. A high percentage of invaded stems were found in some potato fields in which plants had very mild or no symptoms of wilt. Symptomless invasion or mild symptoms were associated with the planting of few or no previous crops of potatoes in the soil, while severe symptoms were invariably associated with a long history of potato culture.

In the three cases there appeared to be a correlation between the severity of wilt and the percentage of roots invaded by Verticillium, and no correlation between stem invasion and the severity of wilt. Knowing Vapam to be an effective soil fungicide it was postulated that the reduction in severity of wilt after soil fumigation might be the result of reducing the inoculum potential of Verticillium, leading to fewer infected roots. If the inoculum potential influenced the numbers of root infections, and if the number of invaded roots was important in determining the severity of disease, the severity of wilt might have been determined by the inoculum potential. This interpretation of Verticillium wilt of potatoes would take into consideration a mild infection in the roots that would allow symptomless invasion of the stems. Two greenhouse experiments were conducted to test the hypothesis that the severity of wilt is primarily determined by the inoculum potential.

The first experiment, using sand-cornmeal inoculum, was set up on December 1, 1956, when air temperatures could be maintained between 65° and 75° F. with greenhouse heating facilities. The second experiment, using cellophane inoculum, was set up on July 1, 1957. Minimum daily air temperatures were adjusted to 75° F. and maximum temperatures frequently exceeded 95° and occasionally exceeded 100° F. In this second experiment, which was conducted during the summer, greenhouse cooling units were not available and maximum temperatures could not be controlled. The first experiment was carried out under temperatures that were near optimum for growth of the pathogen and were near optimum for disease development. The second experiment was conducted under temperatures that are reported as being above optimum for disease development in other crops.

Table 1 shows the treatments that were included in the first inoculum potential experiment. Rates of microsclerotia added to the soil ranged from 179 to 2,932,736 per cc of soil (quantity of air-dried soil required to displace 1 cc of water). Fifteen rates of inoculum were used and 30 single-eye seed pieces were grown in each rate except for the highest rate in which 20 seed pieces were planted. In addition, 30 seed pieces were planted in a soil heavily infested with natural inoculum of Verticillium. Two seed pieces were planted per No. 10 can of soil. Each can contained seven pounds of soil on an air-dry basis. Controls containing similar

TABLE 1. Treatments included in inoculum potential experiment using sand-cornmeal inoculum.

Treatment number	Quantity of sand-cornmeal inoculum added:(gm./35 lbs. soil)	Minimum number of microsclerotia per cc soil:	Number seed pieces planted:
Control	(no Verticillium)		
1.	4.00	0	20
2.	16.00	0	20
3.	64.00	0	20
4.	256.00	0	20
5.	512.00	0	20
6.	1,024.00	0	20
7.	2,048.00	0	20
8.	4,096.00	0	20
Verticillium inoculum			
9.	.25	179	30
10.	.50	358	30
11.	1.00	716	30
12.	2.00	1,432	30
13.	4.00	2,864	30
14.	8.00	5,728	30
15.	16.00	11,456	30
16.	32.00	22,912	30
17.	64.00	45,824	30
18.	128.00	91,648	30
19.	256.00	183,296	30
20.	512.00	366,592	30
21.	1,024.00	733,184	30
22.	2,048.00	1,466,368	30
23.	4,096.00	2,932,736	20
24.	Naturally infested field soil		

quantities of sand-cornmeal, but no Verticillium, were set up to correspond with certain of the Verticillium-infested treatments.

In the second experiment, rates of 0, 10, 100, 1,000, 10,000, 100,000, 1,000,000 and 10,000,000 viable particles of microsclerotia per cc of soil were used. Only one control treatment was necessary

in this experiment because inoculum did not contain the organic material upon which the fungus grew. Fifteen pre-sprouted single-eye potato plants were planted in each treatment with one plant per No. 10 can. Each can contained seven pounds of air-dried soil.

Isolations were made from various plant parts 3, 5, 8 and 12 weeks after planting in the first experiment. Three to six plants were removed at random from each of the inoculated series at each isolating time-interval. Isolations were not made from plants in the second experiment until after the experiment was completed. Wilting data were taken to 113 days after planting in the first experiment and to 112 days in the second experiment.

Time of Infection

Isolations were made from 48 plants growing in soil infested with Verticillium three weeks after planting. Stems of the plants were entirely plated out from the growing tip to the point of attachment to the seed piece. All of the roots, which ranged from two to six inches long, were plated out. At this isolating interval, none of the 48 plants were infected with Verticillium.

Sixty-four plants were removed for isolations at the five-week interval. Isolations were made from about half of the root system, from the stems below the soil level, from the soil level and at two-inch intervals to the tip. None of the 64 plants were infected with Verticillium.

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At the eight-week interval, 48 plants were removed and isolations were made from a small part of the entire root system, from the stem below the soil level, from the soil level and at four-inch intervals to the tip. Verticillium was recovered from below the soil level in 16 of the 48 plants but was not recovered from above the soil level in any of the plants. Infection was found in two of the stems below the soil level and in the remaining 14 infected plants infection was found only in the roots. Many of what were believed to be new infections were found. These were especially abundant in the roots with high rates of inoculum and in the roots of plants grown in naturally infested soil. These new infections were apparently not systemically established in the vascular system because Verticillium grew from the epidermis of sections in localized areas but not from the ends of the sections where vascular tissue was exposed. These local infections probably occurred very recently because no infections were found at the five-week isolating interval and systemic infections in the vascular tissue of the roots were well established at the eight-week isolating interval. Local infections were also found on the stems below the soil level in the higher rates of inoculum and in naturally infested soil.

Since Verticillium was not recovered in isolations from stems and roots plated at the earlier intervals, there apparently was a type of resistance to infection during the earlier stages of plant development. Time of infection was closely correlated with the

time of tuber initiation. Stolon and tuber formation were not ini-
tiated at the three- and five-week intervals, but most plants had
formed stolons and had initiated tubers after eight weeks. In the
higher rates of inoculum and the naturally infested soil, plants
that had not formed stolons were not infected.

At the 12-week interval plants were infected in all inoculated
treatments except the two highest rates of sand-cornmeal inoculum.
The large quantities of sand-cornmeal mixture added to the soil in
these treatments temporarily retarded plant growth and especially
delayed tuber formation. These same effects were present in the
control treatments receiving corresponding quantities of non-
infested sand-cornmeal mixture. Stolons and tubers were not
formed at the eight-week interval in the treatments receiving the
large quantities of infested and non-infested sand-cornmeal mixture.

Table 2 shows the number of plants from which isolations were
made at the various time intervals and the number of those plants
infected by Verticillium.

TABLE 2. Time and frequency of infection of potatoes grown in soil containing different quantities of microsclerotial inoculum.

Minimum number microsclerotia per cc of soil	Ratio of tested to infected plants after:			
	3 weeks	5 weeks	8 weeks**	12 weeks
179	3-0*	4-0	3-0	4-2
358	3-0	4-0	3-0	4-2
716	3-0	4-0	3-0	6-3
1,432	3-0	4-0	3-1	4-2
2,864	3-0	4-0	3-1	4-2
5,728	3-0	4-0	3-0	4-4
11,456	3-0	4-0	3-0	---***
22,912	3-0	4-0	3-2	4-4
45,824	3-0	4-0	3-2	4-3
91,648	3-0	4-0	3-3	5-5
183,296	3-0	4-0	3-2	---
366,592	3-0	4-0	3-1	3-3
733,184	3-0	4-0	3-2	---
1,466,368	3-0	4-0	3-0	3-0
2,932,736	3-0	4-0	3-0	3-0
Field Soil	3-0	4-0	3-2	3-3

*   Figure on the left indicates number of plants from which isolations were made, figure on right indicates number of plants from which Verticillium was recovered.

**   Infections at the eight-week interval were confined to roots and underground parts of stems.

*** Isolations were not made from these treatments.

Time of Symptom Expression

The length of time between planting and the first appearance of symptoms varied between the two experiments. In the experiment with sand-cornmeal inoculum, symptoms of wilt first appeared 69 days after planting. Tubers were formed on these plants between 50 and 60 days after planting, infection occurred at about the time of tuber formation, so symptoms appeared 10 to 20 days after initial infection in the higher rates of inoculum. In the

experiment using cellophane-grown inoculum the time of tuber forma-
tion was not observed, but first symptoms of wilt appeared 27 days
after transplanting pre-sprouted seed pieces to the infested soil.

Table 3 shows the relation of inoculum potential to the time
of wilt development in the first experiment. Wilt symptoms first
appeared after 69 days in those treatments containing 11,456 to
733,184 (minimum) microsclerotia per cc of soil. The highest per-
centage of wilted plants occurred in the treatment receiving
91,648 mocrosclerotia per cc of soil in which 30 per cent of the
plants showed symptoms at 69 days. The data show that under the
conditions of this experiment the time of appearance of wilt
symptoms was largely dependent upon the inoculum potential. This
effect was masked in the highest rates of inoculum, probably due
to factors resulting from the addition of large quantities of
organic matter with the pathogen.

TABLE 3. Relation of inoculum level to time of development of wilt symptoms.

Number micro-sclerotia per cc soil:	Number plants percentage based on:	Percentage of plants showing wilt symptoms at different time intervals (days) after planting:					
		69	75	80	85	90	95
0	92	0	0	0	0	0	0
179	16	0	0	0	0	25	12
358	16	0	0	12	19	12	25
716	13	0	0	15	15	15	46
1,432	15	0	0	7	7	13	27
2,864	14	0	0	7	0	7	43
5,728	10	0	10	40	40	60	70
11,456	16	6	31	37	44	56	75
22,912	14	7	29	79	79	100	100
45,824	16	6	19	56	56	100	100
91,648	15	30	73	93	93	100	100
183,296	19	5	42	58	68	89	100
366,592	17	12	47	59	59	100	100
733,184	20	5	10	30	40	65	95
1,466,368	14	0	0	0	14	14	21
2,932,736	7	0	0	0	0	0	0
Field soil	---*	---	---	---	---	---	---

* Percentage of seed-piece decay was high in field soil, and plants that emerged were used for isolations to determine time of infection by Verticillium.

In the second experiment in which inoculum was relatively free of organic matter, the time of symptom appearance was directly related to the inoculum potential (Table 4). In this experiment the highest rate of inoculum, 10,000,000 microsclerotial particles per cc of soil, was the first to induce symptoms and within 19 days after initial symptoms all 15 of the plants in this treatment showed wilt. None of the plants grown in soil with less than 100,000 microsclerotia per cc showed wilt at the time that 100 per cent of the plants in the 10,000,000 rate showed symptoms.

TABLE 4. Relation of inoculum level to time of development of
wilt symptoms in potatoes.

Number microsclerotia per cc soil	Number of plants showing wilt out of 15 at different time intervals (days) after planting										
	27	31	36	41	46	56	67	77	82	87	97
0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	1	1	1	1	1	1
100	0	0	0	0	0	1	1	1	1	1	1
1,000	0	0	0	0	0	0	0	0	0	0	1
10,000	0	0	0	0	0	0	0	0	1	2	2
100,000	0	0	0	2	3	6	6	7	7	8	8
1,000,000	0	0	5	6	14	14	15	15	15	15	15
10,000,000	4	5	7	12	15	15	15	15	15	15	15

Severity of Wilt and Time of Plant Death

Severity of symptoms was measured by recording the time of death of individual plants and by weighing tuber yields. A plant was recorded as dead when it no longer had any turgid foliage.

Time of plant death was more closely associated with inoculum potential than was the time at which symptoms appeared (Table 5). For example, the treatment with 91,648 microsclerotia per cc of soil induced symptoms of wilt sooner than any other treatment but plants died more quickly in the treatments receiving 183,296, 366,592 and 733,184 microsclerotia per cc of soil even though they developed symptoms later due to the cornmeal affect. When plants in different treatments first showed wilt at the same time, plants grown in the higher rates of inoculum almost invariably died first.

Figure 1 shows plants that began to wilt on the same day. The plants differ in growth and in the severity of wilt. The plants

TABLE 5. Influence of inoculum level on time of plant death using sand-cornmeal inoculum.

Number microsclerotia per cc soil	Per cent of plants dead after:								
	90*	93	95	97	99	102	107	110	113 days
0	0	0	0	0	0	0	0	0	0
179	0	0	0	0	0	0	0	6	6
358	0	0	0	0	0	12	12	25	31
716	0	0	0	0	0	8	23	23	38
1,432	0	0	0	0	7	13	30	47	47
2,864	0	0	0	0	7	29	29	36	36
5,728	0	0	0	0	0	10	20	30	50
11,456	0	0	0	0	0	25	44	50	75
22,912	0	0	0	7	7	29	71	86	86
45,824	0	0	6	6	6	25	37	50	50
91,648	6	6	13	27	30	40	47	80	87
183,296	26	26	37	47	53	63	74	89	100
366,592	35	41	47	53	59	59	71	88	100
733,184	5	10	15	25	25	50	75	85	95
1,466,368	7	7	7	7	7	36	57	64	71
2,932,736	0	0	0	0	29	57	57	57	57

* First day that any plants were dead.

grown in soil containing 91,648 microsclerotia per cc of soil initially showed severe wilting and wilt progressed rapidly until the plants were dead, about two weeks after the photograph was taken. The plant with mild wilt was grown in soil containing 179 microsclerotia per cc of soil. The single leaflet affected unilaterally halfway up the stem was the only symptom of wilt developed by this plant. Two weeks later symptoms had not progressed much beyond this stage and without careful examination these symptoms could easily have gone undetected, if one were looking for more striking signs of infection. In this experiment there were many cases similar to the one in Figure 1 where plants

Fig. 1. Plants with severe and mild wilt grown in soil containing 91,648 and 179 microsclerotia of Verticillium per cc of soil, respectively, 96 days after planting. Mild wilt in plant on right consists of unilateral wilting and yellowing of a single leaflet near middle of plant height. Wilt initially appeared in the plants on the same day.

showing symptoms at the same time, or approximately the same time, were affected much more severely and died sooner in higher rates of inoculum.

The time interval from initial symptoms to death was determined for all plants that were dead 97 days after planting (Table 6). In the only plants that were dead in the lower rates of inoculum, 27 days lapsed between time of symptom expression and death. With higher rates, the time was reduced to 19-22 days and with still higher rates it was reduced to 5-11 days.

Figure 2 shows plants grown in three levels of inoculum beside their corresponding controls 96 days after planting. The plants grown in heavily infested soil were stunted in comparison to the controls. High rates of sand-cornmeal mixture did not influence plant growth except where Verticillium was present and this remained true for all treatments except the two highest rates which temporarily retarded growth of plants in both infested and non-infested soil.

TABLE 6. Relation of <u>Verticillium</u> inoculum level to rapidity of
dying of infected potato plants.

Minimum number microsclerotia per cc soil	Average number of days* from initial symptom expression to death of plants	Number of plants on which average was based
22,912	27	1
45,824	27	1
91,648	22	4
183,296	19	9
366,592	10	9
733,194	11	5
1,466,368	5	1

* Based on plants dead on March 6, 97 days after planting. At this
date, the above treatments were the only ones with dead plants.

Figure 3 shows a representative group of plants grown in soil
containing 179 to 366,592 microsclerotia per cc of soil. This
photograph shows the spectrum of disease severity induced by the
various levels of inoculum. Disease symptoms included stunting,
yellowing of wilted and non-wilted foliage, wilting and death of
plants. Each symptom became more pronounced with increasing levels
of inoculum. At the time that this figure was taken, the controls
all appeared healthy and were uniform in height with all levels of
sand-cornmeal.

In the second experiment, time and frequency of plant death
were directly correlated with the quantity of microsclerotial
inoculum added to the soil (Table 7). Nearly all plants in the
10,000,000 rate of inoculum were dead before those in the 1,000,000
rate began to die. All of the plants in the 1,000,000 rate were
dead before half of the plants in the 100,000 rate were dead.

Fig. 2. The influence of Verticillium-infested and non-
infested sand-cornmeal mixture on plant growth and
disease development 96 days after planting. In each
of the three groups, plants on the left were grown
in Verticillium-infested soil and plants on the right
are the corresponding controls. Diseased plants from
left to right were grown in soil containing 22,912,
45,824 and 766,592 microsclerotia per cc of soil
(32, 64 and 1,024 gm. of sand-cornmeal mixture per
35 pounds soil, respectively).

Fig. 3. Spectrum of disease severity induced by various
levels of microsclerotial inoculum 96 days after
planting. Levels of <u>Verticillium</u> inoculum from
left to right are: 179, 358, 1,432, 5,728,
11,456, 22,912, 45,824, 91,648, 183,296 and
366,592 microsclerotia per cc of soil.

TABLE 7. Influence of inoculum level on time of plant death using cellophane-grown inoculum.

Number microsclerotia per cc of soil	Number of plants dead out of 15 at different time intervals (days) after planting					
	85*	90	95	100	105	110
0	0	0	0	0	0	0
10	0	0	0	0	0	1
100	0	0	0	0	0	1
1,000	0	0	0	0	0	0
10,000	0	0	0	0	1	5
100,000	0	0	1	2	7	9
1,000,000	0	0	1	6	15	15
10,000,000	4	11	15	15	15	15

* First day that plants began to die

Similarly, approximately 50 per cent of the plants were dead in the 100,000 rate before dying began in the 10,000 rate.

The severity of wilt was closely correlated with the quantity of inoculum added to the soil and was less closely correlated with time of initial symptom expression (Tables 4 and 7). About 20 days elapsed between the first appearance of wilt and the time at which all of the plants showed wilt with the 10,000,000 rate of inoculum. Within the first five days after dying began, 73 per cent of the plants in this treatment were dead and within 10 days, 100 per cent were dead.

These data obtained under different environmental conditions confirm the results of the first experiment in that there was a close correlation between the time of symptom expression and the inoculum potential, but there was even a closer correlation between the inoculum potential and the time of plant death or severity of

symptoms. This is further emphasized by the fact that there was one plant showing wilt relatively early in each of the treatments receiving 10 and 100 microsclerotia per cc of soil, but the symptoms were mild and the plants survived until approximately the time at which the controls began to die. The single plant that died in the treatment with 10 microsclerotia per cc of soil was not the same plant that showed symptoms of wilt. Since plants began to die from normal maturity in the controls after 113 days, the death of the two plants in the low rates of inoculum at 110 days may not be significant, although in later isolations both plants were found to be infected by Verticillium.

Occasionally, plants in a higher rate of inoculum first showed symptoms at the same time as a plant in a lower rate. When this occurred, the plant growing in the higher rate of inoculum invariably had more severe wilt and died sooner.

Under the conditions in which this second experiment was conducted, stunting of plants did not occur with any level of inoculum. There was a significant increase in growth of plants at the 10,000,000 and 1,000,000 rates of inoculum shortly after symptoms of wilt appeared but this stimulation in growth is not apparent in the plants shown in Figure 4. As wilt became severe, growth slowed down, but plants in lower levels of inoculum and the controls continued to grow. During early stages of disease development, plants in the two highest rates of inoculum averaged about three inches taller than the controls. In the first

Fig. 4.   Severity of wilt in potato plants 103 days old as
affected by inoculum potential of <u>Verticillium</u> (93
days after transplanting to infested soil).   Levels
of inoculum from left to right are: 0, 10, 100,
1,000, 10,000, 100,000, 1,000,000 and 10,000,000
microsclerotia per cc of soil.

experiment, increasing rates of inoculum caused greater stunting
of plants (Figure 3).

Figure 4 shows a representative group of plants from each
of the treatments in the second experiment. In addition to showing
the severity of wilt, this figure shows some new shoots that
developed from plants in the 1,000,000 rate after symptoms became
severe. This resprouting from axillary buds was common among the
plants in the high rates of inoculum after they were severely
affected with wilt. Resprouting occurred under the conditions of
this experiment but it did not occur in the first experiment.

Tuber Yields

Tuber yields provided another basis for comparing the
severity of wilt with inoculum potential. The yield data are
shown in Tables 8 and 9.

The addition of non-infested sand-cornmeal in the controls
reduced tuber yields at higher rates but rates of 4 to 64 grams
per 35 pounds of soil had very little or no influence on yields
(Table 8). The addition of 256 to 1,024 grams per 35 pounds of
soil reduced the yields considerably and the reduction was in
proportion to the quantity of sand-cornmeal added to soil.

Addition of sand-cornmeal infested with Verticillium
reduced yields in proportion to the quantity of inoculum added
to the soil at rates below those in which non-infested sand-
cornmeal had no affect, or very little affect, on yields (Table 8).

TABLE 8.   Influence of inoculum level on tuber yields using
           sand-cornmeal inoculum.

Weight of inoculum added per 35 lbs.soil	Minimum number microsclerotia added per cc of soil	Grams of tubers in 5 cans selected at random					Average number grams per can	% Reduction in yield
		1	2	3	4	5		
Non-infested sand-cornmeal								
4.00 gm.	0	114	121	138	119	138	126	
16.00 gm.	0	126	118	125	124	139	126	
64.00 gm.	0	124	127	116	113	124	120	
256.00 gm.	0	122	115	109	113	111	114	
512.00 gm.	0	113	113	97	106	113	108	
1,024.00 gm.	0	99	94	90	98	89	93	
Verticillium- infested sand- cornmeal								
.25 gm.	179	135	125	122	129	114	125	00.8*
1.00 gm.	716	116	106	106	91	120	108	14.3*
4.00 gm.	2,864	91	102	109	102	105	102	19.1
16.00 gm.	11,456	82	91	101	119	77	94	25.4
64.00 gm.	45,824	98	64	85	86	104	87	27.0
256.00 gm.	183,296	70	88	57	65	87	73	36.0
512.00 gm.	366,592	61	61	66	63	65	63	41.7
1,024.00 gm.	733,184	51	69	51	63	58	58	37.7

* Based on comparison with the 4.0 gm. rate of non-infected
  sand-cornmeal

The percentage of yield reduction in comparison to the corresponding

controls became greater with an increase in level of inoculum;

however, the highest level examined did not reduce yields as much

as the level just below it. In this respect, tuber yields reflected

the same trends in disease severity as were shown by the data on

time of plant death. Apparently, secondary factors other than

time of infection modified the influence of inoculum potential

with rates of 1,024 or more grams of sand-cornmeal inoculum per 35 pounds of soil.

The average tuber yield of 15 plants per treatment in the second inoculum potential experiment is shown in Table 9. Under the conditions of this experiment, levels of inoculum below 100,000 microsclerotial particles per cc of soil did not significantly reduce tuber yields. With rates of 100,000, 1,000,000 and 10,000,000 microsclerotia per cc of soil, tuber yields were reduced in proportion to the quantity of inoculum added to the soil. In the absence of organic matter added with inoculum, the highest rate of inoculum reduced tuber yields the most with a reduction of 42.3 per cent. This percentage of reduction in tuber yield is comparable to the greatest reduction of 41.7 percent obtained in the first experiment.

TABLE 9. Relation of inoculum level to tuber yields using
cellophane-grown inoculum.

Number microsclerotia per cc of soil	Average tuber yields (grams) of 15 plants	Per cent reduction compared to control
0	207.0	
10	206.9	0.1
100	217.1	(∤4.8)
1,000	199.3	3.8
10,000	198.7	4.1
100,000	179.5	13.3
1,000,000	141.7	31.6
10,000,000	119.6	42.3

Frequency of Infection as Influenced by Inoculum Level

In the first experiment, stems were examined for the presence of
microsclerotia after all of the plants were dead and had dried. Micro-
sclerotia were found in the stems of all plants grown in soil with
Verticillium inoculum. They were not found in the stems of any con-
trol plants. Thus, infection had occurred in 100 per cent of the
plants where Verticillium was present in the soil.

In the second experiment, the stems of all plants were
isolated from at the soil level after the experiment was terminated.
Verticillium was recovered from 100 per cent of the plants in the
treatments receiving 10,000,000, 1,000,000 and 100,000 microsclerotia
per cc of soil. In the 100,000 rate of inoculum, only eight of
15 plants showed wilt during the entire experiment. Six of the
plants in the 10,000 rate produced Verticillium, although only two
showed wilt. None of the plants in the 1,000 rate yielded

Verticillium, but one and two plants produced it with rates of 100 and 10 microsclerotia per cc of soil, respectively. Thus, the results of this second experiment differed from those in the first in that with lower rates of inoculum Verticillium was recovered from a low percentage of plants while the pathogen was found in 100 per cent of the plants in all rates of inoculum in the first experiment.

There was a closer association between stem invasion and symptom expression in the second experiment than in the first. Apparently, conditions of temperature, or some other factor, lessened the frequency of infection but increased the susceptibility of tops after infection occurred in the second experiment. This conclusion agrees with the results of Robinson et al. (94) who were able to obtain wilt in Netted Gem potatoes only when temperatures were 24 to 28° C. Thus, it appears that the internal reactions leading to symptom expression in the Netted Gem potato may be more active at temperatures of 24 to 28° C. Factors leading to root infection appear to operate most effectively at lower temperatures. However, inoculum potential primarily determines the disease severity at any temperature.

TUBER TRANSMISSION OF VERTICILLIUM

The inoculum potential experiments showed that a high population of Verticillium microsclerotia in the soil was a prerequisite to severe wilting of potatoes. In view of the claims in the literature of the importance of tuber transmission of Verticillium wilt, and taking into consideration the methods whereby this importance was determined, it was of interest to determine if the pathogen and disease could be transmitted by tubers under more carefully controlled conditions. If tuber transmission of the pathogen occurred, and if severe disease resulted from such transmission as reported in the literature, this would conflict with the interpretations of results from the inoculum potential experiments.

Three greenhouse experiments were conducted to test tuber transmission of Verticillium wilt. Tubers used in these transmission experiments were obtained from fields that had severe Verticillium wilt. Tubers were stored at temperatures between 55° and 70° F. for at least three but not more than four months before being planted. Unless otherwise stated, they were not treated with fungicides prior to planting. Usually, they were washed in running tap-water and were dried before being planted except in one experiment where it was desired to test the influence of surface contamination in transmission. The experiments were conducted in the greenhouse between December and May.

Experiment 1

Isolations were made from vascular tissue just beneath the surface at the stem-end of 40 tubers. Stem ends were cut from 20 tubers from which Verticillium was recovered and planted in sterilized greenhouse soil. Stems were thinned to one per tuber as they emerged from the soil.

Plants produced by the infected tubers grew vigorously but never showed any symptoms of Verticillium wilt. Adjacent plants grown in an inoculum potential experiment showed severe wilt. Seventy-seven days after planting, four plants were removed and isolations were made from the stems at the soil level. None of these isolations yielded Verticillium. One of the four original seed-pieces was still solidly attached to the stem. Isolations made from the point of union between the tuber and the stem were negative but the pathogen was again recovered from the stem-end seed-piece just beneath the point of the original tuber isolations.

The remaining 12 plants (four of the seed-pieces decayed before producing a plant) were allowed to mature before isolations were made from the stems at the soil level. Verticillium was not recovered from any of the stems at the soil level or from the point of union between stem and tuber in the one plant still possessing a firm seed tuber. Verticillium was recovered from the stem-end of this tuber after it had produced a mature, non-invaded stem. After isolating the pathogen from this tuber a second time, the

tuber was allowed to suberize for a few days and was replanted. Another plant was produced from this tuber but the fungus could not be recovered in stem isolations from the soil level or from the point of union between stem and tuber. Isolations from the stem-end of the tuber produced Verticillium for the third time but neither of the two crops of plants produced by it had become infected.

Experiment 2

One-hundred-fifty tubers were selected for uniformity in size. Fifty tubers were taken at random from the sample and isolations were made from the stem-ends to determine the approximate percentage of infected tubers in the entire sample. Verticillium was recovered from 48 per cent of the tubers.

The following six treatments were set up using the remaining 100 tubers having approximately 48 per cent infection by Verticillium:

1. 50 stem-end halves of tubers

2. 50 eye-end halves of tubers

3. 20 whole tubers inoculated internally with Verticillium

4. 10 whole tubers dipped in a slurry of Verticillium

5. 10 pre-sprouted single-eye plants root-dipped

6. 10 whole tubers grown in infested soil

Fifty tubers were cut into stem-end and eye-end halves and were suberized for several days at room temperature before planting. Twenty tubers were inoculated in the stem-end with

microsclerotia, conidia and mycelium of <u>Verticillium</u>. A hole
1/2-inch deep was made with a transfer needle and inoculum was
forced into the hole which was then sealed with scotch-tape. Ten
tubers were dipped into a slurry of inoculum prepared from two
plates of four-week-old cultures of <u>Verticillium</u>. The contents
of two plates was macerated in a Waring Blendor with 500 ml. water
for three minutes. This inoculum was dark with microsclerotia.

The roots of ten pre-sprouted, single-eye plants were dipped
into the same inoculum slurry used for dipping tubers. The remain-
ing 10 tubers were planted into soil artificially infested with
approximately 10 million microsclerotia per cc of soil. The method
of infesting soil was the same as that used in the second inoculum
potential experiment.

The tubers were planted on January 13 into non-sterilized
soil obtained from an area in which the vegetative cover consisted
primarily of sagebrush and Juniper. Twenty potato plants were
grown in part of this soil earlier and <u>Verticillium</u> was not
recovered from any of the plants in stem isolations made after
they had matured. This soil was from the same source as that used
in the second inoculum potential experiment.

Wilting data were taken at different time intervals and on
April 14 all plants were removed for stem isolations from the soil
level. When stem-ends of the original seed tubers were firm,
isolations were made from them.

During the 92 days of the experiment, all plants in two of the six treatments showed wilt symptoms (Table 10). These were the treatments in which roots were dipped in the inoculum slurry and in which tubers were planted into infested soil. The treatments in which naturally infected tubers were planted, and in which tubers were inoculated internally or externally, produced plants that did not show symptoms of disease. Under the conditions of this experiment, plants grown in infested soil showed far more severe wilt than plants inoculated by root-dipping.

Stem isolations showed that 100 per cent of the plants in the two treatments with wilt became infected by Verticillium (Table 10). One of 10 plants produced by tubers dipped in the inoculum slurry was infected, but this plant had not shown symptoms of wilt. Verticillium was not recovered from any of the 50 plants grown from naturally infected stem-ends of tubers or from any of the 50 plants produced by the corresponding eye-ends of the same tubers. None of the 19 plants produced by the 20 internally inoculated tubers were infected.

Of the 50 stem-end halves of tubers planted, 14 had not decayed at the end of the experiment. Isolations were made from these 14 tubers and six of them (44 per cent) produced Verticillium from just beneath the original point of attachment to the stolon. Therefore, these six tubers were infected with Verticillium during the entire length of the experiment but the fungus had not infected the stems produced by them.

TABLE 10. A comparison of the frequency of wilt in potato plants
grown from naturally infected tubers and plants inocu-
lated by various methods.

Treatment and number of tubers planted	Number plants produced by tubers	Number plants showing wilt*	Number plants from which Verticillium was isolated
50 stem-end halves of tubers with 48 per cent natural infec-tion	50	0	0
50 eye-end seed-pieces (from above tubers)	50	0	0
20 internally inoculated tubers	19	0	0
10 tubers dipped in inoculum	10	0	1
10 pre-sprouted plants root-dip inoculated	10	10**	10
10 tubers planted into artificially infested soil	10	10	10

* Plants were examined for wilt up to 92 days after planting.
** Plants inoculated by root-dip inoculation showed mild wilt
compared to those grown in soil artificially infested with
approximately 10,000,000 microsclerotia per cc of soil.

Experiment 3

Another sample of tubers in which approximately 50 per cent
were internally infected with Verticillium, as shown by isolations,
were obtained. Seventy-five of these tubers were selected for
uniformity in size. These tubers were not washed or cleaned between
the time of harvesting and the time that they were used in this
experiment. Twenty-five of the tubers were surface sterilized in
20 per cent Clorox for two minutes and were then rinsed in running
tap-water. They were cut into stem-end and eye-end halves and were

allowed to suberize several days before planting. The remaining 50 tubers were cut into stem-end and eye-end halves but they were planted without washing or surface-sterilizing. Tubers were planted in greenhouse soil that had not produced infection by Verticillium in preliminary experiments with potatoes.

The numbers of tubers producing plants before decaying were: surface-sterilized stem-ends, 21; surface-sterilized eye-ends, 18; non-sterilized stem-ends, 44; non-sterilized eye-ends, 48. The plants were examined closely for wilt symptoms but symptoms failed to develop on any of the plants between the time that they emerged and the time that they matured.

Isolations were not made from the plants but each stem was examined for the presence of microsclerotia after the plants matured. The presence of microsclerotia on dead stems and the recovery of Verticillium in isolations have been closely correlated in all of the greenhouse experiments reported in this thesis. Absence of microsclerotia from dead stems above the soil level, under greenhouse conditions, has been correlated with absence of infection. Therefore, it was assumed that none of the stems in this tuber transmission experiment were infected with Verticillium because microsclerotia were not found in any of them.

DISCUSSION

Some of the older concepts of Verticillium wilt that are still generally accepted are incompatible with present knowledge of the disease. Earlier workers (70, 71, 86, 91, 95) emphasized the importance of tuber transmission in disease development in the field and believed that a single locus of infection could serve to induce severe symptoms. The roots were not obviously damaged when invaded by Verticillium and it was believed that they served primarily as pathways for the pathogen to gain entrance to the stem. Without sufficient evidence, it was assumed that the host was susceptible to invasion at any stage of development in the field when pathogen and host were in close proximity. Different investigators believed that Verticillium spread from plant to plant during the growing season and that the fungus grew saprophytically through normal field soil. These concepts of Verticillium wilt are in conflict with the results presented in this thesis on the influence of inoculum potential on disease development.

Influence of Inoculum Potential on Disease Severity

The inoculum potential experiments showed conclusively that the level of inoculum in the soil is the principal factor that determines rate of disease development. Under conditions near optimum for disease, severe wilt resulted only with high levels of inoculum and mild symptoms or no symptoms appeared in plants grown in low levels of inoculum. When temperatures were above optimum

for disease development severe wilt still occurred, but only with very high levels of inoculum.

In the first experiment, microsclerotial inoculum was approximately 2,000 times as concentrated in the treatment producing most severe disease as in the one producing the mildest symptoms. Since all plants became infected, there could have been approximately 2,000 times as many infections per plant with the higher level of inoculum.

Assuming that infections were distributed at random throughout the root system, it is possible that with rates of inoculum producing severe wilt the entire root system was invaded soon after the plants became susceptible to infection. Because of the density of inoculum in the high rates, roots would not be capable of outgrowing infections since new infections could occur as rapidly as new root growth appeared. Thus, with a maximum effective level of inoculum a maximum disease severity would result because the entire root system would be invaded quickly after the plant became susceptible to infection. This concept of Verticillium wilt, which takes into consideration the importance of the quantity of root system invaded, is in excellent agreement with results obtained by Keyworth (60) and Talboys (109, 110, 111) and Talboys and Wilson (108) for Verticillium wilt of hops, but they did not associate their results with the importance of inoculum potential.

The severity of Verticillium wilt is a relative phenomenon related to time and the level of inoculum in the soil. The inoculum potential experiments showed that a high level of inoculum produced severe symptoms of wilt relatively early, while progressively lower levels of inoculum initially produced milder symptoms that became severe more slowly. The lowest levels of inoculum induced initial symptoms latest and these had very little effect on the plants. These results are in agreement with those obtained by Robinson et al. (94), although no interpretation of their results was given.

Because little attention has been given to the importance of inoculum potential, the results of earlier pathogenicity experiments with various isolates of Verticillium are of doubtful significance. It is difficult to tell whether the results of these experiments represent differences in inoculum potential, in longevity of isolates within the soil, or in pathogenicity between isolates after infection occurred. Probably the single exception to this statement is the mint wilt pathogen (38, 34, 82, 83, 115) which is well established as being a distinct pathogenic strain of Verticillium.

Pathogenicity of Verticillium should be more clearly defined before comparisons of pathogenicity between isolates are attempted. As pointed out by Talboys (110) different strains have different capacities for successful invasion of vessels in the roots after penetration to cortical tissue is accomplished. In this case, a higher level of inoculum of one strain may be required to cause an

equal number of successful vessel invasions as compared to another strain. Pathogenicity could then be related to the ability of a strain to gain entrance to vessels or it could be related to the damage done after penetration to vessels is accomplished.

A resistant and a susceptible variety of the same host might be rated as equal in susceptibility if a higher level of inoculum was used in testing the resistant host. All comparative tests of varieties for resistance should be carried out in well-designed field plots or in artificially infested soil in the greenhouse where the level of inoculum can be carefully controlled.

Attempts at comparing virulence of different isolates should take into consideration survival of the pathogen in soil. Any factor that will alter survival of Verticillium in the soil will also influence the apparent pathogenicity of that isolate or strain. Nelson and Wilhelm (81) recently showed that environmental factors influenced survival of microsclerotia more with some isolates than with others. Where there is a delay in host invasion, such as occurs with potatoes, environmental factors favoring the survival of one isolate over that of another would show up as differences in pathogenicity between the two isolates whereas there may not be a true difference in pathogenicity. Thus, what appears to be a difference in pathogenicity between microsclerotial and non-microsclerotial white variants of Verticillium (8, 49, 83, 85, 88, 94) may not be a difference in pathogenicity but instead, the differences in apparent

pathogenicity might represent a greater survival of the micro-
sclerotial strains. Since the microsclerotial isolates are more
resistant to adverse environmental conditions, differences in
apparent pathogenicity could be explained by differences of inocu-
lum potential. It is necessary to consider inoculum potential and
survival of _Verticillium_ in any pathogenicity study.

Time of Infection

Isolations from plants in the inoculum potential experiments
showed conclusively that potatoes of the Netted Gem variety are
resistant to infection from microsclerotia in the soil when plants
are young. Time of infection was closely associated with the time
of initial tuber formation. In several subsequent greenhouse and
field trials the time of infection was found to be associated with
the time of tuber formation. Environmental factors that delayed
tuber formation also delayed the time of infection.

Different varieties of potatoes are known to develop tubers
during different periods of the growing season. A comparison of
resistance to Verticillium wilt among such varieties at any given
time should take into consideration possible differences in time
of susceptibility to infection. Differences in time of infection
could easily be mistaken as differences in susceptibility between
varieties. As pointed out in the literature review, there is
reason to believe that hosts other than potatoes have certain
stages of susceptibility to infection.

Since there is a definite stage in the early development of potato plants when they are resistant to infection, it would be of interest to determine the duration of susceptibility. In an experiment not reported in this thesis, an attempt was made to determine the length of susceptibility to infection by isolating from many thousands of inches of potato roots taken from plants grown in naturally infested soil in the field. Roots were susceptible to infection for only a relatively short period of time under the conditions of this experiment.

The occurrence of definite periods of susceptibility of plants to infection is not a new phenomenon. Granger (25) reported two stages of high susceptibility in potatoes to infection by the late blight fungus, Phytophthora infestans. Plants were highly susceptible during early stages of growth, followed by a period of high resistance which was again followed by a period of high susceptibility. Changes in susceptibility were shown to be correlated with changes in carbohydrate levels in the plants. These stages of susceptibility were not related to the presence or absence of inoculum and proper environmental conditions as shown by planting potatoes adjacent to each other at various time intervals.

It is interesting that the stage of development when plants were highly resistant to infection by late blight would correspond to the approximate stage when we found them to be highly susceptible to infection by Verticillium. Conversely, the phases of growth when Granger (25) found plants to be highly susceptible to infection

by late blight would correspond closely to the stages when they were
highly resistant to infection by Verticillium.

Methods of Inoculation

The results from the inoculum potential and tuber transmis-
sion experiments suggest that present methods of inoculation of
plants be reconsidered.

The first commonly used method of inoculation was to insert
a small quantity of Verticillium inoculum into the stem of a plant.
This gave erratic results and isolates of Verticillium obtained
from severely wilted plants frequently failed to produce symptoms.
When symptoms appeared they were generally mild and not typical of
those found under natural conditions (8, 27, 32, 60).

The root-dip inoculation method in which roots of a plant
were immersed in a fungus suspension was a great improvement over
stem inoculations because more severe symptoms of wilt were pro-
duced and severity of symptoms was more uniform between plants.
This method of inoculation appeared so promising that workers over-
looked its limitations. Those roots that are present at the time
of inoculation become infected but generally a majority of roots
develop after inoculation. There are no data to show that
Verticillium spreads from root to root or that it grows to any
extent towards the apex of an inoculated root. Therefore, it would
be expected that a limited part of the root system would become
invaded using root-dip inoculation. With hosts requiring a

relatively large part of the root system invaded for severe wilt to occur, this method of inoculation would not be satisfactory.

In an experiment reported in this thesis, root-dip inoculation using a heavy spore, mycelium and microsclerotial slurry of Verticillium was compared with soil infestation. Root-dip inoculation produced symptoms as early as soil infestation, but symptoms were much milder and more localized with root-dip inoculation. The mild symptoms resulting from root-dip inoculations resembled those produced by plants grown in a low level of inoculum where it was shown by isolations that a small part of the root system was invaded. A heavy infestation of soil with microsclerotia resulted in far more severe symptoms of disease than occurred with root-dip inoculation.

The method of growing microsclerotial inoculum on cellophane proved valuable in more than one way. It was possible to adjust the microsclerotial population to any desired level without the addition of organic matter and without significant effect on the soil microflora. Soil dilutions could be made to follow changes in microsclerotial populations without interference from an increase of saprophytic, heavily sporulating fungi that commonly develop during decomposition of organic matter. The use of sand-cornmeal inoculum was satisfactory for increasing Verticillium in the soil to a certain level, but beyond this level the influence of inoculum potential was reversed. This was probably due to adverse effects on plant development and, possibly, a reduction of inoculum

occurred because of the increased microbial activity stimulated by the high rate of organic matter.

The addition of some organic materials in relatively small amounts is known to result in a reduction of the inoculum potential (118, 134, 136), and the stimulated activity of soil organisms also reduces Verticillium (143, 148). Therefore, it is important not to add large quantities of organic matter to the soil when attempting to build up the population of Verticillium.

Tuber Transmission

According to the literature, there are two types of tuber transmission of Verticillium wilt. In one case Verticillium is present in soil adhering to the surface of tubers (94) and planting of such tubers results in infection of plants, presumably through roots coming in contact with the surface inoculum. However, this type of transmission is relatively unimportant because of the current widespread use of seed-treatment fungicides.

The second type of transmission reported in the literature is that in which Verticillium is present within the tuber, and disease is believed to develop after the pathogen grows through the seed-tuber into the new shoots. This type of transmission was advocated by earlier workers (70, 71, 86, 91, 95), although it was not demonstrated experimentally.

No one has demonstrated conclusively that Verticillium-infected tubers give rise to infected shoots. The entire foundation

of the tuber transmission theory is based on field experiments in which it was always assumed that _Verticillium_ was entirely absent from the soil. Furthermore, methods of analyzing tuber transmission in the field experiments have always been indirect. Methods of recording transmission data included: differences in tuber yields; vascular discoloration; presence or absence of wilt; and percentage of tubers invaded in the crop produced by infected seed. The data of many workers show that none of these methods alone are reliable for determining the presence or absence of _Verticillium_-infection in plants. At present, the only sure way of determining infection of plants is by isolations.

The conditions under which tuber transmission experiments were conducted and the methods used in recording transmission data are reason enough to question the entire tuber transmission theory advocated by earlier workers.

In three separate greenhouse experiments using autoclaved soil and soils that were known to be free of _Verticillium_, transmission from naturally infected tubers that had been stored for from two to four months prior to planting did not occur. Plants grown from naturally infected tubers did not become infected although the pathogen was shown to be present in the seed tubers during the entire growing season unless the seed pieces had rotted. One tuber produced two separate crops of stems, neither of which

became infected and <u>Verticillium</u> was recovered from the tuber before
the original planting and after each crop of stems had matured.

SUMMARY

Severity of Verticillium wilt in Netted Gem potatoes was directly proportional to the quantity of microsclerotia of Verticillium albo-atrum added to soil in which the plants were grown. Symptoms resulting from infection with various levels of inoculum ranged from symptomless invasion with low levels to rapid wilting and yellowing with high levels of inoculum.

Time of appearance of wilt symptoms and time of plant death were related to the level of inoculum, but time of plant death provided a better measure of disease severity. When plants grown in low and high levels of inoculum first showed wilt at the same time, plants in the higher rate of inoculum invariably were wilted more severely and died more quickly.

Plants did not become infected until approximately the time of initial tuber formation even though plants and root systems were well developed long before tubers were set.

In three greenhouse experiments designed to determine the importance of tuber transmission of Verticillium, tuber transmission did not occur. Naturally infected tubers produced healthy, non-infected plants when planted in soil free of Verticillium. The pathogen survived within non-decayed tubers through the entire growing season without causing infection of plants.

In experiments that were not repeated, stunting of potato plants occurred in proportion to the quantity of inoculum added to

soil when air temperatures were maintained between 65 and 75° F.
When the minimum daily air temperatures were maintained above 75° F.
stunting did not occur even with high levels of inoculum that
induced severe wilting and yellowing. With minimum daily air tem-
peratures above 75° F., a significant increase in growth resulted
during early stages of disease development with high levels of
inoculum.

An extensive review of the literature on Verticillium wilt
was presented to show that earlier concepts of the disease which
are still commonly accepted frequently conflicted, due to misinter-
pretation of data. The results presented in this thesis conflict
with the conclusions of many workers, but not with the data upon
which these conclusions were based. The data presented in this
thesis are complementary to earlier reports when the latter are
reinterpreted.

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