Verticillium wilt of hops in Oregon, caused by Verticillium dahliae Kleb., was first reported in 1956. Later, V. albo-atrum Reinke & Berth. was also isolated from infected hop plants, and thus both species of the fungus were shown to be the causal agents of the disease in Oregon.

The purpose of this research was to study temperature relations and host range of hop isolates of Verticillium existing in Oregon, and also to determine infectivity and pathogenicity of these isolates to different varieties of hops.

The temperature relations of two V. dahliae and two V. albo-atrum isolates, recovered from infected hops grown throughout the Willamette Valley of Oregon, were studied by dry weight measurements. Two additional V. dahliae isolates, one from peppermint and
one from potato, were included in the experiments. The isolates were grown in Czapek Dox broth plus yeast extract at ten temperatures between 5°C and 32°C.

The dry weight was affected by the interactions of isolate, temperature, and period of incubation. At temperatures up to 17°C, the dry weights of all isolates were increased by longer incubation periods with no autolysis of the mycelial mass observed in 17 days. Above 17°C, however, the incubation periods required for maximum dry weight production and the onset of autolysis varied, depending on the temperature and the isolate. A faster initial growth rate, caused by increasing temperature, hastened the onset of autolysis, and thus did not result in greater amounts of dry weight production in longer periods. On the other hand, a slow initial growth rate, caused by too high a temperature, resulted in delay of autolysis and production of greater dry weights in longer incubation periods.

_V. albo-atrum_ isolates did not grow at 30°C and evidence was obtained showing that failure to grow was due to thermal death of germinated and ungerminated spore inoculum rather than to inhibition of germination. All _V. dahliae_ isolates grew fairly well at 30°C but failed to grow at 32°C. The latter temperature was lethal to the spore inocula of _V. dahliae_ isolates after 7 to 12 days of incubation.

These results point out a significant physiological difference between _V. albo-atrum_ and _V. dahliae_ and thus support the validity of
considering *V. dahliae* as a distinct species.

In host range studies, one *V. albo-astrum* isolate (No. 138), originally obtained from Fuggle hops, was found to infect and proliferate greatly in stems of potato, and to a lesser extent in tomato and eggplant. Among *V. dahliae* isolates, one originally recovered from Bullion hops (No. 148), proliferated poorly in potato, eggplant, and strawberry. The other (No. 150), obtained from Fuggle hops, proliferated greatly in stems of peppermint and was highly pathogenic to that host.

The conclusion was reached that the hop isolates of *Verticillium* existing in Oregon, are primarily pathogens of other plants, with host ranges that include hops to some extent.

*Verticillium* isolates from infected hops, peppermint and potato, infected a low percentage of plants among 14 hop varieties grown in infested soil. Differently prepared inoculum (laboratory infested soils and straw culture) or different levels of inoculum, did not affect the percentage of infected plants appreciably. All *Verticillium* isolates, regardless of their origin or species, proliferated poorly in stems of infected hop plants and no actual wilting or death of the infected plants was observed.

It was concluded that the strains of *Verticillium* affecting hops in Oregon are not virulent pathogens of this crop and that they are primarily adapted to plants other than hops.
Verticillium Wilt of Hops: Pathogenicity, Host Range, and Temperature Relations of the Causal Agents

by

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INTRODUCTION

The United States leads the world in hop production. In 1967 about 30,000 acres produced some 50 million pounds of this crop (37). Commercial production of hops is confined almost entirely to California, Idaho, Oregon, and Washington (38, p. 538). The brewing industry uses approximately 98 percent of the world hop production in the manufacture of beer and ale (8, p. 3).

Verticillium wilt of hops was first reported in 1927 by Harris (34) in England, and Verticillium albo-atrum R & B was identified as the causal agent. The disease persisted from year to year but fluctuated in severity and hop production was not seriously affected. During late 1930's, a more severe form of the disease was reported in England (32; 51). The new outbreak differed from those of previous years in that it spread rapidly through the affected fields and progressed each year in extent and severity. Issac and Keyworth (47) in 1948 presented conclusive evidence that the "fluctuating" and "progressive" outbreaks were caused by different strains of V. albo-atrum, which were morphologically identical but distinctly different in their virulence to hops. Verticillium dahliae Kleb. also has been reported to cause wilt of hops (11; 51; 101), however, the disease
caused by this fungus is similar to fluctuating outbreaks in extent and severity.

In the United States, Verticillium wilt of hops was first identified in 1956 by Horner (38), who isolated *V. dahliae*, and later, *V. albo-atrum* from infected Fuggle hops grown in Oregon. Close observation of the outbreaks have revealed that strains of *V. albo-atrum* and *V. dahliae* infecting hops in Oregon are much less virulent than the progressive strain of *V. albo-atrum* found in England. The spread of the disease has been relatively slow and hop production has not been seriously affected.

Verticillium wilt is a widespread and destructive disease of many crops in hop producing states. The recovery of *V. albo-atrum* from hops in Oregon has been reason for concern since it causes a devastating wilt disease of hops in England. Because of the potential importance of Verticillium wilt in United States hop production, research is needed to provide information on spread and survival of the causal agent, resistance of hop varieties, and control measures.

The purpose of my research was: 1) to obtain information on the physiological characteristics (temperature relations) of *V. albo-atrum* and *V. dahliae* isolated from hops and other crops; 2) to determine the host preference of hop isolates of *Verticillium*; and 3) to test commercial and newly developed experimental hop varieties for resistance to the disease.
REVIEW OF LITERATURE

Taxonomy and Morphology

The Host Plant

The commercial hop plant, *Humulus lupulus* L., is composed of annual climbing vines arising from a perennial crown. In addition to the aerial stems, the crown also produces true roots and underground stems or rhizomes. Rhizomes bear numerous buds and adventitious roots. The aerial stem portions or vines are herbaceous at first, later become woody, and possess long, hollow internodes which tend to be somewhat angular. The vine twines in a clockwise manner about its support. Hop plants are remarkably vigorous and a growth of 6 to 12 inches in 24 hours has been reported (8, p. 3). The stems may grow 20 to 25 feet long in a growing season and they die back to crown after maturity.

The hop plant is typically dioecious but an occasional plant may bear both staminate and pistillate inflorescences (8, p. 3; 65, p. 2). Clusters of pistillate inflorescences are borne in the axils of the upper leaves, with bulk of the flowers on the long lateral branches. The mature female flowers comprise the so-called "hops" of commerce and are sometimes called strobiles or cones. In the mature hop cone, the outer surface of bracteoles, the perianths, and the
bases of the bracts are covered with numerous bright yellow, multicellular, glandular trichomes. These structures called lupulin glands become filled with a volatile secretion called lupulin (65, p. 3). Lupulin contains a variety of essential oils and resinous substances which impart the characteristic aroma and taste of great importance in the brewing industry (8, p. 3).

**Humulus lupulus** has been placed in a number of plant families over the years. At present it is placed in the Moraceae (mulberry family) by Lawrence (58), and in the Cannabinaceae (hemp family) by Metcalf and Chalk (64) and Benson (2).

**The Pathogen**

In 1879 Reinke and Berthold (72) described a species of *Verticillium* which caused vascular wilt of potato plants in Germany. They named the fungus *Verticillium albo-atrum* R & B, and described and illustrated dark brown cells and dark thickened hyphal masses as the only resting bodies of this organism. In 1913 a similar wilt disease was described in dahlia by Klebahn (56) who isolated and named the causal agent *Verticillium dahliae* Kleb. One of the characteristics of Klebahn's fungus was formation of small, black pseudosclerotia (microsclerotia) which were not present in the type culture of *V. albo-atrum* R & B.

Isaac (39, p. 138–139), in a "comparative study of pathogenic
isolates of *Verticillium*¹, clearly described formation of the two types of resting structures, dark mycelium and microsclerotium. In the dark mycelial types, hyphae become septate and the "cells" so formed thickened to form black resting mycelium. On some media after considerable growth, black specks were seen which resembled microsclerotia. But close examination revealed that they were formed by the intertwining of the hyphae giving rise to mycelial knots. These mycelial knots were never formed from a single hypha and no budding of cells was observed in their formation. In the microsclerotial types, on the other hand, contiguous hyphae became slightly swollen and divided by septa to form new cells. New cells were observed to bud off in all planes, forming a small, almost spherical mass. Soon, commencing from the center of the mass, the walls of the cells began to thicken and darken, eventually forming a microsclerotium. No structure resembling the resting hyphae of the dark mycelial types was ever observed (39, p. 138).

The validity of *V. dahliae* as a separate and distinct species has been questioned by several workers including Presley (71), Rudolph (78), and Wilhelm (102), who have considered that the dark mycelial and microsclerotial isolates belong to one species, *V. albo-atrum*.

Rudolph (78, p. 252-254), in expressing his view about the controversy, maintained that a difference in resting structure should not entitle a fungus to specific rank. Wilhelm, (102, p. 919) from his
experiments on the effect of temperature on microsclerotial forma-
tion, concluded that the resting structures, especially microsclerotia, are not reliable characters upon which to separate species. In his experiments, microsclerotia formed readily in culture at lower tempera-
tures, but above 25°C only sparse microsclerotial formation oc-
curred.

The authors (71; 78; 102) refusing to accept V. dahliae as a dis-
tinct species, however, have never been able to prove that micro-
sclerotial isolates will change to dark mycelial type cultures or vice versa. Moreover, they have made no critical comparisons on the physiological characteristics, i.e., temperature relations, of their isolates with dark mycelial types such as described by Pethybridge (70), Van der Meer (100), Berkeley et al. (3), Chamberlain and Brien (12), Ludbrook (59), Ayers and Hurst (1), and Isaac (39; 46). The degree of microsclerotial formation and the pathogenicity of the iso-
lates used by Rudolph and Presley varied considerably (89), but
neither author described any of the typical characteristics of V. albo-
atrurn, such as blackened bases of conidiophores, dark thickened hyphae, and generally large and occasionally one septate conidia (89).

The significance of the morphological differences of the resting my-
celium in V. albo-atrurn and microsclerotia in V. dahliae has been stressed by many workers (3; 39; 56; 59; 76; 100; 110).

In this literature review, in order to minimize the confusion
regarding the species of *Verticillium* involved, the method proposed by Martinson (60, p. 10) is followed. That is, where there is a reasonable certainty that *V. albo-atrum* was used to designate the microsclerotial type of the fungus, the original author's nomenclature is reported, with (*V. dahliae*) added.

*Verticillium* sp. have shown a very high frequency of variation in colony morphology and amount of resting structure production. Gauger (27) and Presley (71) demonstrated this phenomenon for the colonies originated by single sporing from the same parent colony, which in turn was of single spore origin. They concluded that the ability to produce dark mycelium or microsclerotia was related to the variation of the original type culture, *V. albo-atrum*.

Berkeley et al. (3) discovered that by culturing on sterilized potato plugs, the hyaline variants of both *V. albo-atrum* and *V. dahliae* can be successfully induced to form resting structures characteristic of the original culture. The same kind of observation was made by Robinson et al. (76) who reported that the certain hyaline variants of both species possessed the ability to form their characteristic resting bodies when they were cultured on potato plugs or media containing phenolic substances such as catechol. However, microsclerotial isolates (*V. dahliae*) were never induced to form dark mycelia characteristic of *V. albo-atrum*.

Several researchers have attempted to explain the high degree
of variation in *Verticillium* sp. Fordyce and Green (25) reported evidence for genetic compatibility and recombination of factors between dark mycelial and microsclerotial isolates. Based on their observations, they assumed that heterokaryosis was involved among the dark mycelial and microsclerotial types. Hastie (36) felt that the genetic recombination demonstrated by Fordyce and Green (25) was likely a result of parasexualism, since he could detect no heterokaryotic conidia. Dual phenomenon has been discussed by some workers to explain the variation in imperfect fungi including *Verticillium* (31; 76).

The colony characteristics in *Verticillium* sp. have been shown to vary with external factors, i.e., media and conditions of the culture. Brandt (7) has reported that under identical conditions of temperature, humidity, and medium composition, he could detect significant variations in colony appearance of his homokaryotic isolates of *V. albo-atrum* (*V. dahliae*). He felt that variations in colony appearance were due to the proximity of other *Verticillium* colonies and described the presence and effect of a morphogenic factor.

Johnson (49) has shown that the concentration and type of nitrogenous compounds in the medium can affect the morphology of the colonies in *V. albo-atrum* (*V. dahliae*).

It can be seen that the workers not accepting *V. dahliae* as a separate species were very possibly misled by differences in colony appearance and the high frequency of variations in their cultures.
Talboys (93) has shown that *V. albo-atrum* and *V. dahliae* can be easily distinguished on a medium containing 100 ml concentrated prune extract, 5 g lactose, 1 g Difco yeast extract, 30 g agar plus distilled water to make 1000 ml, with the pH adjusted to 5.8 to 6.0.

Isaac (39; 41; 42; 44) and Keyworth (51, p. 346-357) have accepted *V. dahliae* as a valid species. To support his view, Isaac (39, p. 141-157) has demonstrated that the maximum temperatures as well as pH optima for growth of the two types of *Verticillium* are different. He has reported that *V. dahliae* grew moderately well at 30°C, while *V. albo-atrum* did not. The pH range for the optimum growth of *V. albo-atrum* was 8.0-9.6, while that of *V. dahliae* was 5.3-7.2. Isaac has also presented evidence showing a difference in the preference of carbon sources for optimum growth by the two forms of the fungus.

Ludbrook (59, p. 122-123) also demonstrated that *V. dahliae* and *V. albo-atrum* could be distinguished by the temperature response as well as resting stage morphology. He showed that the upper temperature limit for *V. albo-atrum* was 28 to 30°C. At this temperature range, *V. dahliae* made fair growth and the upper limit was higher.

The information about the effect of temperature on the growth of *V. dahliae* and *V. albo-atrum* will be reviewed in detail in following sections.
In addition to the resting structure differences, attempts have been made to investigate other morphological differences existing between the species of *Verticillium*. Van der Meer (100, p. 1-82), in 1925, stated that in her studies microsclerotial types of the fungus differed from dark mycelial type in spore size. Isaac (39, p. 140) reported that he was unable to find any such constant differences in conidial dimensions. Canadian workers Devaux and Sackston (19, p. 807-808) also could not detect any consistent differences in the size of conidia and thus stated that conidial dimensions can not be used to distinguish between *V. albo-atrum* and *V. dahliae*.

Chaudhuri (13, p. 519), in 1923, reported that size of the conidia of *V. albo-atrum* varies with different media and with the liquid or solid nature of the culture medium.

In a recent paper, however, Smith (89, p. 458-469) reported that there are significant differences in size of conidia formed by *V. albo-atrum* and *V. dahliae* on natural substrate. He indicated that the failure of the previous workers to detect such differences was mainly due to study of the organisms strictly on artificial media rather than on natural host material. His observations show that *V. albo-atrum* conidiophores are typically larger with a dark swollen base and larger conidia, mainly 3.5-8 x 2-3 microns. On the other hand, *V. dahliae* has fewer, smaller, and completely hyaline conidiophores, smaller conidia, mainly 3-6 x 1.5-2 microns. The dark bases of
conidiophores have been described by others as typical of *V. albo-atrum* (3; 19; 39; 59; 77), and their diagnostic significance has been stressed by Smith (89).

Review of the older literature and particularly information accumulated from recent and more critical studies, indicates that there is sufficient evidence and data to support the validity of *V. dahliae* as a distinct species. Many plant pathologists have reported that *V. albo-atrum* is a more virulent pathogen than *V. dahliae*, e.g., in hop (32), lucerne (46), potato (76), strawberry (97), tomato and cucumbers (89). In advising control measures, as stated by Isaac (42), it is therefore essential to know the identity of the pathogen.

The differences in the temperature relations, pathogenicity, host range, and geographic distribution of *V. albo-atrum* and *V. dahliae* will be considered in the following sections. Based on this information, it is clear that these two types of *Verticillium* are quite distinct and that there is considerable advantage in recognizing them as species.

**Temperature Relations**

**In Culture**

There are several methods used for measuring fungal growth. However, as Cochrane (16, p. 11) points out, some methods are
useful only for particular organisms or for special problems; no method is so general that it can be recommended for all. The most satisfactory measurement of growth, within limits, is by determination of dry weight of mycelium. The principal limitation is that weight may reflect accumulation of polysaccharides or other reserve materials rather than synthesis of new protoplasm (16, p. 11). There are several techniques for determination of dry weight; the one most commonly used is filtration of the liquid cultures, using special filter paper and oven drying and weighing the fungal material.

Measuring linear growth on agar media is the least laborious method for estimating growth and has been correspondingly popular. Growth is commonly expressed as the constant rate established after the initial period of slow growth. As regards simplicity the latter method has obviously great advantages. It is possible to carry out experiments on a large scale, a circumstance which allows of abundant repetition of experiment and effective control in each case. There is, further, the great advantage that the growth of any one colony can be followed throughout all its stages, whereas the dry weight method involves the destruction of a culture for each measurement taken. The greatest objection to the method of linear measurement, as Brown (9, p. 109) has indicated, is that "it affords in many cases no indication whatever of the amount of mycelium in the fungal colony." Chaudhuri (13, p. 528) reported that there was little or no
difference in the rate of spread of V. albo-atrum on full, 1/2, 1/4, and 1/16 strength nutrient medium, although there was a marked difference in the amount of mycelium produced.

Another factor which often has been overlooked in linear extension measurements, is that the spread of the fungus is significantly influenced by the depth of the medium. This has been pointed out by several workers (13; 18).

In general the authors that have accepted V. dahliae as a distinct species, have reported that it can tolerate significantly higher temperatures than V. albo-atrum in culture. Table 1 shows this for the isolates obtained from different hosts.

Ludbrook (59, p. 122-123) used 8 cultures of V. dahliae and 37 cultures of V. albo-atrum isolated from widely different host species in his temperature studies. He reported that in every case the maximum temperature for growth of V. dahliae was distinctly higher than that of V. albo-atrum. Thus he demonstrated that the two species could be separated on the basis of temperature response as well as morphological characters. This was confirmed by Isaac (39) who reported essentially the same results.

Smith (89, p. 456) cultured 20 pathogenic isolates of Verticillium, obtained from California and England, at 30°C. None of V. albo-atrum isolates grew at 30°C, while all V. dahliae cultures made fair growth. Robinson et al. (76) reported that more than 25 isolates
Table 1. The maximum temperatures reported for the growth of *V. albo-astrum* and *V. dahliae* in culture.

<table>
<thead>
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<th>Verticillium species and host</th>
<th>Maximum temperature (°C)</th>
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<tr>
<td><strong>V. albo-astrum:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>below 30°</td>
<td>Isaac (39)</td>
</tr>
<tr>
<td>Cucumber</td>
<td>below 30°</td>
<td>Ludbrook (59)</td>
</tr>
<tr>
<td>Hop</td>
<td>below 30°</td>
<td>Isaac (39)</td>
</tr>
<tr>
<td>Hop</td>
<td>below 30°</td>
<td>Ludbrook (59)</td>
</tr>
<tr>
<td>Hop (fluctuating)</td>
<td>below 30°</td>
<td>Smith (89)</td>
</tr>
<tr>
<td>Hop (progressive)</td>
<td>below 30°</td>
<td>Smith (89)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>below 30°</td>
<td>Smith (89)</td>
</tr>
<tr>
<td>Tomato</td>
<td>below 30°</td>
<td>Smith (89)</td>
</tr>
<tr>
<td>Potato</td>
<td>below 30°</td>
<td>Robinson <em>et al.</em> (76)</td>
</tr>
<tr>
<td>Potato</td>
<td>28-30°</td>
<td>Devaux and Sackston (19)</td>
</tr>
<tr>
<td>Potato</td>
<td>28°</td>
<td>Edgington and Walker (21)</td>
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<tr>
<td><strong>V. dahliae:</strong></td>
<td></td>
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<tr>
<td>Sainfoin</td>
<td>above 30°</td>
<td>Isaac (39)</td>
</tr>
<tr>
<td>Hop</td>
<td>above 30°</td>
<td>Isaac (39)</td>
</tr>
<tr>
<td>Hop</td>
<td>above 30°</td>
<td>Smith (89)</td>
</tr>
<tr>
<td>Eggplant</td>
<td>34°</td>
<td>Richardson (73)</td>
</tr>
<tr>
<td>Eggplant</td>
<td>above 30°</td>
<td>Ludbrook (59)</td>
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<tr>
<td>Strawberry</td>
<td>above 30°</td>
<td>Smith (89)</td>
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<td>Cotton</td>
<td>32°</td>
<td>Edgington and Walker (21)</td>
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<td>Cotton</td>
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<tr>
<td>Potato</td>
<td>32°</td>
<td>Robinson <em>et al.</em> (76)</td>
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of *V. albo-atrum* did not make any growth at 32°C after two weeks, while all the isolates of *V. dahliae* grew. They did not make any observations at 30°C; however, from the description of the growth of *V. albo-atrum* at 28°C it can be assumed that no growth could have been expected at 30°C.

The optimum temperatures for growth of *V. albo-atrum* and *V. dahliae*, unlike the maximum, has been reported to be essentially the same or with very little difference, ranging between 20 to 25°C (1; 4; 10; 13; 19; 21; 23; 39; 73; 76; 109).

**Temperature and the Disease Development**

Like all biochemical processes, the development and severity of plant diseases caused by pathogenic microorganisms is influenced by temperature. Relatively few workers have studied the temperature requirements of vascular wilt diseases incited by *V. albo-atrum* and *V. dahliae*.

In 1920 Edson and Shapovalov (23), working on the *Verticillium* wilt of potato, suggested that there were two strains of the fungus; one strain caused potato wilt in the northern regions of the United States and the other in the southern area. The Southern strain, which formed microsclerotia in culture (*V. dahliae*), showed a better adaptation to higher temperatures and grew fairly well in culture at temperatures of 30°C or above, while the Northern strain in which
microsclerotia were absent, adapted itself more readily to lower temperatures and did not grow at 30°C.

Bewley (4, p. 116-134) found that the best air temperature for development of wilt in tomato plants caused by V. albo-atrum, was 16-20°C. An air temperature of 12.5°C was unfavorable for symptom development and symptoms were inhibited at 25°C.

Ludbrook (59, p. 129-146) showed that V. dahliae produced marked disease symptoms in eggplants at soil temperatures from 12 to 30°C, but not at 32°C. With V. albo-atrum no disease symptoms were observed above 28°C. The air temperature was between 19 and 23°C in each case. Symptoms of the disease caused by V. dahliae were very mild at air temperatures of 28 to 31°C and soil temperatures of 22 to 28°C; no disease developed above 28°C soil temperature.

With tomato plants, Ludbrook (59) found that severe disease was caused by V. albo-atrum at soil temperatures ranging from 16 to 24°C. Significant reduction in severity of disease was recorded at soil temperatures of 26 and 28°C, and no disease developed at 30°C. The air temperature was maintained at 20°C in these experiments.

With V. dahliae very slight or no symptoms developed in tomatoes at soil temperatures from 16 to 30°C, although the fungus could be re-isolated from the plants at 26°C and 28°C soil temperatures.

Williams (110, p. 281) presented experimental evidence indicating that the infection of a variety of tomato by V. albo-atrum was
checked by high temperature and humidity much more decisively than that of *V. dahliae*. Low temperatures favored infection by *V. albo-atrum* in cucumbers as well as in tomatoes.

Cartledge (11, p. 211-212) found that *V. dahliae*, the causal agent in Verticillium wilt of hop in Tasmania, caused wilting in inoculated tomato plants at temperatures of 19, 21, 25, 27 and 29°C.

Isaac (39, p. 148-149) in his comparative studies of pathogenic species of *Verticillium* reported that both *V. albo-atrum* and *V. dahliae* caused severe wilt of tomatoes at 21.5°C. At 25 and 27°C no symptoms of the disease were exhibited by the plants inoculated with *V. albo-atrum*, while *V. dahliae* induced severe wilting at these temperatures.

Edgington and Walker (21, p. 595-596) reported that *V. albo-atrum* caused severe disease symptoms on tomato plants at soil temperatures of 20 or 24°C combined with air temperatures of 16, 20, or 24°C. With soil temperature of 28°C the disease was slight at those air temperatures. When the air temperature was raised to 28°C, the severity of symptoms was reduced at soil temperatures of 20 and 24°C. With both the soil and air temperatures at 28°C no symptoms were observed. *V. dahliae*, on the other hand, induced marked disease symptoms on tomato at all combinations of soil and air temperatures tested.

In evaluating the influence of soil and air temperatures on
disease development, Edgington and Walker (21, p. 596-598) suggested that soil temperature was more influential than air temperature.

Robinson et al. (76, p. 21-22) found that in all cases severe wilt of potatoes infected by *V. dahliae* occurred at temperatures of 24 and 28°C; and by *V. albo-atrum* at 16 and 20°C.

Smith (89, p. 469-473) found that *V. albo-atrum* caused more severe disease symptoms in many host plants (including tomato, potato, cucumber, and strawberry) at 20°C than at 25°C. It also was more pathogenic than *V. dahliae* at 20°C.

Griffiths and Isaac (29, p. 264-265) reported that at 30°C no wilt symptoms were observed on tomatoes inoculated with *V. albo-atrum* and that they were unable to reisolate the fungus from plants growing at this temperature. With *V. dahliae*, however, wilt symptoms occurred at this temperature and the fungus was reisolated in pure culture from the plants.

From the review presented above it appears that the workers who distinguish between the two species all report that *V. dahliae* tolerates higher temperatures than *V. albo-atrum*. The ability to grow and attack susceptible hosts at slightly higher temperatures could well account for the prevalence of *V. dahliae* in most parts of the United States and Prairie Provinces of Canada (19, p. 809). The lower temperature tolerance of *V. albo-atrum* may restrict its range to northern North America. Temperature apparently determines its
seasonal occurrence in Connecticut (22). *V. albo-atrum* occurs regularly in the cool Atlantic Provinces of Canada (76).

**The Disease Syndrome**

Verticillium wilt of hops was first reported in 1927 by Harris (34, p. 92-93) in England. From that date up until late '30's new outbreaks were reported in several hop growing locations (51, p. 346). The causal fungus could be isolated easily from infected plants and it was identified as *V. albo-atrum* in careful examinations of the cultures. Young hop plants inoculated with the pathogen developed all the characteristic symptoms of wilt (33). Later, Keyworth (51, p. 347) isolated *V. dahliae* from wilted hop plants and thus both species of the fungus were shown to be the causal agents of the disease.

Verticillium wilt of hops has been reported to occur in New Zealand (14; 15), Tasmania (11; 101), Germany (112), and the United States (38).

**Infection and Symptom Development**

In Verticillium wilt diseases, the pathogen present in the soil infects susceptible plants by penetration of the roots, ramification within the cortex, and subsequent systemic invasion of the xylem (45; 67, p. 65-69; 92, p. 361; 96; 106, p. 363). Direct penetration of root tips occurs (45; 67, p. 66), but the wounds, especially those made by
the emergence of adventitious roots, insects, and tillage operation (67, p. 66) can also be used as the route of entry. However, wounds are not necessary for penetration (45).

After the fungus enters the root vascular system, it causes a systemic infections that progresses upward to the stem apex (67, p. 69; 92, p. 361).

Two minimal requirements have been pointed out by Talboys (92, p. 361) for the development of any vascular wilt diseases. These are: "(1) the pathogen shall enter the vascular system of the host; (2) it shall continue to colonize the vascular system to some minimum extent and with some minimum intensity". It follows that any factor preventing either of these requirements is likely to contribute to the host's resistance.

The overall symptoms of Verticillium wilt of hops, as described by Harris (33, p. 158) and Keyworth (51, p. 346-347), are as follows: affected bines become abnormally thickened and swollen in the basal region. The leaves on infected plants usually develop yellow patches which progressively enlarge until the whole leaf becomes yellow. Necrotic black areas develop on the affected leaves and their edges become desiccated and slightly curved upward. The yellowing and withering of the leaves usually starts in the lower leaves, extending upwards and culminating in wilting and browning of the cones.

The wood cylinder of the affected bines is invariably found to
have a light brown color. This discoloration typically exists in the whole of the wood cylinder but it may be limited to only a sector of the cylinder (51, p. 347). According to Keyworth (51), the browning of the wood is the only constant symptom of the disease.

Until 1938, the Verticillium wilt of hops was considered relatively mild in England, and the outbreaks persisted from year to year without progressively eliminating the affected gardens. In 1942, Keyworth (51) reported a more severe form of the disease which progressed each year in severity and spread. On the basis of these observations he grouped the disease outbreaks into "fluctuating" and "progressive" types. The main characteristics distinguishing the two outbreaks, as described by Keyworth (51, p. 351-352) and Isaac and Keyworth (47, p. 343-344), were as follows:

In the fluctuating type, usually single stems on widely scattered plants showed a somewhat diffuse leaf yellowing late in the season, sometimes followed by wilting. Vascular discoloration was usually limited to the center of the wood cylinder and the thickening or swelling of the bines was often present in all affected plants. Most important feature of this type outbreak was that the diseased plants often recovered in future growing seasons. The spread of the disease was very limited and the affected fields remained in satisfactory production for several years.

In the progressive type outbreak, on the other hand, often all
the stems on affected plants showed severe symptoms early in the
season, culminating in the death of the bines without prior thickening.
Brown discoloration usually was exhibited by the entire wood cylinder,
starting immediately below the cortex. The disease spread rapidly
to the adjacent plants from scattered foci and the infected plants usu-
ally occurred in well-defined groups.

Isaac and Keyworth (47, p. 243-249) studied the comparative
pathogenicity of the isolates from fluctuating and progressive out-
breaks. By using soil inoculation and direct inoculation techniques,
they proved that the two types of wilt were caused by strains of \textit{V. albo-atrum} which were distinctly different in their virulence to hops.
No distinct morphological differences between the two strains were
found in culture.

\textbf{Causes of Symptom Development}

Plant pathologists are not in agreement on the cause of wilting
induced by \textit{Verticillium}. Concepts suggested by different workers to
explain wilting include: (1) toxins produced by the pathogen which
cause wilting (28; 67, p. 94-101; 94, p. 415-427); (2) production of
growth hormones by the fungus in the vascular system which play a
role in wilting by inducing tylosis in the vessels (69); (3) prevention
of water transport by hyphae of the fungus growing in the vessels (6;
99, p. 58); and (4) obstruction of vessels by plugs of pectic or other
materials (67, p. 69; 79; 99, p. 68).

Tyloses are frequently found in hop plants infected with *V. albo-atrum* and they are uncommon in non-infected and undamaged plants. In the formation of tyloses, an increase in volume of the contents of xylem parenchyma cells, accompanied by the addition of new cell-wall material to the distended pit membranes, gives rise to balloon-like structures which protrude into the lumina of the vessels (91, p. 250). Very frequently the nuclei of the parent cells pass through into the tyloses, and starch grains may also accumulate in them. A single xylem parenchyma cell may initiate several tyloses simultaneously, but usually the nucleus passes into one of them, and this one enlarges while the others remain small (91). Tyloses may become so numerous in a vessel that its lumen is completely occluded by them.

Previous observations by several workers, including Talboys (91, p. 250), had drawn attention to the occurrence of tyloses in *Verticillium*-infected plants, and had suggested the possibility that, by causing extensive vascular obstruction, tyloses might induce acute desiccation of the aerial parts of the plant and so give rise to the severe disease symptoms. However, this has not been supported by the extensive evidence obtained by Talboys (91). He examined many plants of both wilt-sensitive and wilt-tolerant hop varieties infected with various strains of *V. albo-atrum*. His observations indicated that there was an inverse relationship between the intensity of tylosis
and the intensity of mycelial growth within the vessels. Acute symptoms (progressive wilts) were usually associated with intense mycelial colonization of the vascular system and slight tyloses. Mild symptoms, on the other hand, were associated with sparse mycelium and intense tylosis (91, p. 250-252). Talboys noted that in plants with mild symptoms the distribution of tyloses may become so extensive that an increasing proportion of the xylem elements would become blocked and therefore not available for water transport. As a result of these processes a situation arises in which plants with slight vascular obstruction show severe symptoms while others in which a large proportion of the vessels are completely occluded by tyloses show only mild symptoms. The reason for this, as explained by Talboys (91, p. 253-254), lies in the fact that extensive tylosis is almost always accompanied by renewed or abnormally prolonged cambial activity leading to formation of additional secondary xylem. This relatively narrow ring of outer vascular tissues is available for water transport and is sufficient to maintain the life of the plant. The characteristic thickening of the stem in fluctuating wilt (33; 51) is the result of this hyperplasia of the xylem (91, p. 253-254).

Leaf necrosis, as evidenced from histological studies by Talboys (91, p. 257), results from the action of fungal toxins, and the processes leading to necrosis seem to be essentially similar in the mild and severe syndromes.
These observations thus suggest strongly that the capacity of the host plant to respond to the presence of the pathogen in the vascular system by formation of tyloses, together with the potential for development of additional xylem, contribute significantly to the disease tolerance of the hop plant.

Obstruction of vessels by plugs of pectic and other materials of high molecular weight has been studied by many workers. Production of pectic enzymes by Verticillium has been shown in vitro (5; 18; 50, p. 325-327; 62, p. 26-76) and in vivo (16; 17). If these enzymes are produced by the pathogen in the host xylem vessels, pectic and other substances of relatively high molecular weight might be released into the vascular sap (20, p. 346). Wood (111, p. 120-139) found that very dilute solutions of undegraded pectic substances, even at concentrations as low as 30 ppm, caused wilting of cut shoots of tomato. Blackhurst (5, p. 79-88) showed that solutions of sodium polypectate induced slightly less water loss than solutions of pectin and were more active in causing wilt of tomato shoots.

McIntyre (62, p. 26-76) suggested that V. albo-atrum produced at least two endo-polygalacturonases in culture. One endo-polygalacturonase preferentially hydrolyzed sodium polypectate to large fragments, the other preferentially hydrolyzed small fragments from the substrate. Kamal and Wood (50, p. 322-340) observed a close relationship between the protopectinase activity of culture filtrates of
Verticillium dahliae and toxicity of these filtrates to parenchyma cells. These results were confirmed by Blackhurst (5) who found that filtrates of the fungus on pectic substances which were known to have a very high polygalacturonase activity, caused rapid wilting of cut tomato shoots, maceration of stem tissue, and vascular browning. Also a solution of commercial pectic enzyme preparation, pectinol 100D known to have a high polygalacturonase and pectin methyl esterase activity, induced wilting and vascular discoloration in both resistant and susceptible varieties of tomato.

Wood (111) found that V. dahliae grew poorly in the xylem sap, presumably because of the shortage of carbon, since when glucose was added growth increased greatly. The fungus was able also to use pectin and polypectate under these conditions, and growth on polypectate was almost as good as on glucose. Wood (111, p. 136) concluded "it seems that pectic enzymes would be produced in xylem vessels provided that relatively small quantities of appropriate substrates were available, from what is known about the structure and composition of xylem elements this is very likely to be the case." McIntyre (62) in 1964 found that the crude enzyme preparation of V. albo-atrum from infected tomato plants yielded pectic fragments as well as monogalacturonic acid. This indicated the presence of an endopolygalacturonase that hydrolyzed small fragments from the substrate.

From these studies it appears that Verticillium produces
polygalacturonase abundantly when it is supplied with pectic sub-
stances. Blackhurst (5) feels this enzyme is the most active compon-
ent of culture filtrates in inducing the disease symptoms. Deese and
Stahmann (17, p. 53-70) studied the pectic enzyme formation of V.
albo-atrum grown on surface-sterilized living stem tissues of resis-
tant and susceptible tomato plants. They found that the fungus formed
a large amount of polygalacturonase on susceptible tissue but little or
none was formed on resistant tissues. They indicated that culture
filtrates from resistant tissues contained a high oxidizing power
which was absent from tissues of the susceptible variety. In resis-
tant varieties the inhibition or suppression of polygalacturonase for-
formation appeared to be associated with this high oxidizing power.
Deese and Stahmann (17, p. 66) from their study proposed the follow-
ing hypothesis in relation to the cause of wilting in infected plants:

The fungus confined to the vascular system of suscep-
tible tomato plants secretes pectic enzymes and pos-
sibly other hydrolytic enzymes which attack the cell
wall to release simple carbohydrates in the transpira-
tion stream which may not cause wilting. These sub-
stances may be used by the fungus to grow and produce
more invading hyphae which block the xylem and petio-
lar vessels. The small amount of PME produced by
the young growing pathogen and that already present in
the susceptible plant demethylates pectin to yield low-
methoxy pectin and pectic acid; this process enhances
the initial polygalacturonase activity. If this process
continues, the combined action of hydrolytic enzymes,
like polygalacturonase, and the concomitant synthesis
of polysaccharides by the invading fungus would re-
duce transport of nutrients in vascular system. The
decrease in nutrient and water transport may produce
dwarfing and wilting of the leaves which become yellow and epinastic.

It is likely that dysfunction of the stem involves the degradation of pectic substances of the middle lamella and cell wall. Since wilting can be induced experimentally by the uptake of compounds of high molecular weight which prevent the upward water flow (5; 50), therefore Blackhurst (5) believes that it is reasonable to assume that the action of pectic enzymes able to hydrolyze compounds of high molecular weight lead to the formation of insoluble pectinates or pectates which, if persisting, will impede the flow of the vascular sap and consequently cause water shortage and wilting.

Influence of the Environment and Plant Nutrition on Disease Severity

The effect of temperature on the development and severity of Verticillium wilt diseases in general, has been reviewed in previous pages. In 1960 Zattler and Chrometzka (112) reported that the severity of wilt of hops in Germany caused by *V. albo-atrum* was strongly influenced by soil temperature. They found that cool soils (20°C) were more favorable for wilt development than warm soils (28°C).

Harris (33, p. 159) in his investigations of the fluctuating Verticillium wilt of hops, concluded that the severity of the disease was primarily related to the fluctuations of environmental conditions, especially soil moisture, from year to year. He stated that the disease
symptoms were most evident during a wet summer. Keyworth (51, p. 352; 55, p. 148), although with reservations, also admitted that variations in severity of fluctuating outbreak were related in some way to local soil conditions such as impeded drainage. However, he could not find any such correlation between soil moisture and the disease incidence or severity in his study of progressive outbreaks (51, p. 352-353).

The effects of the nutrition of plants on their reaction to disease have been studied by many workers. Garret (26) has reviewed the information related to the root infecting fungi causing vascular diseases.

Soil fertility and its relation to Verticillium wilt of many crop plants has been the subject of intensive studies and many of the reports in the literature are conflicting. The normal procedure in these investigations has been the measurement of the effects of fertilizer amendments on the disease severity.

Roberts (74; 75) reported that severity of wilt of tomatoes caused by *V. albo-atrum*, was increased by the application of nitrogenous fertilizers. Reduction in nitrogen supply both reduced the number of infected plants and retarded the development of the pathogen in such plants.

Keyworth and Hewitt (54) performed experiments extending over four years on the effect of *V. albo-atrum* on hops growing in sand
cultures supplied with various nutrient solutions. The plants were grown under conditions involving both deficiencies and excesses of most of the elements necessary for plant growth. They found that a reduction in the nitrogen supply of the plants caused the most marked and consistent reduction in disease incidence and severity. Many of the nitrogen deficient plants from which Verticillium was isolated did not show any external symptoms and the fungus failed to grow farther than the crowns of the plants or lower parts of the stem.

Presley (71) found that Verticillium wilt of cotton increased proportionally by increasing the nitrogen fertilizers. However, when greater amounts of potassium were added to the soil, wilt decreased.

Isaac (40) found that the nutritional status of the inoculum was an important factor in disease development by species of Verticillium. He showed that V. dahliae grown on nitrogen-free agar caused little or no wilt in Antirrhinum majus L., while inoculum grown on a 0.2% NaNO₃ medium caused nearly 100% disease. External organic nitrogen in soil had little effect.

**Dispersal of the Pathogen**

In Verticillium wilt diseases when infected plants die, the fungus readily permeates the surrounding tissues (46, p. 552-558; 51, p. 348; 67, p. 69; 82). After death, resting bodies typical of the species involved form throughout the infected plant tissues (46; 104; 107).
In studying wilt of lucerne caused by different species of *Verticillium*, Isaac (46, p. 552-558) observed that in the initial stages of the disease the fungus is confined to the vessels and tracheids of the xylem. When the shoots were nearly dead, the pathogen grew out transversely from the vessels and tracheids, passing through the pits toward the cortex. After invasion of the cortex, superficial conidiophores developed. When the stem died, hyphae in the wood rays and cortex became black and carbonized and formed either the typical black resting mycelium of *V. albo-atrum* or the black microsclerotia of *V. dahliae*.

*V. albo-atrum* and *V. dahliae* can not survive for long periods of time as mycelium and conidia (45; 80; 85), but microsclerotia and dark mycelia are capable of passive survival for many years (45; 85; 104; 107). Wilhelm (104) found that microsclerotia of *Verticillium* (*V. dahliae*) could survive for 13 years in culture and 12-14 years in soil in the absence of known host plants. *V. albo-atrum* and *V. dahliae* provide perfect examples of the group of soil-borne pathogens known as "soil invaders", for free hyphal growth through the soil does not occur (43; 103; 108).

Isaac (43, p. 630-638) demonstrated that spread of *V. albo-atrum* and *V. dahliae* throughout soil and subsequent infection of the new host plants was related to the ability of these pathogens to kill and cause the decay of roots of the infected hosts with consequent
liberation of the fungus into the soil. He concluded that since these pathogens appear to be incapable of spreading as soil saprophytes, the rapid spread of the disease from the infected plant must be due, not to the growth of fungal mycelium in the soil toward new host, but to the growth of the roots of these potential hosts into the infected soil around the initially diseased plants.

Martinson (60, p. 92-98) showed that V. dahliae is capable of saprophytically colonizing a low percentage of pieces of crop residues introduced into the soil. Isaac (43, p. 635) demonstrated that a progressive reduction in the amount of viable material of the two species occurs when they remain in soil for several months.

V. albo-atrum and V. dahliae are normally transmitted to new areas on propagative material or within infected plant debris (51, p. 350; 63, p. 464; 67, p. 162; 82) and the importance of this, especially in Verticillium wilt of hops, has been clearly proven by several workers (45; 47; 51; 85). Sewell and Wilson (85, p. 193) pointed out that the above-ground dispersal of infected parts of hop plant may occur in two phases: (1) during the growing season when plant infections result in wilting and the rapid shedding of all infected leaves, and (2) after the senescence and death of the annual bines in the autumn. They stated that of these two phases the latter is probably the more important for it has frequently been found that a far greater number of plants are infected but symptomless at the end of growing
season than are wilted during the season. With the onset of natural senescence all above-ground parts of symptomless infected plants may rapidly be colonized and the resulting large quantities of infested tissues dispersed.

*V. albo-atrum* and *V. dahliae* can maintain a high inoculum potential by infecting many different plants. In 1957, Engelhard (24) published a survey of the literature on the host index of these two species. His work showed that *V. albo-atrum* has been isolated (not necessarily pathogenic) from approximately 220 and *V. dahliae* from 60 plant species including trees, shrubs, field crops, and vegetable crops. Russian workers Soloveva and Polyarka (90) found that *V. dahliae* attacked 27 different plants, but cereals were immune.

Martinson and Horner (61) have shown that many plants, including members of the family Gramineae were susceptible to infection by *V. dahliae* without showing any disease symptoms. They indicated that the fungus could form microsclerotia, pathogenic to peppermint, in the roots of these plants.

Sewell and Wilson (87) assayed stems of common weeds grown in a diseased hop garden. They found that *V. albo-atrum* was present in all of four weed species sampled and that the fungus was highly pathogenic to hops.

Smith (89, p. 469-474) found that *V. dahliae* isolated from nine cultivated plant species was pathogenic on eggplants. On the same
series of experiments he showed that *V. albo-atrum* and *V. dahliae* both isolated from potatoes, induced wilt symptoms in tomato, tobacco, eggplant, cucumber, strawberry, and *Antirrhinum*.

Sewell (82) was able to show that *V. albo-atrum* isolated from wilted hops caused infection and root degeneration of tomatoes.

Cartledge (11, p. 211-212), by using several inoculation techniques, found that *V. dahliae*, the cause of wilt of hops in Tasmania, was strongly pathogenic towards tomatoes and potatoes. He suggested that tomato could be used as an indicator plant to check on the presence of hop strains of *V. dahliae* in suspected soils. Later Wade (101, p. 266-267) stated that Verticillium wilt of hops in Tasmania is always associated with previous tomato or potato cultures.

Christie (14, p. 15-16; 15, p. 17-19) found that *V. albo-atrum*, the causal agent of hop wilt in New Zealand, caused severe wilt in potatoes and tomatoes upon inoculation. He suggested that the disease was brought in with infected potato plants.

Information about the sporulation of *Verticillium* is very limited. McKay (63), in 1926, reported sporulation of *V. albo-atrum* (*V. dahliae*) in soil. He buried infected potato stems in soil under field conditions and observed the fungus sporulating freely on the infected plant materials. According to McKay the sporulation continued for at least six months.

Keyworth (51, p. 346-357) working on wilt of hops caused by
V. albo-atrum, observed verticilliate conidiophores and conidia on dead diseased bines and leaves lying on the ground after harvest.

Isaac (46, p. 550-558) and Sewell (82, p. 312-321) also studied the sporulation of V. albo-atrum. Their studies revealed that any fragmentary plant material containing the fungus, when kept under moist conditions, becomes covered by conidiophores, bearing enormous numbers of spores. Sewell (82) found that the sporulation of the fungus on roots of infected tomato plants occurred shortly after the onset of root degeneration in soil. The duration and intensity of sporulation was related to the quantity and rapidity of degeneration of the tissues.

Information on germinability of Verticillium microsclerotia and dark mycelia is meager and conflicting. Thomas (98) was unable to germinate microsclerotia without prior cold treatment; and even then germination percentages were low.

Jackson (48, p. 96-97) and Rovira (77, p. 53-63) demonstrated that seedling roots and root exudates stimulated germination of fungal spores in soil. Schreiber and Green (81, p. 260-264) found that the fungistatic principle of all natural soils prevented germination of both conidia and microsclerotia of Verticillium and this fungistatic effect was overcome to varying degrees by plant root exudates. Root exudate from tomato (a host) overcame fungistasis to a greater degree than that of the non-host (wheat). Fractionation of tomato root
exudate suggested that amino acids or other nitrogen-containing compounds were responsible for overcoming fungistasis.

Lacy (57) showed that the rhizosphere of soils from all plants tested had significantly higher populations of *V. dahliae* than did non-rhizosphere soils. The host plants supported large populations of *V. dahliae* in the rhizosphere, but plants such as wheat, corn, and beans (non-hosts) tended to support fewer numbers of *Verticillium* propagules in the rhizosphere. Lacy felt that the larger fungal population in the rhizosphere was related to the stimulation of reproduction of propagules by roots.

**Control Measures**

Because of the longevity and resistance of the resting structures of the pathogens and also ability to survive by infecting roots of non-host plants, the control of *Verticillium* wilt is a very difficult problem.

Crop rotation in general has given little control of *Verticillium* wilt with most crops. With hops, short term rotation is economically impossible since the plant is a perennial species, and once a garden is established it should be in good production for several years. The only practicable form of rotation would be the growing of an immune crop for some years on wilt-affected ground. In advising crop rotation as the control measure for *Verticillium* wilt, it is essential
to know the identity of the pathogen involved, since V. albo-a atrum and V. dahliae are not affected equally. Sewell and Wilson (86) found that the infectivity of soils infested with V. albo-a atrum (progressive wilt strain), declined rapidly in the absence of dicotyledonous plants. Under a grass cover, the fungus was apparently eradicated after 3-5 years. V. dahliae, on the other hand, was not suppressed with grass cover treatment. The differential response of the two species of the fungus to crop rotation has been reported also in Verticillium wilt of potatoes. Robinson et al. (76) reported control of the disease caused by V. albo-a atrum, by 3-4 years intervening cropping with grain and clover. On the other hand, Guthrie (30) and Nielson (68) obtained no control of potato wilt caused by V. dahliae after 4-7 years intervening cropping with grains and alfalfa.

The possible methods of control of hop wilt have been studied by English workers for many years. These studies have indicated that in general the measures taken to control wilt in any particular garden are influenced by the following factors: 1) the hop variety, 2) the virulence of the pathogen, 3) the geographical location of the affected garden, and 4) the age of disease outbreak.

It appears that the overall control measures recommended fall under two headings: cultural practices and use of tolerant varieties.
Cultural Practices

The highly infective nature of hop waste after harvest and the significance of the infected plant debris in dispersal of the pathogen have been demonstrated experimentally by Keyworth (51), Sewell and Wilson (84) and many others. Thus the importance of field sanitation in reducing or preventing the distribution of the disease has become evident. Sewell and Wilson (85, p. 193) have pointed out that at the end of the growing season a great number of plants may be infected without external symptoms. After the onset of natural senescence all above-ground parts of these plants may rapidly be colonized and result in dispersal of large quantities of infected tissues. With machine-picking these bines will be removed green and intact from hop garden before autumn senescence, and the distribution of Verticillium in the accumulated picking waste by burning, or by efficient composting, can thus check completely the major avenue of spread of the pathogen. Sewell et al. (88, p. 102-106) found that after 21 weeks of composting V. albo-atrum was destroyed in 99% of the infected hop residues. They suggested that in order to achieve the sustained high temperatures necessary to kill all Verticillium propagules, the compost mass should be of minimum surface area, insulated against heat loss, and adequately drained.

It has been proposed (85, p. 193-194) that a grass cover, free
from all broad-leaved plants, can be used to limit the spread of *Verticillium* from localized primary disease outbreaks and to reduce soil infectivity before replanting with tolerant varieties.

Reduction in the amount of nitrogen fertilizers has been reported to cause a considerable reduction of fluctuating wilt and an increase in yield (85, p. 197-198). The best result was obtained by reducing ammonium sulphate applications from nine to three cwt per acre. However, it is clear that the minimal requirements will vary for different farms with the level of organic matter and with local soil characteristics and climatic conditions.

Improvement of drainage in wet soils has also been recommended as a control means in fluctuating type wilt (51, p. 356; 85, p. 197).

Keyworth (51, p. 355), Cartledge (11, p. 212), and Wade (101, p. 267) have suggested that hop gardens should not be established on lands previously planted to potatoes and tomatoes, since the fungus is also the causal agent in *Verticillium* wilt of these crops.

**Tolerant Varieties**

Strict field sanitation and also fumigation with any available material, have proven impracticable on a commercial scale, and in spite of such precautions taken, the pathogen has not always been eradicated (52, p. 99; 101, p. 267). A search for varieties resistant to progressive wilt was started by Keyworth in 1939 (95, p. 177).
Extensive research on breeding and selecting resistant hops with acceptable brewing qualities, has been carried out in East Malling Research Station and Wye College in England, and some varieties thus developed are in commercial use at the present time.

Harris (35, p. 208) has given the description "wilt tolerant" to these varieties. He points out that to be truly resistant to progressive wilt, it would imply that the plant would be immune to the disease by keeping the fungus outside in the soil, and therefore all the propagative material obtained from it would also be free from contamination. But in practice it has been shown that most of these developed varieties tend to become infected as do the sensitive varieties, but differ from the latter in that such infection causes little or no wilting of the affected hill (35, p. 208). The tolerant varieties planted on land infested with the virulent hop strain of the fungus may develop mild symptoms of a fluctuating type in the first year, but the number of affected plants generally declines to negligible proportions after 3-5 years (32, p. 201; 85, p. 194).

The mechanisms of disease tolerance in the newly developed hop varieties has been studied by Talboys (95; 96). He has shown (96, p. 229-232) that initial invasion of the host may be retarded by lignification of cell walls in the epidermal and cortical cells of roots, the extent of which appears to be determined by the virulence of the pathogen.
Prevention of the invasion of pathogen into the vascular tissues seems to depend upon deposition of a layer of suberin in the endodermal cell walls. The extent and rapidity of formation of this layer appears to be the determinant factor in the disease tolerance of a hop variety. In most of the tolerant varieties this process of suberization takes place more rapidly than in similar roots of more sensitive varieties. Thus the central cylinder near the root tip is ensheathed and prevents extensive infection by the pathogen (95, p. 179).

In addition to the varietal characteristics, the rapidity of suberization also depends on the conditions under which the plant is grown. Talboys (95, p. 179) has found that large amounts of nitrogen fertilizers can cause a delay of suberization in an otherwise tolerant variety.
MATERIALS AND METHODS

Verticillium Isolates

Throughout the experiments the following seven isolates of Verticillium were used:

1. No. 95, identified as *V. dahliae*, originally isolated from infected peppermint (*Mentha piperita* L. 'Mitcham').

2. No. 119, identified as *V. dahliae*, originally isolated from wilted potato (*Solanum tuberosum* L.)

3. No. 138, identified as *V. albo-atrum*, isolated from hops (*Humulus lupulus* L. 'Fuggle').

4. No. 146, identified as *V. dahliae*, isolated from 'Fuggle' hops.

5. No. 148, identified as *V. dahliae*, isolated from 'Bullion' hops.

6. No. 149, identified as *V. albo-atrum*, isolated from 'Fuggle' hops.

7. No. 150, identified as *V. dahliae*, isolated from 'Fuggle' hops.

The hop strains of the pathogen were isolated from diseased bines grown in different yards throughout the Willamette Valley of Oregon.

Stock cultures of all the isolates were of single spore origin and
were maintained on potato dextrose agar (PDA) in screw cap tubes. To minimize the morphological variations within each isolate, successive transfers of the cultures were carried out every three or four weeks, and only the cultures producing uniform dark resting structures were used in experiments.

Throughout the thesis, I will use DM and MS to distinguish dark mycelial *V. albo-atrum* and microsclerotial *V. dahliae*, respectively.

**Temperature Relations**

**Media Preparation**

The culture medium used in temperature studies was prepared by dissolving 35 grams of Czapek Dox broth and 5 grams of Difco yeast extract in one liter of distilled water. As indicated by the Difco Laboratories, 35 grams Czapek Dox broth in one liter of water provides 30 g/l sucrose, 3 g/l sodium nitrate, 1 g/l dipotassium phosphate, 0.5 g/l magnesium sulfate, 0.5 g/l potassium chloride, and 0.01 g/l ferrous sulfate. The yeast extract was used as the source of B vitamins.

The prepared medium was distributed in clean 125 ml Erlenmeyer flasks in 30 ml quantities. The flasks were then cotton plugged and autoclaved for 18 minutes at 15 psi. The final pH of the prepared media was 7.01.
Inoculation and Incubation

A conidial suspension of the isolates was used to inoculate the flasks. This was done in the following manner: a small portion of the mycelial mass of each isolate was transferred into small prescription bottles containing 15 ml of PDA. The cultures were incubated at room temperature in the dark. After five days of growth, the conidia produced on the surface of the colony were harvested by adding 20 ml of distilled sterile water to each bottle and shaking gently. Spore concentration was obtained by hemocytometer readings and adjusted to 100,000 spores per ml with distilled sterile water. Inoculations were made by transferring 1 ml of spore suspension into each flask containing autoclaved medium. The flasks were immediately cotton plugged and incubated at desired temperatures.

Strict aseptic techniques were used in all steps of the experiments to prevent contamination. The incubators used in these studies were B-Line Refrigerated Incubators, Model 12, manufactured by Forma Scientific Inc. Growth of the isolates was studied at ten temperatures: 5, 10, 15, 17, 20, 23, 25, 28, 30, and 32°C. Temperatures were checked frequently with a calibrated mercury thermometer and variation never exceeded ±0.5°C.
Data Collection

Dry weight of fungal material produced in the cultures was used as the criterion of growth at given temperatures. Dry weight measurements were made after 7, 12, and 17 days of growth, by the following method:

Five flasks of each isolate were removed from the incubator and were examined microscopically for purity of the cultures. Mycelium and spores were collected by filtering the culture through pre-weighed, dry, 7 cm glass filter pads (Whatman GF/A) placed in a Buchner Funnel mounted on a filtering flask. A gentle suction was provided to aid filtration.

In the process of filtration, special care was taken to remove all fungal materials adhering to the sides of the flasks, using a rubber policeman and distilled water. The filtration was completed by a final rinsing of the collected mycelial mass with 30 ml of distilled water.

Drying was done in a thermostatically controlled laboratory oven set at 96°C. After 24 hours, the dried samples were placed in a desiccator for 4 hours. The dry weight was determined to the nearest mg by weighing on a Mettler balance.
Data Analysis

The data were statistically analyzed as a three-factor factorial experiment (6 isolates x 10 temperatures x 3 incubation periods), using the analysis of variance method.

To establish relationship between the growth (dry weight) and the period of incubation at given temperatures, stepwise multiple regression analysis, with the aid of a high speed computer was used.

Host Range Studies

Test Plants

In these experiments the proliferation of hop isolates of *Verticillium* in six plant species was tested. These were:

1. Potato (*Solanum tuberosum*), Variety 'Red Warba'
2. Tomato (*Lycopersicon esculentum*), Variety 'Bonnys Best'
3. Eggplant (*Solanum melongena*), Variety 'Black Beauty'
4. Peppermint (*Mentha piperita*), Variety 'Mitcham'
5. Strawberry (*Fragaria chiloensis*), Variety Northwest'
6. Geranium (*Pelargonium hortorum*), Variety 'Cardinal'

Tomatoes and eggplants were obtained by sowing the seeds in a greenhouse bed. In order to obtain vigorous eggplants, an air temperature of 27-29°C was required at the seedling stage. This was provided by placing the seedlings in a thermostatically controlled
heat chamber. Peppermint plants were uniform cuttings made from the vigorous shoots produced on rhizomes planted in the greenhouse. Potatoes were grown from seed tubers by planting a portion of the tuber containing a healthy "eye". Uniform geranium plants were obtained from a nursery near Corvallis, and the strawberry runner plants were provided by the Department of Horticulture.

Inoculation

One DM isolate (No. 138) and two MS isolates (No. 148 and No. 150) were used. The necessary inoculum of each isolate was prepared by culturing the fungus on wheat straw by the following technique:

Forty grams of wheat straw amended with 80 ml of a one percent Czapek Dox solution were placed in one-liter Erlemeyer flasks. The flasks were then cotton plugged and autoclaved for 30 minutes at 15 psi. Shake cultures, five to seven days old prepared in a medium containing 35 g/l Czapek Dox and 5 g/l yeast extract, were used to inoculate the straw. The inoculation was done by adding 4 ml of the shake culture of the given isolate into each flask followed by vigorous shaking and agitation of the straw to provide a uniform distribution of the inoculum. The prepared flasks were incubated at room temperature in the dark. Extensive colonization of the straw and production of the resting structures, characteristic of each isolate, normally
occurred during the incubation periods ranging from 4-8 weeks. After this period the contents of the flasks were removed, air dried at room temperature and then stored at 4°C until used.

Soil infestation was done by uniform distribution of 4 grams of straw inoculum on the soil surface in partially filled pots. Root systems of the test plants were placed directly on the layer of inoculum and then the pots were filled with clean soil. Five plants of each species were inoculated with each isolate. Five uninoculated controls of each plant species were also included in the experiments. The plants were kept in the greenhouse under favorable conditions for growth.

Observations and Assays

Plants were observed frequently for the development of disease symptoms. Records were maintained on the dates of appearance of the initial symptoms and also symptom characteristics in individual plants. About 60 days after inoculation, the plants were rated visually for the disease severity on the following arbitrary scale: 1 = no symptoms, 2 = mild symptoms, 3 = moderate symptoms, 4 = severe symptoms, 5 = dead plant. The plants were then harvested by cutting the stems at the soil line.

Individual plants in each species, including the controls, were assayed in the laboratory to determine infection and the quantity of
Verticillium propagules in the stem tissues by the following procedure:

One gram of the basal portion of the stem was surface sterilized for 3 minutes in a 1.05% sodium hypochlorite solution. In most cases the "bark" of the stem sample was peeled off in order to remove saprophytes and to aid the wetting of the surface by the sterilant. Following surface sterilization, the sample was chopped into several segments with a sterile razor blade and was further fragmented in 100 ml of distilled water with a high speed Omni-Mixer.

Two dilutions of the stem fragments, 1:100 and 1:1000, were plated by transferring 1.0 ml or 0.1 ml of the prepared suspension into sterile petri plates. The plates were then covered with 10 ml of melted, cooled (42°C) culture media. PDA amended with 100 ppm streptomycin sulfate was used for the assay of dark mycelial isolate. The microsclerotial isolates were assayed on ethanol-streptomycin agar containing one percent sodium polypectate, described by the author in 1965 (113). The latter medium has been shown to enhance microsclerotial production by V. dahliae.

The plates were incubated at room temperature in the dark for two weeks. At the end of this period the average number of colonies in three plates were determined for each plant sample. The number of Verticillium propagules per gram of stem tissue was calculated by multiplying the obtained average by the proper dilution factor.
Pathogenicity Tests on Hops

Test Varieties

In these experiments, the reaction of 14 hop varieties in response to inoculation with different isolates of Verticillium was studied in field plots for two successive years. The test plants, which included commercial varieties as well as newly developed hop strains are described below:

1. Fuggle: first introduced into the United States from England during the 19th Century. It is an early maturing variety with a considerable resistance to downy mildew caused by Pseudoperonospora humuli. This variety is mainly grown in Oregon and to some extent in Washington. In 1959 it was estimated to occupy about ten percent of the total U.S. hop acreage (8). In England Fuggle is highly susceptible to Verticillium wilt.

2. Fuggle-H: a clonal selection of Fuggle, first selected in 1948 as a single hill in a commercial Fuggle yard near Corvallis. In most respects it resembles the standard Fuggle but it has an advantage of a 15 percent increased yield.

3. Brewers Gold; 4. Bullion: these two varieties were developed at Wye College in England and were introduced into
the United States during the 1930's. They are both vigorous and high yielding varieties but moderately susceptible to downy mildew. In 1959 they occupied about two percent of the total hop acreage in the U.S. (8). Both varieties are susceptible to Verticillium wilt in England.

5. Late Cluster; 6. Early Cluster: these are the most widely grown hop varieties in the United States. In 1959 they occupied approximately 88 percent of the total hop acreage. Early Cluster originated in Oregon in 1908 as an early-maturing bud sport in a yard of Late Cluster. Both are vigorous and high yielding varieties and are used interchangeably by the brewing industry. They are, however, both susceptible to downy mildew.

7. Janus; 8. Density: these two varieties were developed in 1962 in Wye College in England. They are both reported to be resistant to progressive wilt caused by a virulent strain of *V. albo-atrum*. They are not planted in the U.S. except for experimental purposes on a limited scale.

8. OB-826; this strain was developed at Oregon State University through breeding and is still in experimental stages. It is a sterile triploid and thus always seedless. It is comparable to Bullion in quality and resistance to downy mildew and it has some inheritance from Fuggle and Early
Cluster.

10. OB-831: developed at Oregon State University through breeding, as a replacement for Fuggle. It is made up of about 32% Fuggle germ plasm. It has good resistance to downy mildew and yields much higher than Fuggle.

11. OB-835: this is a Cluster type hop bred at Oregon State University and contains some Bullion and Fuggle germ plasms. It is resistant to downy mildew and yields about twice as much as Fuggle.

12. Yakima Cluster (L-1): this variety was selected from Late Cluster in Washington. In most respects it resembles Late Cluster except it is somewhat earlier maturing and free from serious virus diseases.

13. E-2: a clonal selection from Early Cluster which was made in Washington. It is very similar to standard Early Cluster in most respects.

14. 50-S: this strain which is still in experimental stages, has been developed from open pollinated seed of English "Sunshine" variety.

In the first year (1966) of the experiments, the necessary numbers of plants of each variety were obtained from soft wood cuttings taken usually from a single hill growing in experimental plots near Corvallis. Cuttings were placed in a 20 ppm solution of indolebutyric
acid (IBA) for 24 hours, then planted in sand in a propagation bed equipped with artificial light and automatic mist apparatus. After sufficient root development, the cuttings were transferred to small peat pots containing clean greenhouse soil and kept in the greenhouse until they were planted in the field.

Planting material for the 1967 tests consisted of crowns and rhizomes produced by symptomless plants in 1966. These were dug in November, washed and stored at 4°C then replanted in the spring.

Verticillium Isolates

Three hop isolates (Numbers 138, 146, and 148), one mint isolate (No. 95), and one potato isolate (No. 119) were used to infest field plots. In another experiment which involved injection of Verticillium conidia into the stems, three hop isolates, numbers 138, 148, and 150, were tested.

Inoculum Preparation

Inoculum for the 1966 field test was produced by growing the Verticillium isolates in a shake culture medium containing 35 g/l Czapek Dox broth and 5 g/l yeast extract. After one week of growth, the cultures were used to infest a screened and dried Chehalis sandy loam soil. The infested soil was stored at room temperature until completely dry then screened through a 14-mesh screen. This
constituted the stock inoculum of each isolate. The soils infested in this manner were assayed by serial dilution to determine the number of the viable propagules of each isolate. This level was adjusted to 2.5 million propagules per gram, by dilution of the stocks with the necessary amount of non-infested field soil.

Inoculum for the 1967 experiments was prepared by growing the isolates on wheat straw as described earlier.

**Plot Layout, Soil Infestation, and Planting**

The experiments were carried out on the Botany and Plant Pathology Farm near Corvallis, which has a silty loam type soil.

Soil was infested in the first year by placing 50 grams of the inoculum (laboratory-infested soil) in the planting holes. In the second year, a uniform layer of straw inoculum (approximately 4 grams per plant) was distributed on the bottom of an 8-inch deep trench, dug down the planting rows.

Plants were set so that the roots or crowns were directly in contact with the layer of inoculum. Five or more hills of each variety were planted in each row infested with a given isolate, using a completely randomized plot layout.

**Stem Injections**

In one experiment the plants grown on non-infested soil were
inoculated by injecting a conidial suspension of the isolates into the pith cavity of the stem. Conidia were washed from PDA-cultures and their concentration was adjusted to 5 million spores/ml with sterile water. Inoculations were made by injecting 0.2 to 0.5 ml of the spore suspensions into the pith cavity, eight inches above ground. In each variety two to four plants were inoculated with each isolate. Control plants were injected with distilled sterile water.

**Observations and Assays**

Throughout the growing seasons cultural practices necessary for favorable plant growth were maintained. Plants were observed frequently for symptoms of disease. At the end of the growing season (September), bines were harvested by cutting them 6 to 8 inches above the ground level. Individual stems were examined for the presence of vascular browning characteristic of the disease. Samples were taken from the basal portion of each stem and assayed quantitatively. The number of propagules per gram of stem tissues was determined using the procedures described earlier.
RESULTS

Temperature Relations

The following six isolates of *Verticillium* were used in a comparative study of the effect of temperature on growth:

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate No.</th>
<th>Resting Structure</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. albo-astrum</em></td>
<td>138</td>
<td>DM</td>
<td>Hops</td>
</tr>
<tr>
<td><em>V. albo-astrum</em></td>
<td>149</td>
<td>DM</td>
<td>Hops</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>95</td>
<td>MS</td>
<td>Peppermint</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>119</td>
<td>MS</td>
<td>Potato</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>148</td>
<td>MS</td>
<td>Hops</td>
</tr>
<tr>
<td><em>V. dahliae</em></td>
<td>150</td>
<td>MS</td>
<td>Hops</td>
</tr>
</tbody>
</table>

The criterion for growth was the dry weight of fungal material produced in Czapek Dox broth plus yeast extract. Harvests were made at each temperature after 7, 12 and 17 days. Average dry weights for each isolate-temperature combination are shown in Table 2.

Analysis of the variance of the data showed that:

1. Incubation period affected growth.

2. Isolates possessed inherently different growth abilities.

3. Temperature affected growth.
Table 2. Effect of temperature on growth (mg dry weight) of five replications of six *Verticillium* isolates (Average of five replications).

<table>
<thead>
<tr>
<th>Isolate No. and Type</th>
<th>Days of Incubation</th>
<th>Temperature (°C)</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>17</th>
<th>20</th>
<th>23</th>
<th>25</th>
<th>28</th>
<th>30</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>95 (MS, mint)</td>
<td>7</td>
<td>0</td>
<td>4</td>
<td>15</td>
<td>20</td>
<td>155</td>
<td>178</td>
<td>227</td>
<td>235</td>
<td>145</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>119 (MS, potato)</td>
<td>7</td>
<td>0</td>
<td>9</td>
<td>66</td>
<td>112</td>
<td>137</td>
<td>169</td>
<td>199</td>
<td>236</td>
<td>149</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>148 (MS, hops)</td>
<td>7</td>
<td>0</td>
<td>8</td>
<td>25</td>
<td>54</td>
<td>176</td>
<td>185</td>
<td>201</td>
<td>233</td>
<td>157</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>150 (MS, hops)</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>23</td>
<td>51</td>
<td>115</td>
<td>134</td>
<td>213</td>
<td>227</td>
<td>67</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>138 (DM, hops)</td>
<td>7</td>
<td>0</td>
<td>10</td>
<td>71</td>
<td>81</td>
<td>123</td>
<td>135</td>
<td>138</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>149 (DM, hops)</td>
<td>7</td>
<td>0</td>
<td>17</td>
<td>68</td>
<td>87</td>
<td>133</td>
<td>144</td>
<td>116</td>
<td>67</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

L. S. D. at the 5% level = 15 (Valid only for the dry weights of the different isolates produced in the same incubation period, at a given temperature.)
### Analysis of Variance

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of incubation</td>
<td>2412015</td>
<td>2</td>
<td>1206007</td>
<td>7969</td>
</tr>
<tr>
<td>Isolate</td>
<td>261184</td>
<td>5</td>
<td>52236</td>
<td>345</td>
</tr>
<tr>
<td>Temperature</td>
<td>9085870</td>
<td>9</td>
<td>1009541</td>
<td>6671</td>
</tr>
<tr>
<td>Period x isolate</td>
<td>209258</td>
<td>10</td>
<td>20926</td>
<td>138</td>
</tr>
<tr>
<td>Period x temperature</td>
<td>1475887</td>
<td>18</td>
<td>81994</td>
<td>542</td>
</tr>
<tr>
<td>Isolate x temperature</td>
<td>1620973</td>
<td>45</td>
<td>36022</td>
<td>238</td>
</tr>
<tr>
<td>Period x isolate x temperature</td>
<td>634695</td>
<td>90</td>
<td>7052</td>
<td>47</td>
</tr>
<tr>
<td>Error</td>
<td>108960</td>
<td>720</td>
<td>151</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15808842</td>
<td>899</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*All significant at 1% level.

Further analysis of interactions showed that the following were significant:

1. Incubation period x isolate
2. Incubation period x temperature
3. Isolate x temperature
4. Incubation x isolate x temperature

None of the isolates had grown after 7 days at 5°C. However, when the cultures were transferred to room temperature, they all grew vigorously. After 14 days at 5°C, isolates 95 and 138 still had not grown while the remaining four isolates exhibited a trace of growth. After 17 days all the isolates had produced some growth. Thus it appears that the minimum temperature for the growth of the tested isolates was about 5°C. Isaac (39, p. 142) has reported a minimum temperature of 4.5°C in his temperature studies of
Verticillium isolates.

In order to establish a refined relationship between the growth of isolates at given temperatures throughout the periods of incubation, the growth data was analyzed by the "stepwise multiple regression" technique, using a high speed computer. For the regression model the following 3rd degree multinomial was chosen:

\[ Y = \alpha + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 + E \]

where

\[ Y = \text{dry weight in mg} \]
\[ X = \text{period of incubation in days} \]
\[ \alpha = \text{constant} \]
\[ \beta = \text{regression coefficient} \]
\[ E = \text{error} \]

Dry weight was selected as the dependent variable and period of incubation as the independent variable in the analysis. An equation of the above order was obtained for each isolate at a given temperature and growth curves were constructed by using the predicted dry weights for the days of incubation within the range of 7 to 17 days. Details of the regression analysis are given in Table 1 of the appendix and the growth curves of the isolates at temperatures of 20 through 30°C are presented in Figures 1 through 5. In the discussion of the
Figure 1. Growth of *Verticillium* isolates at $20^\circ$C.
Figure 2. Growth of *Verticillium* isolates at 23°C.
Figure 3. Growth of *Verticillium* isolates at 25°C.
Figure 4. Growth of Verticillium isolates at 28°C.
Figure 5. Growth of Verticillium isolates at $30^\circ$C. Isolates No. 138 and No. 149 failed to grow at this temperature.
data "maximum growth" means the highest dry weights measured for the isolates under specified conditions of temperature and duration of incubation, and not necessarily the highest dry weight recorded throughout the experiment for each isolate.

Table 2 indicates that increasing temperature up to 28°C resulted in an increase in the growth rate of all V. dahliae isolates in the earlier periods of incubation (seven days). The two V. albo-atrum isolates, however, showed the same trend up to only 23°C. At 25°C, the initial growth rate of isolate No. 138 remained essentially unchanged, while that of No. 149 was decreased. Both isolates exhibited a very slow initial growth rate at 28°C and their dry weights in seven days were about one-half of those produced at 25°C. The most striking difference between the behavior of V. dahliae and V. albo-atrum isolates occurred at 30°C. At this temperature all V. dahliae isolates grew fairly well while neither V. albo-atrum isolate showed any growth in 17 days. The V. albo-atrum isolates, when transferred to room temperatures after 17 days, failed to recover, indicating a thermal death of the inoculum.

An experiment was performed to determine whether the lack of growth of V. albo-atrum at 30°C was due to inhibition of spore germination or to thermal death. Spores of isolates 138 and 149 were germinated at 22°C in an identical medium used in temperature studies. These pregerminated spores were used to inoculate flasks
which were then incubated at 30°C. Both isolates again failed to grow in 17 days and did not recover at room temperatures. Thus it was concluded that the maximum temperature for the growth of hop isolates of *V. albo-atrum* in culture was below 30°C and that both germinated and ungerminated spores were killed at that temperature.

At 32°C none of the *V. dahliae* isolates grew regardless of the incubation period. When transferred to room temperature after seven days, all started to grow with the exception of No. 95 which failed to recover. After 12 days of incubation at 32°C only isolate No. 148 survived and resumed growth at room temperature, but it failed to recover after 17 days of incubation at 32°C.

Dry weight production was affected by the interactions of isolate, temperature, and period of incubation (Table 2 and Figures 1 to 5). At temperatures up to 17°C the dry weights of all isolates were increased by longer incubation periods with no autolysis observed at 17 days. Above 17°C, however, the incubation period required for maximum dry weight production and the onset of autolysis varied, depending on the temperature and the isolate (Figures 1 through 5). This is summarized in the following table:
Table 3. Days of incubation required for the maximum growth of Verticillium isolates at different temperatures.

<table>
<thead>
<tr>
<th>Isolate No. and type</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>95(MS)</td>
<td>15</td>
</tr>
<tr>
<td>119(MS)</td>
<td>15</td>
</tr>
<tr>
<td>148(MS)</td>
<td>15</td>
</tr>
<tr>
<td>150(MS)</td>
<td>17</td>
</tr>
<tr>
<td>138(DM)</td>
<td>17</td>
</tr>
<tr>
<td>149(DM)</td>
<td>17</td>
</tr>
</tbody>
</table>

Increasing temperatures affected the growth pattern of isolates No. 95, 119 and 148 similarly. Their maximum dry weight production, and consequently the onset of autolysis, were hastened by increasing temperatures up to 28°C. At 30°C their growth rate was decreased and thus a longer incubation was required at 30°C to reach maximum growth than at 28°C (Table 3). Isolates No. 150, 138, and 149 responded in a much less pronounced manner to the increase in temperature in comparison to other isolates (Figures 1 to 5 and Table 3).

Table 2 and Figures 1 to 5 show that the highest dry weight values recorded for each isolate throughout the experiment varied with the isolate, temperature and the days of incubation. It must also be considered that a faster initial growth rate of the isolates, caused by increasing temperature, did not necessarily result in greater amounts of dry weight production in longer periods. On the contrary, it caused the onset of an early autolysis of the mycelial mass. At
28°C, *V. albo-atrum* isolates showed a slow and limited initial growth but at longer incubation periods both recovered and produced dry weights comparable to those at lower temperatures favorable for rapid initial growth (Figure 4). This was true for isolate No. 150 at 30°C (Figure 5).

Certain differences were present in temperature response of the isolates within each species. In general, isolates No. 95, 119, and 148 (*V. dahliae*) showed the same growth pattern at all temperatures, except No. 95 grew slower than others at lower temperatures (10 to 17°C). Isolate No. 150 (*V. dahliae*), on the other hand, behaved differently, especially at temperatures above 20°C, with the most striking differences at temperature range of 25 to 30°C (Figures 3 to 5).

Isolates No. 138 and 149 (*V. albo-atrum*) showed closely related growth pattern at all temperatures; however, they produced different total dry weights.

**Host Range Studies**

Three hop isolates of *Verticillium* were used to inoculate six plant species to determine if the isolates were adapted to hosts other than hops. The isolates were one DM type (*V. albo-atrum*, No. 138) and two MS types (*V. dahliae*, No. 148 and 150). The test plants were susceptible varieties of tomato, potato, eggplant, geranium, peppermint and strawberry. Plants were grown in soils infested with a
straw culture of the isolates containing abundant resting structures. Five plants per host species were inoculated with each isolate. However, due to non-pathological causes, some plants were lost in the greenhouse and thus the number of replications ranged from three to five (Table 4).

Planting in infested soil was completed April 10, 1967. Fluorescent lighting of 16 hours daily duration was provided for the first six weeks after planting. On June 8, disease symptoms were recorded and laboratory assays were made to determine the amount of *Verticillium* propagules in the infected stems. Degree of symptom expression was judged on the basis of comparison of the inoculated plants with uninoculated controls, and was rated on an arbitrary scale of one to four, one being no visible symptoms, and four being severe symptoms. However, the recorded symptoms were not always a reliable indication of infection by *Verticillium* isolates. Infection was determined by isolation of *Verticillium* from the stem of each plant, irrespective of symptoms. Proliferation, expressed as propagules per gram in the host vascular system, was used to determine host preference of each isolate. The results are presented in Tables 4 and 5.

The infection data (Table 4) shows that all three isolates infected potato, eggplant and strawberry. Tomato plants were susceptible to infection only by isolate No. 138 and the fungus was isolated from
Table 4. Infection of six plant species by three isolates of *Verticillium* from hops.

<table>
<thead>
<tr>
<th>Host Species</th>
<th>Isolate No. 138 (DM)</th>
<th>Isolate No. 148 (MS)</th>
<th>Isolate No. 150 (MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of plants tested</td>
<td>Number infected&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Number of plants tested</td>
</tr>
<tr>
<td>Tomato</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Potato</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Eggplant</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Geranium</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Peppermint</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Strawberry</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

<sup>a</sup>/Based on reisolation of *Verticillium* from stems.

Table 5. Relative host preference of three hop isolates of *Verticillium* in stems of different host plants, as measured by the average number of propagules per gram.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Isolate No. 138 (DM)</th>
<th>Isolate No. 148 (MS)</th>
<th>Isolate No. 150 (MS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>24,866</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Potato</td>
<td>138,750</td>
<td>11,640</td>
<td>5,560</td>
</tr>
<tr>
<td>Eggplant</td>
<td>65,000</td>
<td>16,000</td>
<td>16,350</td>
</tr>
<tr>
<td>Geranium</td>
<td>700</td>
<td>0</td>
<td>5,300</td>
</tr>
<tr>
<td>Peppermint</td>
<td>0</td>
<td>0</td>
<td>274,500</td>
</tr>
</tbody>
</table>
three out of four inoculated plants. With geraniums, three out of five inoculated plants were infected by isolate No. 138, none by No. 148, and only one by isolate No. 150. Peppermint was not infected by isolates No. 138 and 148, but was highly susceptible to infection by isolate No. 150.

The relative host preference of the isolates in tested plants is shown in Table 5. Isolate No. 138 (*V. albo-atrum*) proliferated best in potato stems. The data suggest that No. 138 is specially adapted to potato, less adapted to eggplant and tomato, and not adapted to geranium or peppermint.

Isolate No. 148 failed to infect tomato, geranium and peppermint, and proliferated poorly in the stems of potato and eggplant, suggesting that this isolate is not adapted to any of the tested host species.

Isolate No. 150 proliferated greatly in peppermint suggesting that it is specially adapted to that host. The infected plants, however, were symptomless. To confirm the host preference of No. 150, ten peppermint cuttings were inoculated by dipping the roots in a concentrated spore and mycelial suspension. For comparison, a similar number of plants were inoculated with a virulent peppermint strain of *V. dahliae* isolated from severely infected plants. The inoculated plants and uninoculated controls were planted in clay pots and kept in the greenhouse. About two weeks after the inoculation, characteristic
symptoms of Verticillium wilt appeared in all inoculated plants. No difference was observed in the onset or severity of symptoms caused by each isolate. Symptoms became progressively more severe, causing eventual death of all inoculated plants. Therefore, it is concluded that _V. dahliae_ isolate No. 150, originally obtained from infected hops, is specialized for pathogenicity on peppermint.

With strawberry plants, the usual procedure for the determination of propagules was not used. Instead, relative proliferation was assayed by isolation from five randomly selected mature leaf petioles from each inoculated plant, and was expressed as the percent of leaf petioles infected. Isolates No. 138 and 150 proliferated in strawberry equally, infecting 88 percent of the leaf petioles. Isolate No. 148 was recovered from only 40 percent of the petioles of the infected plants.

These experiments demonstrate that distinct differences in infectivity and host preference exist, not only between dark mycelial (_V. albo-atrum_) and microsclerotial (_V. dahliae_) types, but also within the microsclerotial types of _Verticillium_, all isolated from hops. Two of the three tested hop isolates (No. 138 and No. 150) appear to be potentially pathogenic on other host species. From the results of pathogenicity tests on hops, presented in the following sections, it is possible to suggest that hop isolates of _Verticillium_ existing in Oregon, are primarily pathogens of other plants, with host ranges that
include hops to some extent.

Pathogenicity Tests on Hops

Infested Soil

The reactions of 14 varieties of hops grown in soils infested with five isolates of Verticillium were studied in two successive years. The test plants included commercial varieties as well as newly developed types still in experimental stages. Four isolates of V. dahliae (No. 95 from peppermint; No. 119 from potato; No. 146 from hops; and No. 148 from hops) and one isolate of V. albo-atrum (No. 138 from hops) were tested.

In the first year (1966), soil infested with shake cultures of each isolate was used as the inoculum. In the second year, (1967), a straw-culture of each isolate containing abundant resting structures was used.

Number of replications varied with the isolate and the year of the experiment. In 1966, 12 plants per variety were tested with isolates No. 95 and 119 and six plants with the remaining three isolates. In 1967, five plants of each variety were tested with all isolates, except variety OB-831 which had ten plants. Records were taken on symptom development during the growing season. At the end of the season, individual bines were examined for vascular discoloration,
and stem sections from each plant were assayed in the laboratory to determine the number of Verticillium propagules in the infected stems.

Foliar symptoms and vascular discoloration were not reliable indicators of infection. Therefore, the infection by a given isolate of Verticillium was determined by isolation of the fungus from the stems of inoculated plants. The varietal preference of the isolates was determined by estimation of the number of Verticillium propagules per gram of stem. The data on the infection and proliferation of the isolates are presented in Table 6.

Table 6 shows that in both years of the experiments some varieties became infected with the tested isolates. However, the varieties showing infection in the first year were not necessarily infected in the second year and vice versa. The percentage of infected plants within the infected varieties was very low with all the isolates.

More varieties were infected by V. dahliae isolates of hops (No. 146 and 148) than that by V. albo-atrum (No. 138).

Soil infestation with differently prepared inoculum (laboratory infested soils or straw-culture of the isolates), did not affect the percentage of infected plants appreciably. However, with the straw-inoculum more varieties of hops were infected by isolates No. 146 and 148.

The effect of different levels of inoculum on infection and
<table>
<thead>
<tr>
<th>Hop Varieties</th>
<th>Isolate No. 95</th>
<th>Isolate No. 119</th>
<th>Isolate No. 138</th>
<th>Isolate No. 146</th>
<th>Isolate No. 148</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuggle</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fuggle-H</td>
<td>8</td>
<td>1,000</td>
<td>20</td>
<td>1,200</td>
<td>0</td>
</tr>
<tr>
<td>Brewers Gold</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bullion</td>
<td>8</td>
<td>11,200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Late Cluster</td>
<td>8</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Early Cluster</td>
<td>8</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Yakima Cluster</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>Janus</td>
<td>8</td>
<td>200</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Density</td>
<td>8</td>
<td>400</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OB-826</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>1,000</td>
<td>-</td>
</tr>
<tr>
<td>OB-831</td>
<td>8</td>
<td>1,400</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>OB-835</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>E-2</td>
<td>8</td>
<td>3,800</td>
<td>20</td>
<td>300</td>
<td>0</td>
</tr>
<tr>
<td>50-S</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>300</td>
<td>0</td>
</tr>
</tbody>
</table>

\(a/\) not tested in 1966.
proliferation of isolates No. 138, 146 and 148 was studied in another experiment. The hop varieties tested were Fuggle, Brewers Gold, Late Cluster and Janus. Five plants of each variety were planted in one-gallon cans, containing soils infested with 20,000, 100,000 and 500,000 propagules per gram of given isolates. The inoculum was prepared by artificial infestation of soil with shake cultures of the isolates and the desired levels of the inoculum were obtained by dilutions of infested soil with fresh greenhouse soil. Planting in infested soil was done on June 15 and assays for the number of propagules in the stems of individual plants were made on October 5.

No *Verticillium* was recovered from any of the test plants indicating that all three isolates had failed to infect hops regardless of the inoculum level.

**Direct Inoculation**

Reaction of nine hop varieties to direct stem inoculation with isolates No. 138, 148 and 150 was studied in a field experiment. Two to four plants of each variety were inoculated with a conidial suspension of the isolates injected into the pith cavity of the stems. Isolations for infection and assays for vascular proliferation were made at the end of the growing season. The results are shown in Table 7.

Isolates No. 138 and 148 infected all nine varieties tested. The two isolates were recovered from all the inoculated plants of Fuggle-H,
Table 7. Infection and proliferation of three *Verticillium* isolates in hop varieties by direct stem inoculation.

<table>
<thead>
<tr>
<th>Hop Variety</th>
<th>Number of plants tested (I); number of plants infected (II); number of propagules/gm (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Isolate No. 138 (I) (II) (III)</td>
</tr>
<tr>
<td>Fuggle</td>
<td>3 3 100</td>
</tr>
<tr>
<td>Fuggle-H</td>
<td>3 3 733</td>
</tr>
<tr>
<td>Brewers Gold</td>
<td>4 4 162</td>
</tr>
<tr>
<td>Bullion</td>
<td>3 3 5,166</td>
</tr>
<tr>
<td>Early Cluster</td>
<td>2 1 21,000</td>
</tr>
<tr>
<td>Janus</td>
<td>2 1 200</td>
</tr>
<tr>
<td>Density</td>
<td>4 3 1,100</td>
</tr>
<tr>
<td>OB-831</td>
<td>3 3 617</td>
</tr>
<tr>
<td>50-S</td>
<td>3 2 325</td>
</tr>
</tbody>
</table>
Brewers Gold, Bullion and OB-831; from one-half of Early Cluster and Janus plants; and from two of three inoculated plants of 50-S. Isolate No. 150 failed to infect Bullion and Early Cluster, but different numbers of the plants of other varieties were infected.

No variety was immune to infection by all three isolates with this method of inoculation.

Isolate No. 138 proliferated best in Early Cluster and Bullion. Isolate No. 148 increased most in Density, Fuggle and Bullion; and No. 150 did not reach high numbers in any variety.
Controversy among different workers on recognition of *Verticillium dahliae* as a species distinct from *V. albo-astrum* has existed for a long time and very likely will continue. As Isaac (42) recently stated, "the confusion is increasing to such an extent that different people from the same institute are publishing results of research in which they give different names to what is obviously the same organism". The failure to differentiate between these two species has retarded recognition of the distinct ecological and pathological aspects of the wilt diseases they cause. Many researchers have reported *V. albo-astrum* as a more virulent pathogen than *V. dahliae* in several crops (32; 46; 76; 89; 97), and the measures useful for control and eradication of one species are not necessarily applicable to the other. The separation of the two species, in addition to the differences in pathogenicity, morphology and geographical distribution, can also be justified on physiological grounds such as temperature requirements for growth.

Reports in the literature differ markedly on the effect of temperature on the growth of *Verticillium* in culture. This is particularly true about the maximum temperature for growth. It is reasonable to assume that these conflicting findings are due, in part, to failure to distinguish between *V. albo-astrum* and *V. dahliae* by some workers.
The inconsistencies can also be attributed to the use of different media and different measuring techniques. In most cases the nature and composition of the media employed has not been adequately described. Chaudhuri (13, p. 525) found that with \textit{V. albo-atrum} maximum temperature for growth varied with different nutrients, but the optimum always remained the same. Cochrane (16, p. 13), in reviewing the literature on the effect of temperature on growth of fungi, notes that the effect of temperature on final dry weight of \textit{Phycomyes black-esleeanus} can be obscured if the nitrogen supply is made limiting.

Authors who accept \textit{V. dahliae} as a distinct species have reported that it can tolerate higher temperatures in culture than \textit{V. albo-atrum} (Table 1). These reports have been confirmed by the results presented in this thesis. Four \textit{V. dahliae} isolates obtained from hops, peppermint and potato grew fairly well at 30°C, while the two tested \textit{V. albo-atrum} isolates (from hops) failed to grow at that temperature (Table 2). Further experiments demonstrated that the lack of growth of \textit{V. albo-atrum} isolates at 30°C was due to thermal death of both germinated and ungerminated spores rather than to inhibition of germination. The maximum temperature for the growth of \textit{V. dahliae} isolates was between 30°C and 32°C. At 32°C none of these isolates showed growth, and their spores were not viable after 7 to 12 days.

The optimum temperature for growth is usually defined as the
temperature in which the fungus produces the maximum amount of mycelium. Many workers have used measurements of linear extension of fungal colonies on agar media as the criterion of growth in temperature studies. They have determined the optimum as the temperature in which the test organism exhibits the greatest expansion of the colony diameter in a given period of incubation. This technique, however, in most cases provides no indication of the actual amount of mycelium produced by the test fungus (13). Experiments involving dry weight measurements, on the other hand, provide information on the actual amount of fungal material produced under given conditions. However, three factors must be considered in interpretation of the results of temperature studies by dry weight measurements. These are: a) the rate of growth (increase in the dry weight) under given conditions of temperature and periods of incubation; b) the maximum dry weights produced under specified conditions of temperature and periods of incubation; and c) the highest total dry weight produced by the fungus among different conditions of temperature and periods of incubation. Because of autolysis of the mycelium, which occurs after maximum growth of fungi in liquid media, use of a single incubation period will often yield results that can not be considered representative of potential growth.

The data presented in Table 2 and Figures 1 through 5 indicate that dry weight production was affected by the interactions of isolate,
temperature, and period of incubation. The onset of autolysis appeared to be affected directly by both temperature and the initial growth rate of the isolates. A faster initial growth rate caused by increasing temperatures (up to 28°C for V. dahliae and 25°C for V. albo-atrum), did not necessarily result in greater amounts of dry weight production; to the contrary, it caused the onset of an early autolysis of mycelium. On the other hand, a slow initial growth rate, caused by too high a temperature (30°C for V. dahliae and 28°C for V. albo-atrum), resulted in delay of autolysis and production of greater dry weights in longer incubation periods (Table 2 and Figures 3 to 5). For these reasons, no temperature can be determined conclusively to fit the definition of the optimum temperature for the growth of tested isolates.

The failure of dark mycelial isolates to grow at 30°C indicates a significant physiological difference between V. albo-atrum and V. dahliae, and thus supports the validity of considering V. dahliae as a distinct species. Within V. dahliae isolates, the different temperature response of isolate No. 95 at 10 to 17°C and No. 150 at 25 to 30°C, demonstrate differences in physiological characteristics of the strains.

The data on the infection and proliferation of hop isolates of Verticillium in plants other than hops (Tables 4 and 5) suggest complex host-pathogen relationships in these isolates. V. albo-atrum
isolate (No. 138) was found to infect and proliferate greatly in stems of potatoes, and to a lesser extent in tomatoes and eggplants. The data suggested that this isolate, originally recovered from infected Fuggle hops, is specially adapted to potatoes. Information furnished by the owner of the hop yard from which the original infected hop stems were obtained, indicated that the land had a previous cropping history that included potato.

One _V. dahliae_ isolate (No. 148), which was originally recovered from Bullion hops grown on land with a previous history of strawberry culture, proliferated poorly in potato, eggplant and strawberry, the data suggested that this isolate was not specially adapted to any of the tested host plants. The other _V. dahliae_ isolate (No. 150), which was originally obtained from infected Fuggle hops, proliferated greatly in stems of peppermint and was highly pathogenic to that host. No difference was observed in the pathogenicity of this isolate and that of a virulent peppermint strain of _V. dahliae_. Information on the cropping history of the hop yard from which the isolate was obtained, indicated that the yard had been in hop production for 20 years. However, further inquiry revealed that the entire hop yard was surrounded by fields which were previously planted to peppermint, but that had gone out of production because of Verticillium wilt caused by _V. dahliae_. Thus, the special adaptation of isolate No. 150 to peppermint can be explained.
When 14 hop varieties were inoculated with *Verticillium* isolates from infected hops, peppermint and potato, a very low percentage of plants became infected. The varieties showing infection in 1966 were not necessarily infected in 1967 and vice versa (Table 6). Soil infestation with differently prepared inoculum (laboratory infested soils or straw cultures) and different levels of inoculum, did not affect the percentage of infected plants appreciably.

In general, all *Verticillium* isolates, regardless of their origin or species, proliferated poorly in the stems of infected hop plants (Table 6).

Direct stem inoculation by injection of spores of hop isolates into the pith cavity, resulted in infection of a high percentage of plants (Table 7). Isolates No. 138 (*V. albo-atrum*) and No. 148 (*V. dahliae*) infected all nine tested varieties, and isolate No. 150 (*V. dahliae*) infected seven of the tested hop varieties. However, no large differences in proliferation of the isolates in different varieties were obtained.

*Verticillium albo-atrum* and *V. dahliae* causing vascular wilt of susceptible hosts, normally enter through the roots and then invade systemically the xylem tissue of the root and stem. The extent of colonization of stem and the disease severity seems to be proportionally related to the extent of the root infection (53). The high percentage of infected hop plants obtained by stem injection compared to the
low percentage obtained by growing plants in infested soil, suggests that the tested isolates of *Verticillium* are not specialized for invasion of the hop root.

No actual wilting or death of the infected hop plants was observed throughout the experiments. Leaf yellowing and vascular discoloration were exhibited by the test plants, however these symptoms were not always reliable indicators of infection and pathogenicity of the isolates.

On the basis of the data obtained, no definite conclusion regarding the resistance of tested hop varieties to *Verticillium* wilt is possible. It is concluded that the strains of *Verticillium* affecting hops in Oregon are not virulent pathogens of this crop. The evidence obtained in host range studies also suggests that these strains are primarily adapted to plants other than hops.

These findings do not overrule the necessity and importance of continued research on the problem. Although surveys of outbreaks for several years have indicated that disease has not seriously affected hop production in Oregon (38), recovery of *V. albo-astrum*, in particular, from infected plants points out the potential importance of hop wilt in the United States. As Horner (38) has stated

...*Verticillium* wilt in England was considered a disease of minor consequence for about 10 years, when a progressive killing form of the disease caused by a more virulent strain of the pathogen occurred, and subsequently caused severe losses.
BIBLIOGRAPHY


Table A-1. Predicted equations obtained by the stepwise multiple regression analysis of the data on temperature relations of *Verticillium* isolates.

<table>
<thead>
<tr>
<th>Isolate and temperature</th>
<th>Predicted equation</th>
<th>$R^2$</th>
<th>DF</th>
<th>SSE</th>
<th>F*</th>
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<tr>
<td>20</td>
<td>$Y = -358 + (90.7)X - (2.92)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>1360</td>
<td>156</td>
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<tr>
<td>23</td>
<td>$Y = -443 + (120)X - (4.5)X^2$</td>
<td>0.96</td>
<td>12</td>
<td>3285</td>
<td>154</td>
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<tr>
<td>25</td>
<td>$Y = 149 + (2.56)X^2 - (0.1385)X^3$</td>
<td>0.78</td>
<td>12</td>
<td>3727</td>
<td>33</td>
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<tr>
<td>28</td>
<td>$Y = 170 + (11.02)X^2 - (0.0349)X^3$</td>
<td>0.83</td>
<td>12</td>
<td>1877</td>
<td>10</td>
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<tr>
<td>30</td>
<td>$Y = -260 + (80)X - (3.17)X^2$</td>
<td>0.98</td>
<td>12</td>
<td>567</td>
<td>443</td>
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<td>$Y = -323 + (84.98)X - (2.74)X^2$</td>
<td>0.96</td>
<td>12</td>
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<tr>
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<td>$Y = -441 + (117.65)X - (4.35)X^2$</td>
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<td>$Y = 173.25 + (10.6)X - (0.033)X^3$</td>
<td>0.70</td>
<td>12</td>
<td>3213</td>
<td>7</td>
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<td>$Y = -250 + (78.3)X - (3.036)X^2$</td>
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<td>12</td>
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<td>$Y = -266 + (82.2)X - (2.73)X^2$</td>
<td>0.98</td>
<td>12</td>
<td>1596</td>
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<tr>
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<td>$Y = -407 + (113.6)X - (4.16)X^2$</td>
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<td>12</td>
<td>264</td>
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<td>$Y = -178 + (74.67)X - (2.93)X^2$</td>
<td>0.84</td>
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<td>4137</td>
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<tr>
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<td>$Y = 165 + (11.27)X - (0.033)X^3$</td>
<td>0.86</td>
<td>12</td>
<td>1050</td>
<td>18</td>
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<td>$Y = -223 + (74.5)X - (2.89)X^2$</td>
<td>0.87</td>
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<td>3721</td>
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<td>$Y = -191 + (51.65)X - (1.148)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>1269</td>
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<tr>
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<td>$Y = -462 + (111)X - (3.66)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>1508</td>
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<tr>
<td>25</td>
<td>$Y = -281 + (94.4)X - (3.4)X^2$</td>
<td>0.99</td>
<td>12</td>
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<td>$Y = -123 + (66.96)X - (2.44)X^2$</td>
<td>0.94</td>
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<td>$Y = -551 + (113.8)X - (3.65)X^2$</td>
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<td>$Y = -252 + (67.52)X - (2)X^2$</td>
<td>0.94</td>
<td>12</td>
<td>6379</td>
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<td>$Y = -489 + (119.2)X - (4.28)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>758</td>
<td>603</td>
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<tr>
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<td>$Y = -341 + (90.95)X - (3.21)X^2$</td>
<td>0.91</td>
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<td>7169</td>
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<td>$Y = -555 + (116.6)X - (3.82)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>644</td>
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<td>30</td>
<td>$Y = 0$ (no growth)</td>
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<td>20</td>
<td>$Y = -126 + (44.15)X - (1)X^2$</td>
<td>0.97</td>
<td>12</td>
<td>2590</td>
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<tr>
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<td>$Y = -479 + (57.6)X - (1.64)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>992</td>
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<td>$Y = -238 + (64.49)X - (1.99)X^2$</td>
<td>0.99</td>
<td>12</td>
<td>1027</td>
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<td>$Y = -429 + (88.83)X - (2.57)X^2$</td>
<td>0.95</td>
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<td>30</td>
<td>$Y = 0$ (no growth)</td>
<td>-----</td>
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</tbody>
</table>

* All significant at 1% level.