AN ABSTRACT OF THE THESIS OF

ROBERT CHARLES CARLSTROM for the Ph. D.
(Name) (Degree)

in PLANT PATHOLOGY presented on 1-20-69
(Major) (Date)

Title: SURVIVAL OF VERTICILLIUM DAHLIAE IN SOIL

Abstract approved: Redacted for Privacy
Dr. R. L. Powelson

Three Oregon soil types (Chehalis silt loam (CSL), an alluvial soil from the Willamette Valley; Ayre sandy loam (ASL), a volcanic pumice soil from the Central high desert; and Klamath fine sandy loam (KFSL), a basaltic mineral soil from the Klamath Basin) were collected adjacent to fields which had been removed from potato production due to Verticillium wilt caused by the soil-borne fungus Verticillium dahliae kleb.

The soils were infested with V. dahliae inoculum composed of individual microsclerotia (MS) or microsclerotia imbedded in stem pieces (SPMS). The infestation level was 100, 000 propagules/g soil. The inoculum was derived by fragmenting and screening field-infested potato stems.

Each infested soil type was transported to, and incubated at, each of the above geographic locations. Changes in the V. dahliae populations were studied over a five-year period. The inoculum potentials of the surviving V. dahliae populations in soils which had

been infested with 1000 propagules/g were bioassayed with eggplants after five years.

V. dahliae microsclerotia freshly added to moist soils showed an initial flush of sporulation with a subsequent population decline. Population lows were reached after 71 or 256 days incubation, depending on soil type and geographic location. After reaching lows of 14-45 percent of the original population, all V. dahliae populations showed gradual increases through the remainder of the five-year test period. Final populations were 34-63 percent of the original population.

CSL soil and the Corvallis environment exerted a strong influence on V. dahliae sporulation. V. dahliae populations in all three soil types infested with MS inoculum showed periodic population fluctuations of up to four-fold at the Corvallis location through the five-year study. ASL and KFSL soil types suppressed sporulation of V. dahliae at the Redmond and Klamath Falls locations.

Organic matter breakdown and subsequent release of V. dahliae propagules from stem piece inoculum was related to soil type and geographic location. After 20 months incubation, organic matter breakdown ranged from 7 percent in the CSL soil at the Corvallis location to 88 percent in the ASL soil at the Klamath Falls location. After five years incubation the inoculum potentials varied with soil type and geographic location. Only 20 percent of the

eggplants grown in the CSL soils from Redmond showed symptoms, whereas 100 percent of the plants grown in the KFSL soil from the Corvallis and Klamath Falls locations showed symptoms.

The three soil types at three levels of infestation were studied over a 290 day period under controlled conditions of soil moisture and temperature in the laboratory. Upon initial infestation with microsclerotia there were population increases in direct proportion to the level of infestation. Unlike the field experiments, no periodic population fluctuations occurred during the 290 day incubation period. The percent V. dahliae survival after 290 days was inversely proportional to the initial infestation levels in the three soils, while percent survival between soil types was in the order of CSL> ASL> KFSL.

A linear regression analysis of Verticillium wilt symptom development in eggplants grown in the three soil types, infested at three levels showed a significant difference between the lowest infestation level and the two higher levels and also between soil types. Symptom development in the KFSL soils were significantly slower and less severe.

The number of infection loci/g root was not proportional to the inoculum density in the soil. Infection loci/1000 propagules/g of soil were 4.0 in KFSL soils and 2.4 in ASL soils. Eggplants grown in V. dahliae infested soils showed increased fresh weights at each increased infestation level.

Survival of *Verticillium dahliae* in Soil

by

Robert Charles Carlstrom

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1969

APPROVED:

Redacted for Privacy

Associate Professor of Plant Pathology
in charge of major

Redacted for Privacy

Head of Department of Plant Pathology

Redacted for Privacy

Dean of Graduate School

Date thesis is presented 1-20-69

Typed by Donna L. Olson for Robert Charles Carlstrom

ACKNOWLEDGMENTS

The author wishes to thank Dr. R. L. Powelson for his guidance throughout this study and for aid in preparation of this manuscript. Thanks are given also to Dr. M. E. Corden for constructive criticism of the manuscript.

Appreciation is also expressed to Dr. R. V. Frakes for technical assistance in the statistical analyses.

Financial support of the Oregon Potato Commission is gratefully acknowledged.

Special appreciation goes to my wife, Joyce, for patience during the course of this study and aid in the preparation of this thesis.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

SURVIVAL OF VERTICILLIUM DAHLIAE IN SOIL

INTRODUCTION

Although the annual acreage of potatoes in Oregon has changed little in the last 68 years (i.e., 40,000 acres/yr.), many areas have been forced to abandon potato culture due to the soil-borne vascular wilt fungus Verticillium dahliae. Verticillium wilt has been a limiting factor in Oregon potato production since 1921 when McKay first reported experimental studies he had conducted since 1916 on this disease in the Northern Willamette Valley. With the development of new irrigation projects, potato culture expanded into Central Oregon, the Ontario area and the Klamath Basin. Little Verticillium wilt was initially found in these newly developed areas.

Verticillium dahliae is a comparatively weak pathogen and disease severity is directly proportional to the amount of fungus in the soil and the number of root infections. Once a locus of V. dahliae infestation has been established in a field the normal cultural practice of incorporating potato plant residues into the soil after harvest introduces large quantities of V. dahliae into the field.

After V. dahliae has been established in a soil, the fungus is capable of long-term survival under either fallow conditions or

rotations to supposedly non-host crops. The investigations presented in this thesis were initiated to: (1) determine the long-term survival of naturally cultured V. dahliae microsclerotia in three soil types from, and at, three geographic locations with major climatic differences; (2) determine the survival of microsclerotia in three soil types at three levels of infestation under controlled temperature and moisture conditions; and (3) determine by eggplant bioassay tests the inoculum potential of V. dahliae propagules in soils using different inoculum types at different inoculum densities through differing periods of incubation.

LITERATURE REVIEW

Taxonomy

The genus Verticillium was described in 1816 by Nees von Esenbeck. Corda described the genus Acrostalagmus in 1838 to accommodate the fungus A. cinnabarinus, which differed from Verticillium by formation of spores in a head at the tip of each conidiophore phialide. Hoffman showed in 1854 that no real difference was demonstrable in the mode of spore production by the two supposedly different genera.

In 1879 Reinke and Berthold published the first description of a fungal vascular parasite in plants. They isolated a fungus from potato plants which, on the basis of Hoffman's findings, they classified as V. albo-atrum. In 1913 Klebahn isolated a fungus from dahlia plants which he considered sufficiently distinct from V. albo-atrum to be designated V. dahliae. He pointed out that the chief difference between V. albo-atrum and V. dahliae is the production of microsclerotia by the latter and the complete absence of such structures in the former. Klebahn's differentiation of these two species in 1913 has been the basis for a continuing taxonomic controversy.

Rudolph (1931) questioned the validity of V. dahliae because he found that production of microsclerotia was not sufficiently constant to be used as a criterion in differentiating the two species.

Reinke and Berthold describe "Sklerotien", but their illustrations of V. albo-atrum depict masses of dark resting mycelium, not microsclerotia. Microsclerotia, as formed by V. dahliae, consist of swollen, almost spherical cells that multiply by divisions in all planes, forming a small, generally spherical mass of cells that progressively develop thick walls and darken from the center of the mass outward (Isaac, 1946).

Wilhelm (1948), Nelson (1950) and Presley (1950) concluded that resting structures such as microsclerotia, dauermycelium, and sclerotien are not reliable characters for species separation. An excellent discussion of the history and development of the V. albo-atrum vs. V. dahliae controversy has been presented by Isaac (1957).

Many workers consider Verticillium to be a highly variable genus and believe that all the pathogenic variants should be included in V. albo-atrum (Wollenweber, 1929; Rudolph, 1931; Presley, 1941; Wilhelm, 1948; Van den Ende, 1958; and Brandt, 1964). Others consider the dauermycelial (dark mycelial) and microsclerotial types sufficiently different to be assigned separate rank (Klebahn, 1913; Pethybridge, 1916; Van der Meer, 1925; Berkeley et al., 1931; Ludbrook, 1933; Van Beyma thoe Kingma, 1940; Isaac, 1949; Robinson et al., 1957; and Smith, 1965).

Isaac (1967) notes that in the rising confusion different people from the same institution are publishing Verticillium research

reports in which they are giving different species names to the same organism. Smith (1965) observed that even if V. albo-atrum and V. dahliae are variants of the same species their differences in geographical occurrence, host range and pathogenicity are sufficiently important to justify specific recognition. Many authors have used V. albo-atrum when reporting on work using the microsclerotial form of this fungus. Thus, to clarify further citations used in this thesis, the notation (MS) will be inserted following the name V. albo-atrum where it is reasonably certain that the author was working with the microsclerotial type.

Hosts

Since Reinke and Berthold's first description of Verticillium as a vascular wilt pathogen in 1879, the host range of this fungus has been extended to a great number of plant species (Rudolph, 1931; Engelhard, 1957; Parker, 1959; Martinson, 1964; Isaac, 1967). Green (1951) tested eight crop and 21 weed species for susceptibility to Verticillium. V. albo-atrum was reisolated from both the stems and roots of Mentha rotundifolia L. and Solanum melongena L. var. Black Beauty (eggplant), but only the roots of Capsicum annuum L. var. Calwonder (red pepper). Horner (1954) tested the common weeds Arctium minus Bernh. Cirsium arvensae (L.) Scop., Chenopodium album L., Malva rotundifolia L., Amaranthus

retroflexus L., and Lycopersicon esculentum Mill (tomato) for susceptibility to infection by the peppermint strain of V. albo-atrum (MS). He recovered the fungus from all plants tested. He also found that Verticillium isolates from maple, peony, potato, tomato, and raspberry could infect peppermint but producted no typical disease symptoms.

Wilhelm and Thomas (1952) report that the symptomless weed host Solanum sarachaoides Sendt (South American nightshade) increases inoculum densities of V. albo-atrum (MS) in strawberry fields. Sewell and Wilson (1958) tested a number of weeds growing in a hop garden showing severe Verticillium wilt. They isolated V. dahliae from Solanum vulgaris, and V. albo-atrum from Chenopodium album L., Senecio vulgaris L., Solanum nigrum L. and Urtica urens L.

Martinson (1964) reported root invasion by V. dahliae in 20 of 21 crop plants tested. Included in his tests were the following graminacious species previously thought to be non-hosts: Triticum aestivum L. (wheat), Sorghum vulgare Pers. (sorghum), Avena sativa L. (oats), Zea mays L. (corn) and Hordeum vulgare L. (barley). None of these "non-hosts" showed foliar symptoms after seven weeks growth in V. dahliae infested soil.

Inoculum Potential

Garrett (1963) defined inoculum potential as "the energy of growth of a fungus (or other microorganism) available for colonization of a substrate at the surface of the substrate to be colonized." Dimond and Horsfall (1960) defined inoculum potential as "the resultant of the action of the enviornment, the vigor of the pathogen to establish an infection, the susceptability of the host and the amount of inoculum present." The latter definition fits only pathogenisis, whereas Garrett's definition adequately covers both pathogenic and saprophytic potentials.

Environment and nutritional factors have a great influence on the inoculum potential of any given member of the soil microflora. Davey and Papavisas (1963) temporarily reduced the inoculum potential of Rhizoctonia solani Kuehn as a stem and root rot pathogen of snap beans by the addition of high C:N ratio amendments to soils. Attenuation of the parasitic ability of Rhizoctonia was probably caused either by an increase in CO_2 content of the soil atmosphere, or by a scarcity of available nitrogen in the soil solution. Both of these effects are attributable, however, to a rapid increase in microfloral activity and their concurrently increased respiration rates.

When fresh uncolonized organic substrates are added to natural

soil, the inoculum potential of a microorganism to invade the substrate depends on its competitive saprophytic ability, which in turn is determined in part by the following four factors (Garrett, 1950, 1956 and 1963): (1) high growth rate of hyphae, and rapid germination of spores, (2) rapid synthesis of enzymes, (3) production of antibiotic toxins, and (4) tolerance of antibiotics produced by other microorganisms.

Garrett (1965) states:

> ...in saprophytic competition for a suitable substrate in the soil the success of a particular fungus will be conditioned as much by the inoculum potential at which it happens to be present at the surface of the substrate as by its intrinsic competitive saprophytic ability.

Although Garrett (1963) reports that Ophiobolus graminis, Helminthosporium sativum and Cercosporella herpotrichoides are all poor competitive saprophytes, investigations by Butler (1953), Lucas (1955) and Macer (1961) indicate that, if the inoculum potential of these fungi is sufficiently high compared with other saprophytic organisms at the surface of a grain straw particle in the soil, then each is capable of successful colonization of this substrate.

Blank and Leyendecker (1951) added Verticillium infected cotton stems to field soils each month from December through April. The plots were planted to cotton and wilt symptoms recorded through the growing season. Disease expression was directly correlated with the time the infested stems were in the soils. The

plots infested in December produced the greatest percentage of plants with wilt symptoms, while those infested in April produced fewer wilted plants. Increased disease is probably due to increased Verticillium propagules released into the soils by decomposition of the cotton stems (Evans, Wilhelm and Snyder, 1967).

V. dahliae is considered a poor saprophytic competitor (Wilhelm, 1951, 1955). Martinson (1964) found that V. dahliae is capable of a limited saprophytic invasion of host and non-host stem particles added to Verticillium infested soils. V. dahliae was isolated from significant numbers of stem particles 176 days after burial. The duration of survival of V. dahliae in these saprophytically invaded stem particles was not studied.

Isaac (1957) found that the nutrient status of inoculum influenced its potential. He grew V. dahliae on Dox's agar containing 0%, 0.2%, and 2.5% $NaNO_3$. Stems of Antirrhinum majus were inoculated with Verticillium and the plants were grown in infested soils adjusted to deficient, normal and excessive organic nitrogen levels. V. dahliae grown on nitrogen deficient agar produced little or no wilt. Inoculum grown on 0.2% $NaNO_3$ media produced wilt symptoms in nearly all of the plants tested. The levels of soil nitrogen had no effect on disease expression. Baker (1965) cites unpublished work by D. J. Phillips who demonstrated that severity of Fusarium scab resulting from inoculation of grain hosts with conidia

of Fusarium roseum f. cerealis grown on high carbon media was significantly higher than with propagules produced on low carbon media.

Measurement of inoculum density can often be used to estimate the inoculum potential. Tolmsoff (1959) demonstrated that logarithmic increases in inoculum densities of V. albo-atrum (MS) produced the following effects on Verticillium wilt in potato plants: (1) earlier wilt symptoms, (2) earlier death of plants, (3) a shorter time interval between symptom expression and death of plants, and (4) decreased tuber yields.

Martinson and Horner (1962) found only 10-40 viable V. dahliae propagules/gram of soil from a heavily wilted potato field, while in Tolmsoff's (1959) experiments 23,000 to 91,000 propagules per cc of soil were required to produce severe leaf wilt of potato plants. Martinson (1964) infested Chehalis silt loam with 0, 100, 316, 1000, 3162 and 10,000 propagules of microsclerotial inoculum from naturally infested mint stem pieces and found that 316 propagules/gram soil were required to produce mild symptoms. He also noted significant differences in green plant weight and degree of fungal invasion of stems due to differences in inoculum density. Inoculum densities above 1000 microsclerotia/gram and soil temperatures between 20 and 25°C produced the greatest disease expression.

Population Fluctuations

A number of authors have recorded V. dahliae or V. albo-atrum soil population fluctuations over varying periods of time. Lacy (1965) infested a silt loam with microsclerotia from infected peppermint plants and recorded V. dahliae fluctuations under controlled temperature conditions in the greenhouse. His data indicate wide population changes through a six month period. In a greenhouse study Schreiber and Green (1962) infested a mineral soil with V. albo-atrum (MS) microsclerotia produced on artificial media. Soil samples were bioassayed with tomato plants at five to 12 week intervals for 82 weeks following infestation. Isolations from diseased plants showed that percent infection varied from 41% to 100% between the 77th and 82nd week. Three other fluctuations in infection potential after the 39th week were of similar magnitude.

In a field study of naturally infested cotton soils, Evans et al., (1967) found that V. albo-atrum (MS) microsclerotial populations in the upper 10 cm of the soils increased through the winters and decreased in the summers of two years. McKay (1962a) buried V. albo-atrum (MS) infested potato stem pieces in field soil. In separate observations over a 22-month period he found varying degrees of Verticillium sporulation on the stem pieces at different times of the year.

In a laboratory study of V. dahliae survival in a fine sandy loam, Menzies and Griebel (1967) found that propagule densities generally decreased over a 351 day period at all levels of soil infestation tested. A second laboratory study over 118 days showed only minor fluctuations in V. dahliae populations after 42 days and a slight rise in population at 118 days.

Martinson (1964) incubated V. dahliae infested silt loam in flasks in the laboratory for 176 days. The inoculum was microsclerotia produced on sterile barley straw in the laboratory. His data show a general decline in V. dahliae populations through 72 days with a slight rise between 72 and 176 days.

Inoculum Type vs. Verticillium Survival

The earliest report of research on Verticillium wilt of potatoes in Oregon was by McKay in 1921 concerning work he had done since 1916.

McKay (1926a) buried V. albo-atrum (MS) infested potato stem pieces in field soil at depths from one to six inches. Seven months later tests showed the shallow buried stem pieces contained little V. albo-atrum (MS) while those buried deeper had a high degree of survival as evidenced by profuse sporulation on the surface of stem pieces. After 22 months burial, he detected no sporulation and an almost complete disintegration of the stem pieces. PDA

cultures of "sclerotium-like bodies" recovered from the buried stem pieces yielded no Verticillium. Infested stem pieces which had been stored in the laboratory for one year and then cultured on PDA failed also to yield Verticillium.

McKay mentions observing "sclerotium-like bodies" imbedded in potato stems which may lead one to believe that he was working with V. dahliae, but his comparative short term survival data suggests V. albo-atrum. Heale and Isaac (1963) found that V. albo-atrum resting mycelium contained in buried lucerne stem pieces survived only seven months. Horner (1963) and Evans et al. (1966) found viable microsclerotia of V. dahliae imbedded in buried host tissue assayed 20 to 24 months after burial.

Horner (1963) compared the field survival of V. dahliae microsclerotia recovered from naturally infected mint stems with microsclerotia of V. dahliae produced in culture. After two years burial in soils the field cultured inoculum showed eight percent survival while only 0.8% of microsclerotia produced in culture survived. Lacy (1964) infested silt loam field soils with fine screened microsclerotia recovered from naturally infected mint stems. The soil plots were kept fallow. Assays conducted after 24 months incubation in the soil showed 35% of V. dahliae propagules survived.

Powelson (1968) observed that a large proportion of V. dahliae propagules produced in culture (commonly designated as

'microsclerotial' by authors) are conidial units rather than microsclerotia. Microscopic observations of the conidia in soils revealed that they were short lived. Observation of microsclerotial inocula extracted from naturally infected plant materials shows these preparations to contain principally discrete microsclerotial units (Evans et al., 1966).

Table I outlines 12 sets of data pertaining to the survival of V. dahliae propagules cultured under a variety of conditions and subsequently buried in soils for different periods of time before recovery and assay. On a comparative survival basis the data indicate that: (1) cellophane cultured microsclerotia survive poorly in soils, (2) laboratory grown inocula cultured on natural organic substrates survive only moderately well in soils, and (3) field cultured, naturally infected, inocula maintain comparatively high survival percentages after prolonged incubation in soils.

Temperature and Moisture Effects and Survival

Nadakavukaren (1960) found that microsclerotial survival was poor in soils held above 25°C and best in soils held at 5 to 15°C with moisture levels at 50 to 75% field capacity. Flooding soils caused a rapid decline in Verticillium population at all temperature levels. This effect he attributed to anaerobic conditions.

High temperature survival has been studied by Wilhelm (1950)

Table 1. Reports concerning the survival of Verticillium dahliae in soil.

Author	Inoculum Source	Container	Period Studied	% survival
Field studies				
Horner (63)	Peppermint stem pieces	Nylon sacks	24.0 mo.	64.5
Horner (63)	Peppermint-microsclerotia	Nylon sacks	24.0 mo.	8.0
Horner (63)	Peppermint, cellophane cult.	Nylon sacks	24.0 mo.	0.4
Lacy (64)	Peppermint-microsclerotia	Buried soil box	24.0 mo.	35.0
Evans, et al. (66)	Cotton stem pieces	Cultivated field soil	20.0 mo.	63.0
Greenhouse studies				
Nadakavukaren (60)	Peppermint, cellophane cult.	1 gal. jars, 7 lbs.	6.0 mo.	1.7
Martinson (64)	Peppermint-microsclerotia	900 g, in jars	2.2 mo.	60.0
Lacy (64)	Peppermint-microsclerotia	1.5 pint jars	2.0 mo.	66.0
Laboratory studies				
Nadakavukaren (60)	Peppermint, cellophane cult.	Plastic box, 300 g	6.0 mo.	1.1
Martinson (64)	Barley straw-microsclerotia	Poly capped flasks	5.9 mo.	10.0
Martinson (64)	Peppermint-microsclerotia	Poly capped flasks	2.5 mo.	12.0
Menzies & Griebel (67)	Potato stem-microsclerotia	Poly capped jars	1.7 mo.	36.0

who found that microsclerotia can withstand drying and a constant temperature of 49°C (120°F) for several months. Dry propagules of V. albo-atrum (MS) resist high temperatures far better than moist propagules (Nelson and Wilhelm, 1958).

Menzies and Griebel (1967) conducted a series of laboratory experiments on the survival of V. dahliae in soils. Inocula were grown on autoclaved potato stems and also recovered from naturally infested potato stems. As had been reported by a number of other workers (Nadakavukaren, 1960; Lacy, 1964; Martinson, 1964), Menzies and Griebel noted an initial flush of V. dahliae growth in the first seven to 17 days after soil infestation with a subsequent decline in populations through 351 days. In soil drying and rewetting experiments they report that drying infested soils for three days apparently killed mycelium and conidia of V. dahliae. On rewetting the soils, a second, but attenuated increase in the population occurred. Longer moist incubation periods rendered the microsclerotia less able to withstand dessication in dry soil. Assays conducted 114 days after infestation and wetting, showed that soils given a 50-day moist incubation period prior to infestation maintained populations seven times greater than did soils air dried and stored for two years prior to infestation and wetting. Soils infested with microsclerotial densities of less than 1,000 propagules/g generally increased in propagule densities through 241 days, while those soils

infested at densities greater than 1,000 propagules/g decreased in propagule densities through this period. Population decreases were generally proportional to initial infestation levels. The authors conclude that prolonged moist incubation of microsclerotia weakens their ability to withstand dessication through soil drying. Drought sensitivity increased to a point where few propagules were able to survive air drying the soil to a moisture level under which freshly added microsclerotia could survive for years.

CO_2 and O_2 Effects on Survival

Nadakavukaren (1960) recorded 79.1 to 100% reductions in the V. dahliae population after five to six months burial in soils. His data suggest that the environmental conditions of his experiment were adverse for the survival of V. dahliae. In the best survival treatment, 25% of moisture holding capacity and 10°C, the V. dahliae population dropped from 23,000 propagules/g soil to 4,623 propagules/g soil within six months (figures adjusted for the author's 100X error in assay calculations). Subsequent studies (Horner, 1963; Martinson, 1964; Lacy, 1965; Menzies and Griebel, 1967) indicate generally higher levels of microsclerotial survival over far greater periods of time. I question Nadakavukaren's usage of screw capped glass containers and taped plastic boxes in these studies. He reports that the soil containers were opened only weekly. This practice

would most certainly place the microflora in these soils under intermittent oxygen stress throughout the duration of the experiment. Alexander (1961) points out that gaseous O_2 disappears from water logged soils within two days following inundation. Wilhelm (1950) and Green (1958) found that the apparent vertical distribution of V. albo-atrum (MS) decreased with increasing depth in the soils. While no mention is made of O_2 limitations, Green reported a sharp decline in infection incidence in peppermint plants grown in subsoils taken from a depth of 12 to 18 inches. These soils were described as "...the lower limits of the partially oxidized topsoil...". Wilhelm believed that the vertical distribution was governed by factors other than root penetration, soil type, or climatic environment. Burges and Fenton (1953) studied a number of fungi (Verticillium not included) and found that tolerance to CO_2, rather than low O_2 tensions, determined the vertical distribution of fungi.

Development of Resting Structures

Wilhelm (1954) placed V. albo-atrum (MS) infected tomato stem pieces on and into moist natural soil and observed the formation of numerous conidial heads after a few days incubation. Subsequently the conidia became anastomatized, melanized and formed aerial microsclerotia. Single conidia were observed which had become swollen, dark and carbonized. Wilhelm considered this process an

asset to survival in soil.

Sewell (1959) used glass walled observation boxes to directly observe the behavior of V. albo-atrum on infected hop stem pieces and living tomato roots in soil. At points where young, extending tomato roots came into contact with the inoculum pieces, dark mycelium formed on the root surface. Development of dark mycelium required at least three weeks of contact and did not spread extensively over the root surface. These resting mycelial loci were visible throughout the test period of 18 weeks. Generally, dark mycelium was not seen on roots of plants which later proved to be extensively infected. Sporulation was observed on roots of recently dead plants. Following sporulation, dark resting mycelium was observed in the cortical tissues in sufficient amounts to render these tissues blackened.

Evens et al.(1966) studied conidial and microsclerotial development in naturally infected cotton plants. Observations of decaying cotton leaves laying on the soil surface showed that copious amounts of Verticillium conidia are produced over the leaf surface and, in time, numerous microsclerotia are produced in the leaf petiole and mesophyll near the leaf base. Further formation of microsclerotia was believed to be prevented by competition from invading saprophytes such as Alternaria and Stemphylium spp. In a damp chamber in the laboratory, some leaves became filled with an estimated

200,000 individual microsclerotia. The authors estimate that the formation of microsclerotia under field conditions is limited by saprophytic competitors to an average of 200 to 400 units/leaf and thus, a single cotton plant may return upward of 250,000 microsclerotia to the soil.

In Vitro Germination of Microsclerotia

Until rather recent years it had not been demonstrated that V. dahliae microsclerotia were capable of germination. Snathhorst (1962) considered the microsclerotial cells incapable of germination and stated that the growth of fragments of hyaline mycelium closely associated with the microsclerotia led to the misconception that these structures were capable of germination. Gordee and Porter (1961) demonstrated that microsclerotia are composed of thick-walled melanized cells and thin-walled hyaline cells. Germination of sonically oscillated microsclerotia revealed that germ tubes developed from only the thin-walled hyaline cells. Isaac and MacGarvie (1962) physically crushed microsclerotia and observed that only the thin-walled hyaline cells germinated. They maintained that a 6 to 20 hour water soaking was necessary to induce germination. In a later paper Isaac and MacGarvie (1966) observed germination of thin-walled lightly pigmented sclerotial cells. Thin-walled, hyaline, branching germ tubes produced conidia after 24 hours growth on

nutrient agar.

Nadakavukaren (1962) presented electron micrographs which supported Gordee and Porter's (1961) observations in the light microscope of thick and thin-walled cells in the microsclerotium. The electron micrographs showed that only the thin-walled cells in the microsclerotia are capable of germinating. Nadakavukaren agrees with the postulation of Gordee and Porter (1961) that the thick-walled cells serve a double role of protecting and supplying food for the thinner-walled cells.

Powelson (1966) found that a limited number of V. dahliae conidia which had been enclosed in sucrose amended silica gel blocks and buried in soil for 30 days either germinated and formed secondary conidia or developed, thickened, lightly pigmented conidial cell walls, or developed into microsclerotia. Emmatty and Green (1967) amended V. albo-atrum (MS) infested soils with a number of mono- and disacharides and observed two types of microsclerotial germination, one by narrow hyphae and the second by formation of chains of chlamydospore-like cells or production of small secondary microsclerotia. They found these secondary microsclerotia to be readily separated from the original propagule and they were viable.

Inoculum Potential Bioassays

A number of workers have utilized bioassays to measure the

inoculum potential of soil-borne Verticillium propagules. Schreiber and Green (1962) infested mineral soils with laboratory grown microsclerotial and mycelial-conidial inocula. Using tomato plants they studied inoculum potential of the soils over an 82-week period. Initial and terminal bioassays yielded 100% V. albo-atrum (MS) infected plants from the microsclerotia infested soil, although intervening bioassays yielded as low as 41% V. albo-atrum (MS) infected plants. Mycelial-conidial infested soils failed to produce infected plants after 36 weeks.

Wilhelm (1955) bioassayed a field soil at nine and 14 years following a single tomato crop. The nine year bioassay yielded 50% V. albo-atrum (MS) infected tomato plants and by the 14th year only 5.5% of the tomato plants yielded Verticillium. Wilhelm considers that this data indicates a considerable reduction in inoculum potential of Verticillium in the ninth through 14th years. Also reported in this paper were tests showing viability of laboratory shelf cultures of Verticillium albo-atrum known to be 13 or more years old.

METHODS AND MATERIALS

Inoculum Source

This investigation was initiated in the fall of 1962 when 16 bales of dried V. dahliae infected potato vines were obtained from a potato field near Redmond, Oregon, prior to harvest. The plants in this field had shown severe Verticillium wilt symptoms. To facilitate development of microsclerotia within the potato stems these vines were spread three inches thick over fallow soil for seven months (December, 1962 to June, 1963) at the Botany and Plant Pathology Experimental Farm at Corvallis, Oregon. During this time a high percentage of the stems became ramified with microsclerotia.

The vines were air dried, macerated using a Wiley Mill (1/8" screen) and screened through a Tyler Standard Sieve Series.

Those stem particles passing the 48 mesh screen and collected on the 200 mesh screen were designated SPMS (stem piece microsclerotia). The material passing the 200 mesh screen were designated MS (microsclerotia). The SPMS material contained microsclerotia embedded in the stem pieces, while the MS material contained individual microsclerotial units.

Collection of Test Soils

Soils were collected from each of three Oregon locations: the

Oregon State University Botany and Plant Pathology Farm, Corvallis (Chehalis silt loam, CSL); The F. Shumway farm, Powell Butte (Ayre sandy loam, ASL); and The C. Hill farm, Klamath Falls (Klamath fine sandy loam, KFSL). All soils were taken from fields which had not been monocropped to potatoes, but were adjacent to fields where Verticillium wilt of potatoes had become a serious problem. The soils were taken from the top six inches of the A horizon and passed through a 5/8" screen to remove stones, clods and large pieces of organic matter. The three soils were tested at the Oregon State University Soil Testing Laboratory for pH, milli equivalents P and K, total nitrogen, and percent organic matter.

Soil Infestation

Each lot of newly collected CSL, ASL and KFSL field soil was air dried for seven days prior to infestation with V. dahliae. Four thousand g lots of the air dried soils were uniformly infested with V. dahliae inoculum at 100, 000 propagules/g soil (oven dry basis). Two 4000 g lots of each soil type were infested, one with MS inoculum, and the other with SPMS inoculum. All infested lots were assayed for initial V. dahliae propagule density as described in a following section.

Twenty g portions of each infested soil type were enclosed in perforated nylon bags sewn at one end with nylon thread and

closed at the other with an aluminum staple (Figure 1B). The bags were fabricated from 16 mm diameter 1.0 mil nylon tubing (Sierra Engr. Co., Sierra Madre, Cal.). Each bag had 12 lines of perforations 1 mm apart produced on a zig-zag sewing machine. Two lengths of bags were produced: 9 cm long for the MS infested soils, and 12 cm long for the less dense SPMS infested soils.

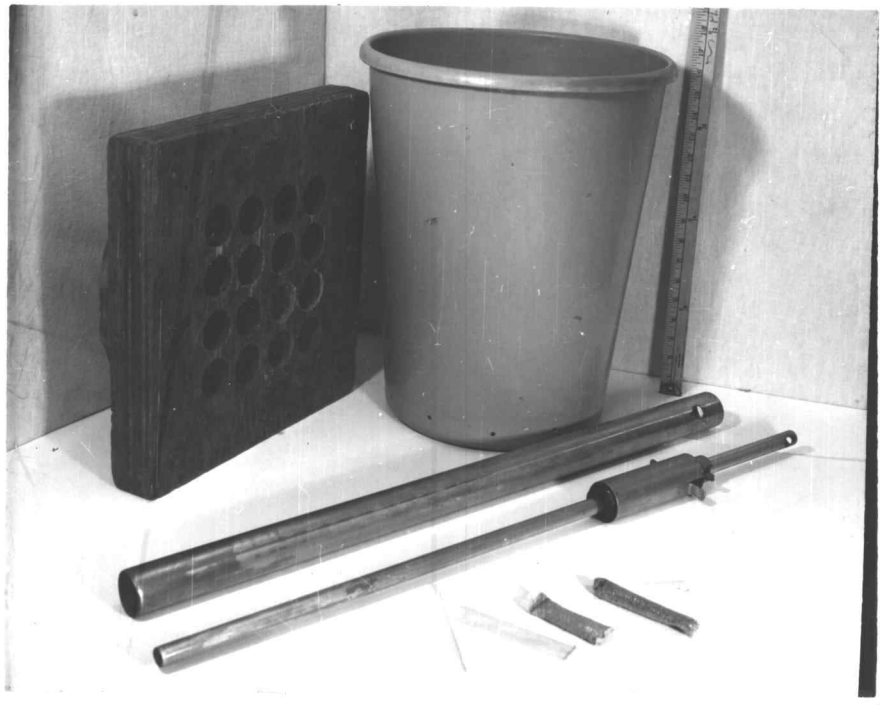
Establishment of Field Plots

Soils were collected at three geographic locations and 56,000 g lots were interchanged between these locations. Small nylon sacks filled with V. dahliae infested soil were buried in each soil type. These sacks were then removed over a five-year period and assayed for V. dahliae to determine levels of survival. Separate lots of each soil type were infested at a low level with V. dahliae and bioassayed after five years with eggplants.

Field plot containers. Soils collected at each of the three locations were placed in plastic waste baskets 34 cm top x 25 cm base x 43 cm high (Figure 1). Each container had been drilled with 10 side (4 cm from bottom) and four bottom drain holes and contained 3" of pea gravel in the bottom. Each container held 56,000 g of soil which, upon settling, came to within two inches of the container lip.

Pea gravel was laid at the bottom of each ground hole so as to

Figure 1. Field plot apparatus, A) plastic soil container with template and sack insertion and extraction tubes, B) perforated nylon sacks used to contain Verticillium infested soil during incubation in field plots.



A

B

enclose the lower five inches of the containers and extended six inches out in three directions from the container bottom (Figure 2). The remainder of the ground hole was refilled with native soil. Two inches of the container extended above ground line. All soil lots were irrigated after field implantation of the containers.

Sack placement in soils. A wooden template was constructed to facilitate implantation of the inoculum sacks in each container of soil (Figures 1 and 2). After alignment of the template over the container, a metal tube (18 cm O.D.) was inserted through an adapter sleeve placed into one of the 16 holes of the template and pressed deeply enough into the soil to facilitate placing each sack six inches below the soil surface (Figure 2). This soil was then removed with the tube and an inoculum sack dropped into the resulting hole and the hole refilled with soil.

Two opposite holes were drilled through the top lip of each plastic container into which corresponding pegs on the template fit. These holes were compass oriented north and south. This arrangement allowed for exact realignment of the template when sampling and insured against loss of orientation if the plastic lip should be destroyed.

Sampling of infested soils in buried sacks. At each sampling date the wooden template was aligned over the soil container. A metal tube was then inserted through a template hole, pressed into

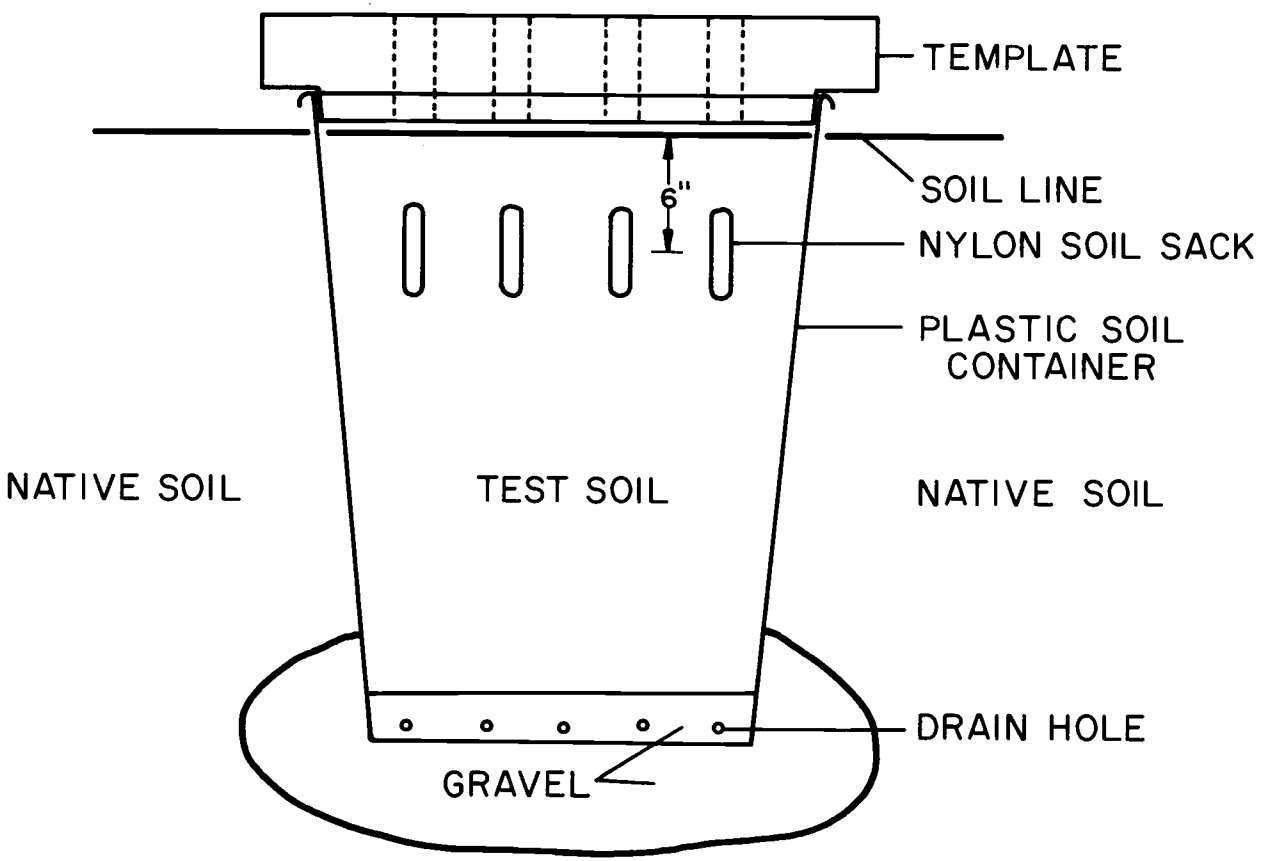


Figure 2. Placement of soil container and nylon soil sacks in field plots.

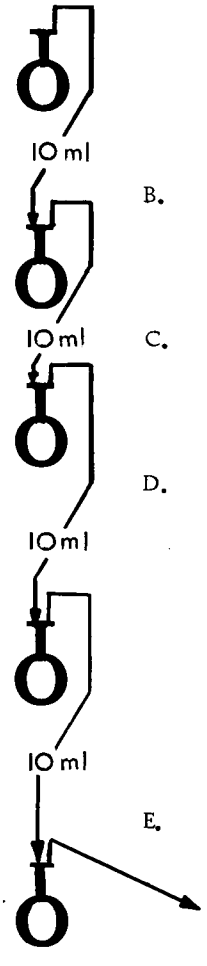
the soil to a depth below the lower edge of a buried soil sack and then removed along with the contained soil and sack (Figures 1 and 2).

Each sack was then tagged, wrapped tightly in aluminum foil and all sacks of the same soil and inoculum type were placed in a plastic sack and stored in a cooler for transport to the laboratory.

Soil dilution assay. At the laboratory each sack was slit lengthwise, the contents removed and uniformly mixed in a sterile petri plate. The standard soil dilution assay was employed to determine V. dahliae propagule densities in the soils (Figure 3).

The dilution procedure is diagrammed in Figure 3. Two subsamples were taken for each assay from each soil, one sample of five g for dilution assay, and one sample of 10 g for oven drying to determine percent of soil moisture. The five g sample was placed in a 500 ml erlenmeyer flask containing 100 ml of distilled water. This suspension was agitated at a setting of 5 (200 oscillations/minute) on a Burell DD wrist-action shaker. After 10 minutes agitation, 100 ml of 1% sodium carboxylmethyl cellulose-water mixture were added to the soil-water mixture as a thickening agent. This viscous mixture suspended the soil more homogeniously in the liquid and delayed settling of small particles. After 10 more minutes of agitation, the flask was removed to a bench. After a 10 second settling period to allow heavy particles to sink (sand, gravel),

I. Soil dilution



		g/soil/ ml	total g soil	water: soil	dilution factor/ 100 ml
A.	1. 5 g soil + 100 ml water	.05	5.0		
	2. 10 min shake[a]				
	3. 100 ml 1% CMC	.025	5.0	40:1	2
	4. 10 min shake				
	5. 10 ml to B		.25		
B.	90 ml water + 10 ml A	.0025	.25	400:1	20
	1. Agitate				
	2. 10 ml to C		.025		
C.	90 ml water + 10 ml B	.00025	.025	4000:1	200
	1. Agitate				
	2. 10 ml to D		.0025		
D.	90 ml water or molten bacti-agar + 10 ml C	.000025	.0025	40,000:1	2,000
	1. Agitate				
	2. If agar, then pour 7-10 petri plates[bc]	.000025	.0025	40,000:1	2,000
	3. If water, then 10 ml to E		.00025		
E.	90 ml bacti-agar + 10 ml D	.0000025	.00025	400,000:1	20,000
	1. Agitate				
	2. Pour 7-10 petri plates[bc]	.0000025	.00025	400,000:1	20,000

II. Soil drying and calculations for propagules/g dry soil.

$$\frac{\text{Total plate count } (D_2 \text{ or } E_2 \text{ above}) \times \text{dilution factor}}{\frac{\text{10 g soil dried 24 hrs at } 100^{\circ}\text{C}}{2}} = \text{propagules/g dry soil}$$

[a] Burrell DD wrist action shaker set at 5 (200 oscillations/min.)

[b] Add 1.6 ml streptomycin (100 ppm) ethanol (7,500 ppm) before adding 10 ml aliquot (mix well).

[c] Incubate plates 20-30 days in dark before reading.

Figure 3. A dilution method for assaying Verticillium dahliae propagules.

a 10 ml aliquot was removed from the center of the mixture with a pipette. The aliquot was then emptied into 90 ml of sterile distilled water, the mixture agitated by hand to evenly disperse the soil particles, and again, a 10 ml aliquot was removed to a second 90 ml of distilled water, agitated and finally, 10 ml were removed and added to 90 ml of molten (42°C) 1% ethanol-streptomycin-agar (Nadakavukaren and Horner, 1959). Just prior to the addition of the last 10 ml aliquot, 1.6 ml of streptomycin sulfate-ethanol mixture was added to the molten 1% bacto-agar (see CMC and ESA preparation). Fifteen ml of the agar-soil mixture were poured to each of seven 90 mm petri plates. The cultures were incubated at room temperature in the dark. After 20-30 days the V. dahliae colonies were readily identified by profuse but confined production of microsclerotia in the agar around the locus of each V. dahliae propagule.

To determine the moisture contained in the original five g assay sample, the 10 g sample was oven dried for 24 hours at 100°C, the sample dry weight was divided by two, and this resultant figure was divided into the product of the sum of the V. dahliae colonies in the seven plates times 2000 (the dilution factor). This final figure represents the propagules of V. dahliae per g oven dry soil (Figure 3, II).

CMC (sodium carboxymethyl cellulose) preparation. The CMC mixture was prepared as follows: 30 ml of distilled water were

added to a 1000 ml blender jar on a Waring Blendor base with the blender set at low speed; 1.0 g of CMC powder was slowly poured into the agitating water, 70 ml of distilled water were then quickly added, the jar capped and the blender speed set on high. The resultant viscous mixture contained myriad air bubbles and had to be set out at room temperature for 24 hours (or autoclaved 20 minutes) to remove the air bubbles.

ESA (ethanol streptomycin agar preparation. The streptomycin sulfate-ethanol-water was prepared as follows: 2.7 g of streptomycin sulfate (Agri-Strep, type A, 37% active, Merk and Co., Mfg. Chemists, Rahway, N. J.) was dispersed in 80 ml of distilled water, then 80 ml of 95% ethanol was added and the mixture agitated. When 1.6 ml of this mixture was added to 100 ml of 1% agar, the resultant medium contained 100 ppm active streptomycin and 7,500 ppm ethanol.

Inoculum Assay

SPMS. SPMS inoculum was processed by soaking 10 g of the material in 250 ml of sterile water for one hour, blending in an Omni-mixer for one minute, then adding 250 ml of 1% CMC and again mixing for one minute. Five ml of this mixture was added to 100 ml of water and 95 ml of 1% CMC and agitated on a wrist-action shaker for 20 minutes. A 10 ml aliquot was then taken through

steps I, A to D, of the soil dilution technique resulting in a 2000: 1 dilution factor (Figure 3).

MS. The MS inoculum was processed by adding 10 g of the material to 250 ml of water plus 250 ml of one percent CMC. This mixture was dispersed by agitation on a wrist-action shaker for five minutes. Five ml of this mixture was diluted by the soil dilution technique, step A, 1, and run through to step E, 2, resulting in a 20, 000: 1 dilution factor (Figure 3).

Field Bioassay Plots

No information was available on the comparative long-term survival of pathogenic *V. dahliae* populations in different Oregon soil types under different environmental conditions after initial infestation with a low level of *V. dahliae* inoculum. Therefore, an experiment was included in the field plots at Corvallis, Redmond, and Klamath Falls where four plastic containers of each soil type (56, 000 g soil) at each location were infested with 1000 propagules/g soil (oven dry basis) of SPMS inoculum.

After five years incubation at each field plot location, the four replicates of each soil type were homogenized. A soil dilution assay was subsequently performed on each of the nine resultant soil samples (three from each location). In the greenhouse, sets of six clay pots (6") were filled with each infested soil type from each field

location and four eggplant seedlings (1.5 inches tall) from a sand culture were transplanted to each pot of soil. Verticillium wilt symptoms were recorded until only one apical leaf remained symptomless.

The Black Beauty variety of eggplant (Solanum melongena L.) selected for the bioassay study is readily propagated in the greenhouse, highly susceptible to Verticillium wilt, and expresses wilt symptoms at a relatively early stage of growth.

Laboratory Survival Studies

An experiment was initiated in the laboratory to determine V. dahliae survival in soils under controlled temperature and moisture conditions. Microsclerotia were incorporated into three soil types. Three inoculum types at three levels of infestation were used. The soils and inoculum materials were identical with those used in the field survival experiments.

The three inoculum types consisted of (1) MS, (2) SPMS, and (3) SF, a combination of sufficient propylene oxide sterilized SPMS material to equal that quantity used in the SPMS inoculum, plus equivalent amounts of MS material to equal those V. dahliae levels used with MS infestation.

All soils were brought to 50% moisture holding capacity immediately following infestation. Three hundred g lots of each

infested soil were firmly packed in 16 oz. plastic cups, covered over the top with clear polyethylene sheets (Dobecknum, 2 mil) which were each secured with a heavy rubber band. The soils were incubated in dark laboratory cabinets at room temperature of 72°F.

Certain of the soil dilution assays of SPMS infested soils were preceded by mixing the soil samples (indicated in results section) with an Omni-mixer. The procedure was as follows: A five g soil sample was macerated in a 250 ml Omni-mixer tube containing 100 ml distilled water. A two-minute mixing thoroughly fragmented the stem piece residues in the soil. The resulting 100 ml mixture was poured into a 500 ml erlenmeyer flask and constituted part I, A, of the soil dilution technique (Figure 3).

RESULTS

Survival of V. dahliae in the Field

A number of reports have indicated that microsclerotia of V. dahliae are capable of extended survival in field soils (Wilhelm, 1949, 1955; Horner, 1963; Lacy, 1965; Evans, et al., 1966). However, no data are available concerning the comparative survival of V. dahliae in different soil types under varying climates over an extended period of time.

Soils. The three soils selected for this study came from areas which had prior histories of Verticillium wilt in potato crops. An analysis of the soils was conducted at the Oregon State University Soil Testing Laboratory (Table 2).

Table 2. Analysis of three Oregon soil types.

	P me/100g	K me/100g	Total N%	OM %	pH	100% moisture holding capacity ml/100g
Chehalis silt loam	36.50	1.40	.171	4.32	6.0	32.72
Ayre sandy loam	17.75	1.08	.092	2.67	6.2	31.11
Klamath fine sandy loam	28.25	1.49	.080	1.71	6.6	24.24

Chehalis silt loam is a river bottom soil from the Willamette Valley, Oregon. Ayre sandy loam is a light pumice volcanic soil from Central Oregon. Klamath fine sandy loam is a basaltic mineral soil from the foothills of the Klamath Basin.

Plot locations and climate. The choice of field plot locations was based upon diversity of soil types and climates. Rainfall and temperature data for the three areas are given in Table 3.

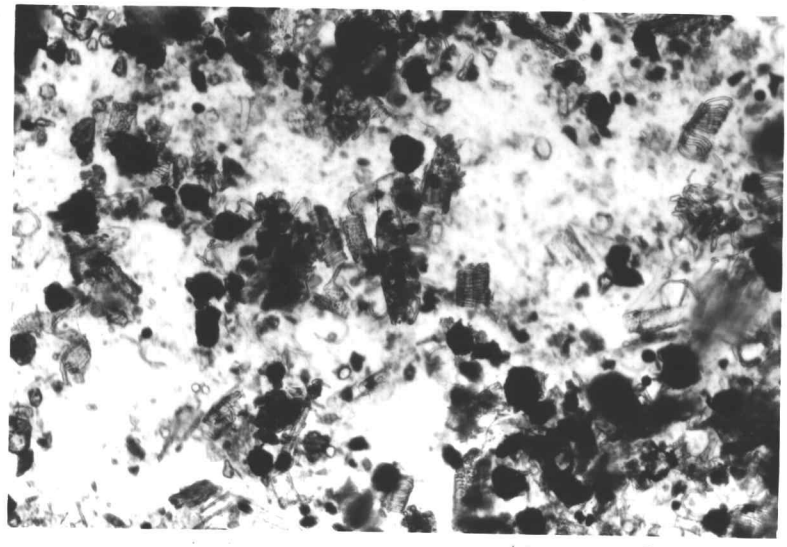
Table 3. Climates of three Oregon geographic areas.

	Ann. Precipt. (inches)	Contingent frost-free days/yr.
Willamette Valley	40	200
Central Oregon	8	12
Klamath Basin	12	100

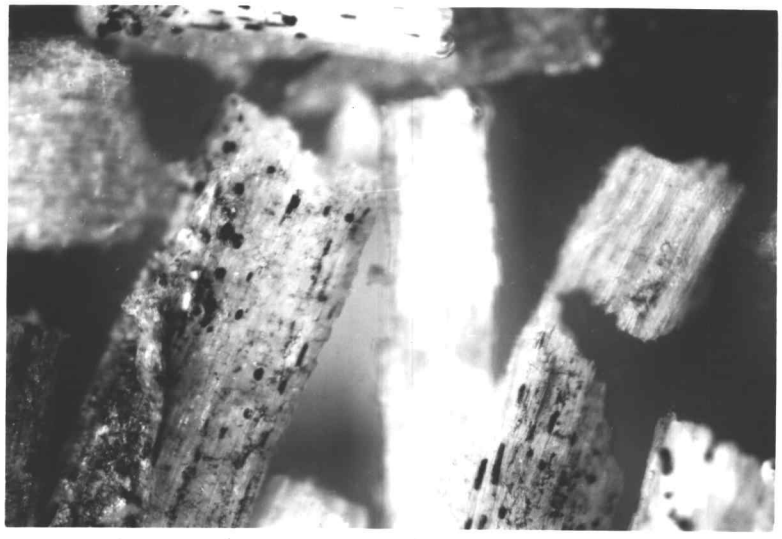
The precipitation patterns are similar for the three locations with 72% of the rain or snowfall occurring from October through April.

Source of V. dahliae inoculum. These experiments utilized inoculum of two types: (1) microsclerotial (MS), consisting of individual microsclerotia separated from infested potato stem tissue by milling and screening as previously described, and (2) stem piece microsclerotia (SPMS), consisting of microsclerotia imbedded in small potato stem pieces (.074-.295 mm) (Figure 4).

Figure 4. A) Microslerotia of Verticillium dahliae harvested from stems of infected potato plants (x100). B) Microslerotia within infected potato stem pieces (x40).



A



B

Dilution assays of the MS inoculum showed it to contain 65.4×10^4 V. dahliae propagules/g. Dilution assays of macerated SPMS inoculum showed it to contain 13.2×10^4 V. dahliae propagules/g.

Microsclerotial (MS) Inoculum

Population development. Substantial microsclerotial germination and sporulation occurs after fresh inoculum is added to soils. A soil dilution assay performed 22 days after infestation showed that an initially heavy flush of V. dahliae sporulation had occurred in the three soils (Table 4). While the initial level of infestation was calculated to be about 100,000 propagules/g soil, population levels at 22 days had increased to 192,000 propagules/g soil in the CSL soils, 188,000 propagules/g soil in the ASL soils and 145,000 propagules/g soil in the KFSL soils. Similar population increases following soil infestation with fresh V. dahliae inoculum have been reported by Tolmsoff (1957), Nadakavukaren (1960), Martinson (1964), Lacy (1965), Menzies and Griebel (1967), and Powelson (1968).

The V. dahliae population in CSL soils at all locations reached a low after 256 days incubation (Table 4). V. dahliae populations in ASL and KFSL soils at the Corvallis location followed a similar pattern to the populations in the CSL soils, reaching a low after 256 days. The populations in the ASL and KFSL soils showed strikingly

Table 4. The influence of soil type and climatic location on the development of soil-borne Verticillium dahliae populations through 256 days.

Location	Soil type[a]	Propagule densities after various days incubation[b]			
		22[c]	71	144	256
Corvallis	CSL	192[d]	78	74	31
	ASL	188	88	58	45
	KFSL	114	36	31	18
Redmond	CSL	192	88	55	14
	ASL	188	16	19	17
	KFSL	114	16	12	16
Klamath Falls	CSL	192	73	53	25
	ASL	188	26	25	24
	KFSL	114	27	25	23

[a]CSL = Chehalis silt loam; ASL = Ayre sandy loam; KFSL = Klamath fine sandy loam.

[b]Initial V. dahliae density was 100,000 microsclerotia/g soil.

[c]Samples assayed at 22 days had not been buried in the field plots. All samples at 71, 144 and 256 days were extracted from the indicated field plots.

[d]Propagules/g soil in thousands.

different survival patterns at the Redmond and Klamath Falls locations. At both of the above locations the V. dahliae populations in these soils reached population lows after only 71 days.

Population fluctuations. Under the influence of the Corvallis environment, after an initial flush of V. dahliae growth and a subsequent decline, all three soils showed wide fluctuations in the V. dahliae population through the five years of this study (Figure 5). The soil environmental influence of CSL soils was evident at both the Redmond and Klamath Falls locations (Figures 5 and 6) where CSL soil type took precedence in its effects on V. dahliae populations over the climatic influences of these locations. While the CSL soils generally maintained high populations, the ASL and KFSL soils showed sharp population declines with subsequent minor fluctuations through five years under the influence of the Redmond and Klamath Falls climates.

CSL soils, independently of their climatic location, influenced the level and fluctuations of V. dahliae populations, whereas V. dahliae populations contained in ASL and KFSL soils were greatly influenced by the climatic location.

Population increases. In all soils at all locations the V. dahliae population trends after the first year were toward increasing numbers (Figures 5, 6, Table 5). Population increases in all soils at all locations after five years incubation tended to be inversely

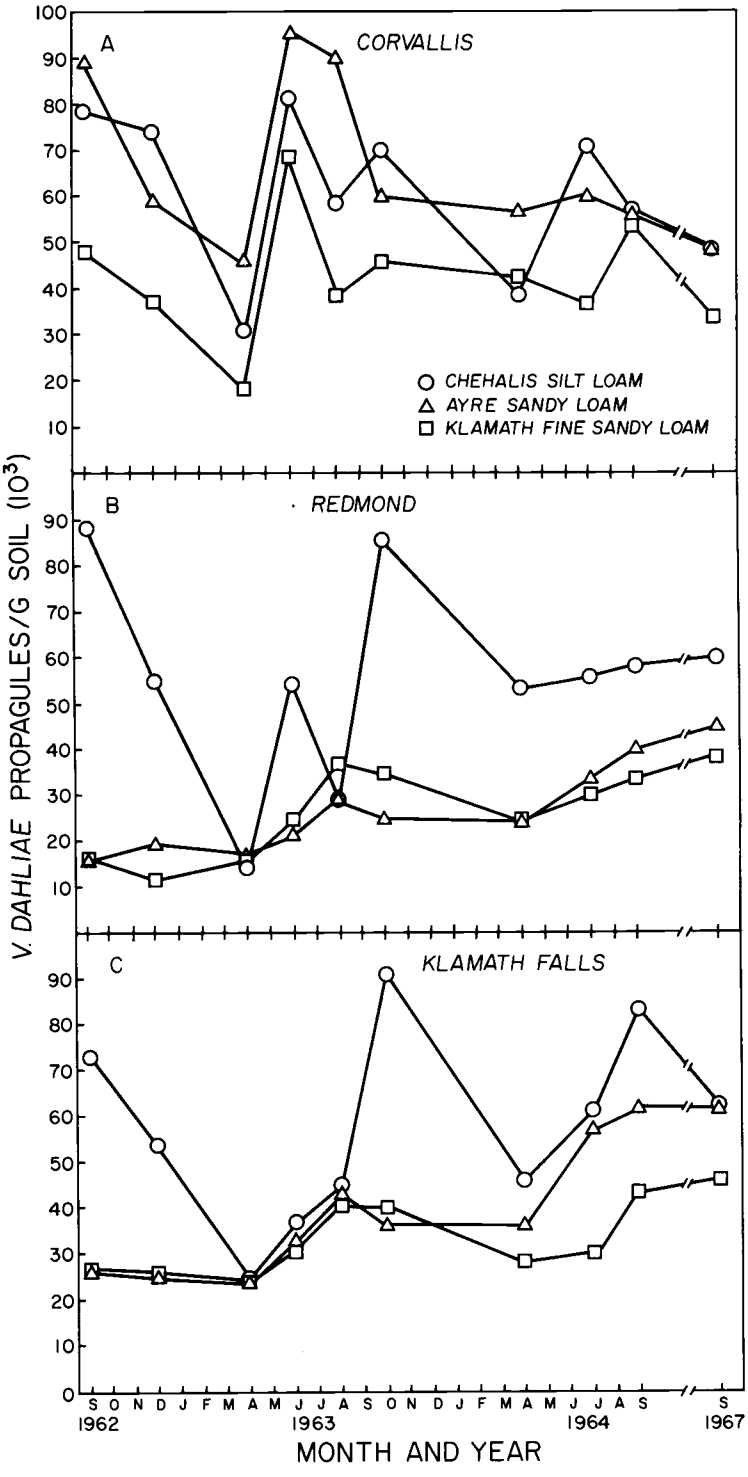


Figure 5. The influence of climatic location on the development of soil-borne Verticillium dahliae populations through five years (microsclerotial inoculum).

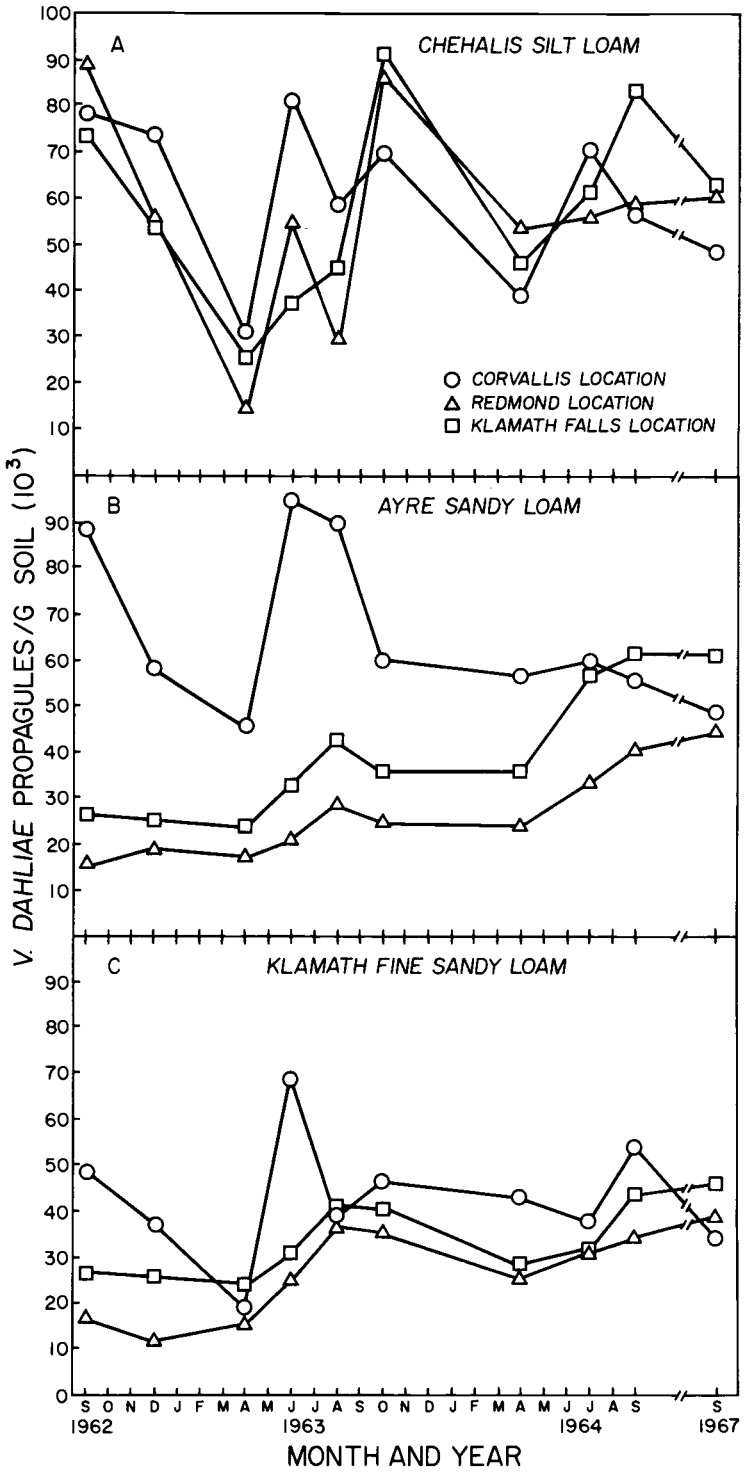


Figure 6. The influence of soil type on the development of soil-borne Verticillium dahliae populations through five years (microsclerotial inoculum).

Table 5. The influence of soil type and climatic location on Verticillium dahliae populations after various periods of incubation.

Location	Soil type	Period of incubation			% increase
		71 d.	256 d.	5 yrs.	
Corvallis	CSL[a]		31[b]	49	58
	ASL		45	48	7
	KFSL		18	34	89
Redmond	CSL		14	60	329
	ASL	17		45	165
	KFSL	16		38	138
Klamath Falls	CSL		25	63	152
	ASL	24		62	158
	KFSL	23		46	100

[a] CSL = Chehalis silt loam; ASL = Ayre sandy loam; KFSL = Klamath fine sandy loam.

[b] Thousands of V. dahliae propagules/g soil (oven dry basis) microsclerotial inoculum (MS).

proportional to initial population lows at either 71 or 256 days. The V. dahliae population in the ASL soil at the Corvallis location showed the least comparative population drop at the 256 day low and after five years incubation had increased only 7%, while the V. dahliae population in the CSL soil at the Redmond location showed the greatest population drop at its 256 day low and after five years incubation had increased 329%.

After five years incubation under field conditions, V. dahliae populations were highest in CSL soils at all locations and highest in all soils at the Klamath Falls location (Figures 5, 6, and Table 5).

SPMS Inoculum Survival

In the standard soil dilution procedure used in these assays, the stem pieces floated to the liquid's surface during the 10 second settling period before the first 10 ml sample aliquot was removed for dilution. Therefore, the V. dahliae propagule counts of the SPMS soil dilution assay measured, almost exclusively, those propagules which were freed into the soil from the stem pieces during the dilution procedure, and propagules which had been freed into the soil by natural means prior to assay by either decay of the stem pieces or the formation of new propagules on the surface of the stem pieces.

Unlike the wide population fluctuations recorded for MS

inoculum under the influence of CSL soils and Corvallis environmental conditions, the V. dahliae variations in SPMS inoculated soils were relatively minor (Figure 7a). Notable is the fact that only the inoculum under ASL and KFSL soils showed more than minor population variations. SPMS inoculum under the influence of CSL soil showed only minor V. dahliae population variations and generally maintained the lowest population levels at all locations through the five years test. V. dahliae populations in ASL soil maintained the highest level.

From population lows at 77 days, all populations tended to increase slightly over the five year test period (Figure 7).

Stem piece disintegration. The extent of stem piece breakdown and numbers of stem pieces retaining V. dahliae after 20 months burial was determined (Tables 6 and 7). Stem piece inoculum was washed from soil using a 48 mesh screen and filtered out on a #1 Whatman filter paper. The recovered material was air dried for 24 hours, weighed, and 0.1 g portions added to molten (42^{o}) ESA for V. dahliae assay.

Stem piece inoculum buried in CSL soils had the least amount of organic breakdown at all three locations (Table 6). The original weight of inoculum added to the soils was 1.515 g/20 g soil. The weight of stem particles recovered from CSL soils at the Corvallis location was 1.344 g/20 g soil, a 93% recovery, while the weight of

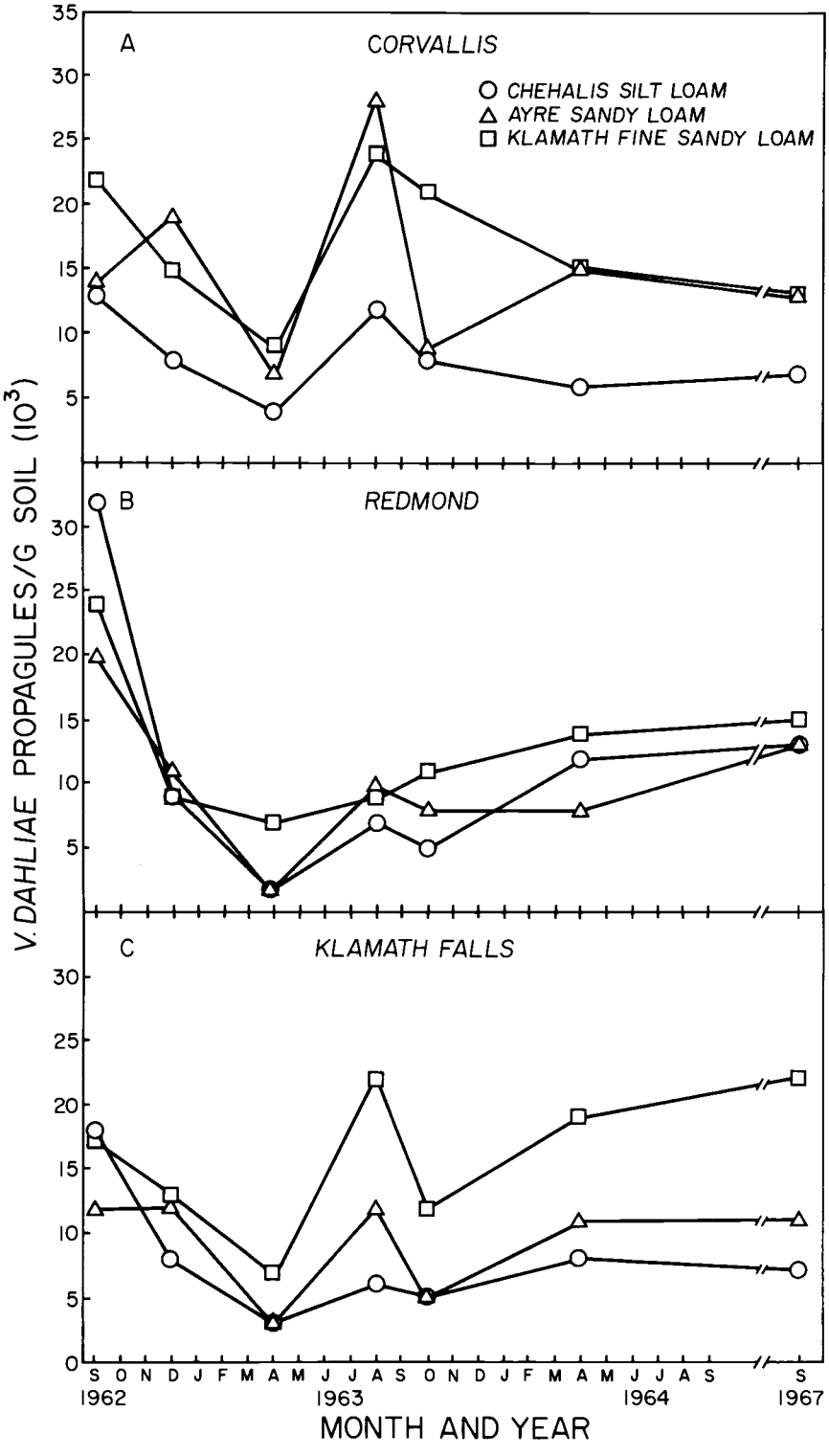


Figure 7. The influence of soil type and climatic location on the development of soil-borne Verticillium dahliae populations through five years (stem piece inoculum).

Table 6. The influence of soil type and climatic location on the decomposition of Verticillium dahliae infected potato stem pieces after 20 months incubation.

Soil type	Location					
	Corvallis		Redmond		Klamath Falls	
	g. recovered	% of initial[a]	g. recovered	% of initial	g. recovered	% of initial
CSL[b]	1.344[c]	93	.436	30	.568	39
ASL	.260	18	.232	16	.388	27
KFSL	.208	14	.168	12	.300	21

[a]Initial additions to soils 1.515 g SPMS/20 grams soil.

[b]CSL = Chehalis silt loam; ASL = Ayre sandy loam; KFSL = Klamath fine sandy loam.

[c]Each figure is the mean value of four replicates.

Table 7. The influence of soil type and climatic location on the numbers of Verticillium dahliae infected potato stem pieces and independent V. dahliae propagules/g soil recovered after 20 months incubation.[a]

Soil type	Location								
	Corvallis			Redmond			Klamath Falls		
	nos/g soil		Ratio	nos/g soil		Ratio	nos/g soil		Ratio
	Infested stem pieces	V. dahliae		Infested stem pieces	V. dahliae		Infested stem pieces	V. dahliae	
CSL	538	6,136	11	412	9,909	21	443	7,727	17
ASL	171	15,014	88	138	7,562	55	233	11,175	33
KSL	104	14,570	140	96	14,209	148	230	18,711	81

[a]Initial level of infestation was 100,000 microsclerotia/g soil.

stem particles recovered from the KFSL soil at the Redmond location was only .168 g/20 g of soil, a 12% recovery.

The order of stem piece breakdown was, as to soils, KFSL > ASL > CSL (Table 6). This order of decomposition and subsequent release of V. dahliae propagules into the soils is reflected in Figure 7 and Tables 7 and 8 where it may be noted that the ratios of V. dahliae infested stem pieces to independent V. dahliae propagules/g soil and the numbers of independent V. dahliae propagules/g soil both follow the order KFSL > ASL > CSL.

Although the weights and numbers of stem pieces recovered were diverse, the percentages of V. dahliae infested stem pieces recovered from all soils at all locations were similar (Table 8).

Bioassay

At the termination of the field survival experiment in September of 1967, soil samples were removed from each of the four bioassay replicates for each soil type at each field location. The soils of the four sub-samples were thoroughly homogenized in a cement mixer. Black Beauty eggplant seedlings (14 days old) were planted (4 each) in 500 g lots of each infested soil type from each field location. Development of wilt symptoms were noted through a 50 day growing period in the greenhouse (Table 9).

Those soils incubated at the Klamath Falls location produced

Table 8. Survival of Verticillium dahliae inoculum within infected stem pieces.[a]

Soil type	Stem pieces/g soil: Total			V. dahliae infected			Percent V. dahliae infected stem pieces		
	Corvallis	Redmond	Klamath Falls	Corvallis	Redmond	Klamath Falls	Corvallis	Redmond	Klamath Falls
CSL[b]	1,902	963	1,388	538	412	443	28	43	32
ASL	560	545	665	171	138	233	31	25	35
KFSL	440	362	639	104	96	230	24	26	36

[a]Stem pieces recovered from soils after 20 months incubation at each location.

[b]CSL = Chehalis silt loam; ASL = Ayre sandy loam; KFSL = Klamath fine sandy loam.

the greatest number of V. dahliae wilted eggplants, and KFSL soils produced the greatest number of wilted plants at all locations.

Table 9. The influence of soil type and climatic location on the inoculum potential of Verticillium dahliae populations as assayed with eggplants.[a]

Soil type	Plants showing Verticillium wilt symptoms[b]		
	Location		
	Corvallis %	Redmond %	Klamath Falls %
Chehalis silt loam	54	20	58
Ayre sandy loam	58	50	70
Klamath fine sandy loam	100	70	100

[a]Soils were originally infested at 1000 propagules/g soil five years prior to bioassay.

[b]Plants were greenhouse grown in the soils for 50 days.

Survival of V. dahliae in Soil in the Laboratory

Five soil dilution assays to determine populations of V. dahliae propagules were conducted over a 290 day period.

MS inoculum. Five days after soil infestation, the V. dahliae populations had increased in all soils, and population increases were in proportion to the initial level of infestation (Figure 8).

After 70 days incubation all V. dahliae populations in CSL and ASL soils continued to show increases, except those initially infested

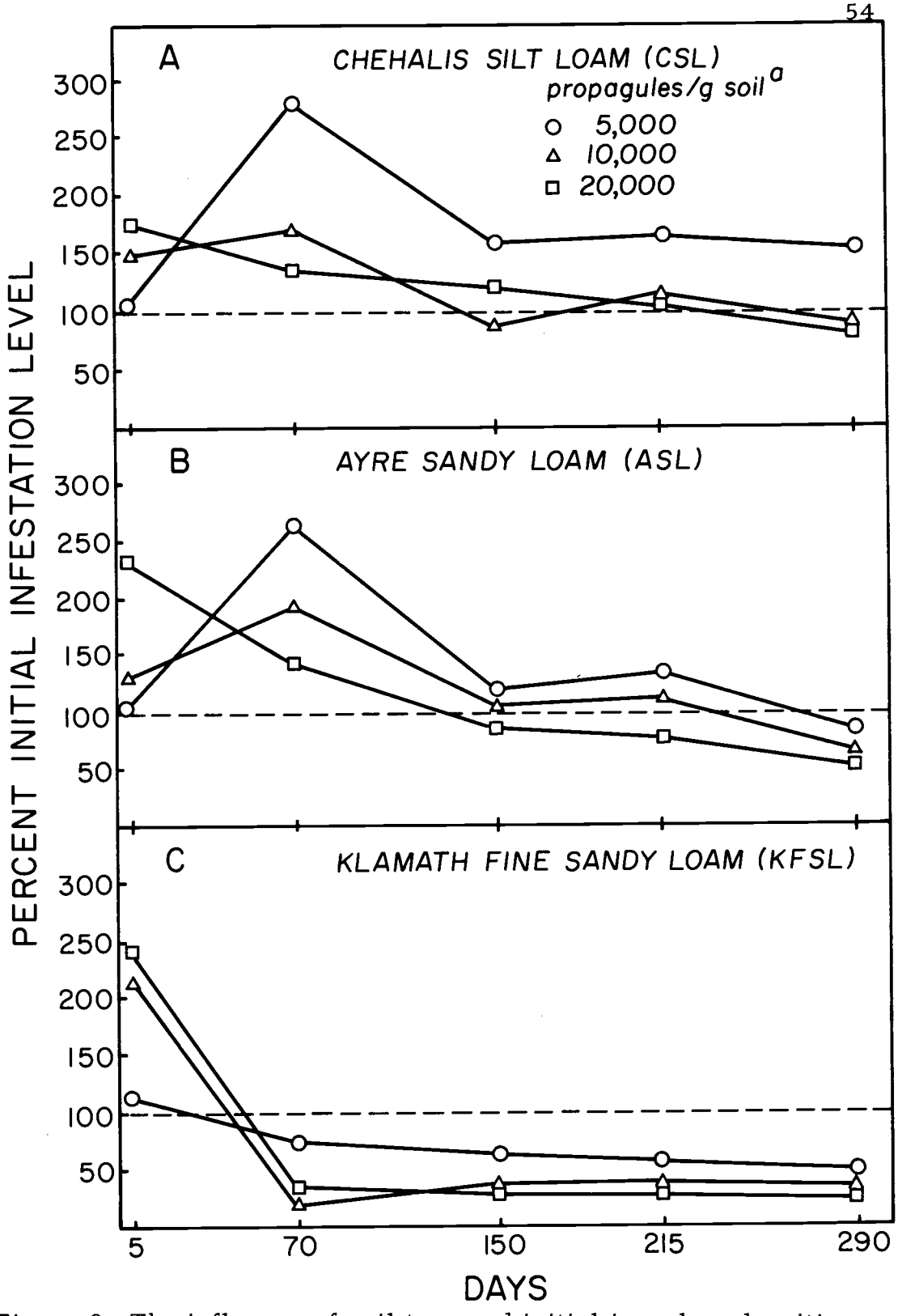


Figure 8. The influence of soil type and initial inoculum densities on the development of Verticillium dahliae populations through 290 days incubation in the laboratory.

at the 5,000 propagules/g soil, which had begun population declines. From 70 days to 290 days all the V. dahliae populations in CSL and ASL soils showed gradual population decreases to below the initial infestation levels, except in the CSL soil initially infested at 5,000 propagules/g soil which maintained a V. dahliae population well above the initial infestation level.

All V. dahliae populations in the KFSL soils showed population declines to below the initial infestation level after 70 days incubation, thereafter these populations maintained a relatively constant level through the 290 day incubation period.

The percent V. dahliae survival after 290 days was inversely proportional to the initial infestation levels in the three soils, while percent survival between soil types was in the order of CSL > ASL > KFSL (Table 10).

Table 10. The influence of soil type, level of soil infestation and inoculum type on the survival of Verticillium dahliae populations in soils after 290 days incubation.

Soil type	Percent survival					
	5,000[a]		10,000		20,000	
	MS[b]	SPMS[c]	MS	SPMS	MS	SPMS
Chehalis silt loam	152	52	86	35	82	37
Ayre sandy loam	84	48	72	37	61	35
Klamath fine sandy loam	50	53	42	40	32	34

[a]Initial infestation level (propagules/g soil).

[b]Inoculum types: MS = microsclerotia; SPMS = stem piece microsclerotia.

[c]Assayed after two minutes omnimixing.

SPMS inoculum. After 290 days incubation the V. dahliae survival levels were inversely proportional to the initial levels of infestation (Table 10 and Figure 8). Menzies and Griebel (1967) found the same inverse order of survival with V. dahliae infestation levels from 700 to 65,000 propagules/g soil after 351 days incubation. No differences in V. dahliae survival were detectable between SPMS infested soil types at any level of infestation.

A comparison of the percent survival of V. dahliae propagules in MS and SPMS infested soils (Table 10) reveals that, while the MS V. dahliae survival was in the order of CSL > ASL > KFSL at all levels of infestation, the SPMS V. dahliae survival generally showed no difference between soil types. The low order of differences in percent survival of V. dahliae in SPMS infested soils as compared to the wide differences in percent survival in MS infested soils may indicate the differential environment afforded the independent microsclerotia as compared to the environment of the microsclerotia enclosed in the stem piece material.

SF inoculum. V. dahliae population counts for both the MS and SF infested soils were nearly identical throughout the assay series. The increased organic matter added with the SF inoculum did not modify the population trends observed when MS inoculum was added alone.

Bioassay of Inoculum Potentials

Bioassay studies by a number of workers have shown that V. dahliae populations in certain soils maintain high inoculum potentials over extended periods of time while in other soils V. dahliae inoculum potentials diminish or disappear in a relatively short period of time (Wilhelm, 1950; Green, 1960; Schreiber and Green, 1962; Lacy, 1965). Information was lacking concerning comparative inoculum potentials of V. dahliae populations in the three Oregon soils used in these experiments. Therefore, an experiment was performed to determine the inoculum potentials of V. dahliae in three Oregon soil types infested with different types of V. dahliae inoculum at three levels of infestation.

After 71 days incubation of V. dahliae infested soils in the laboratory, the four 300 g replicates of each infested soil type were each placed in six inch clay pots and each soil planted with two 14-day-old eggplant seedlings. First wilt symptoms appeared 19 days after planting. Symptom development was recorded through a 23 day period at which time all plants had developed symptoms in at least one leaf per plant. Wilt consistently followed advanced chlorosis by one day, but chlorosis did not necessarily precede wilt. In no case did an entire plant wilt and die, but rather the wilt and death of one lateral leaf was accompanied by the development of a new terminal leaf.

Statistical Analyses of Symptom Development

Factorial analysis. A factorial analysis (Little, 1963) was used to evaluate symptom development (Table 11). Significant differences were found between soil types and infestation levels and no significant differences were found between inoculum types as these factors affected Verticillium wilt symptom development in eggplants.

Table 11. A factorial analysis of the influence of soil type, Verticillium dahliae inoculum type and soil infestation level on the development of Verticillium wilt symptoms in eggplant.

Source of variation	Observed F	Required F 1% level	Significance 1% level
Soil types (S)	23.42	4.66	+
V. dahliae inoculum types (V)	1.78	4.66	-
Infestation levels (L)	64.99	4.66	+
SXV	29.21	3.36	+
SXL	11.83	3.36	+
LXV	9.06	3.36	+
S x L x V	12.00	2.55	+

Linear regression analysis[1]. A factorial analysis is useful in elucidating significant differences between treatments in general but does not indicate specific differences and which treatments are significantly different. Therefore, in order to clarify the differences between individual treatments as indicated by Verticillium wilt symptoms, a linear regression analysis was applied to the symptom data.

The data presented graphically in Figure 9 are interpreted as follows: (1) For each unit increase in time (X axis), there is a unit increase (R) in plants with wilt symptoms (Y axis); (2) The correlation coefficient 'b' measures the closeness of the observations to the mean of the observations. A calculated 'b' value of .978 indicates that 97.8% of the observations are within one standard deviation of the mean of observations; (3) The stippled areas surrounding the regression lines (L values) indicate that area in which the data are significantly alike [at $t_{.05}$ (Snedecor, 1948)], any overlap of these areas indicates that observations of both overlapping areas must be

---

[1] In the log conversion method of Van der Plank (1963) those symptom readings taken in the initial and terminal lag phases tend to be too highly weighted and therefore only the readings taken during the logarithmic phase of symptom development are utilized in the analysis.

Calculations for the regression coefficient (slope) R and the correlation coefficient b followed the method of Van der Plank (1963), while the standard deviation r, fiducial limits L and variance s_b were computed following Snedecor's (1948) methods.

Figure 9. A linear regression analysis of the influence of soil type, Verticillium dahliae inoculum type and infestation level on the development of Verticillium wilt in eggplant.

a. Soil types: CSL, Chehalis silt loam; ASL, Ayre sand loam; KFSL, Klamath fine sandy loam.

b. Inoculum types: MS, microsclerotia; SPMS, potato stem pieces with imbedded microsclerotia; SF, a combination of MS and sterile SPMS.

c. Regression coefficient (Slope) R =

$$\frac{\Sigma xy - \frac{(\Sigma x \Sigma y)}{n}}{\Sigma(x^2) - \frac{(\Sigma x)^2}{n}}$$

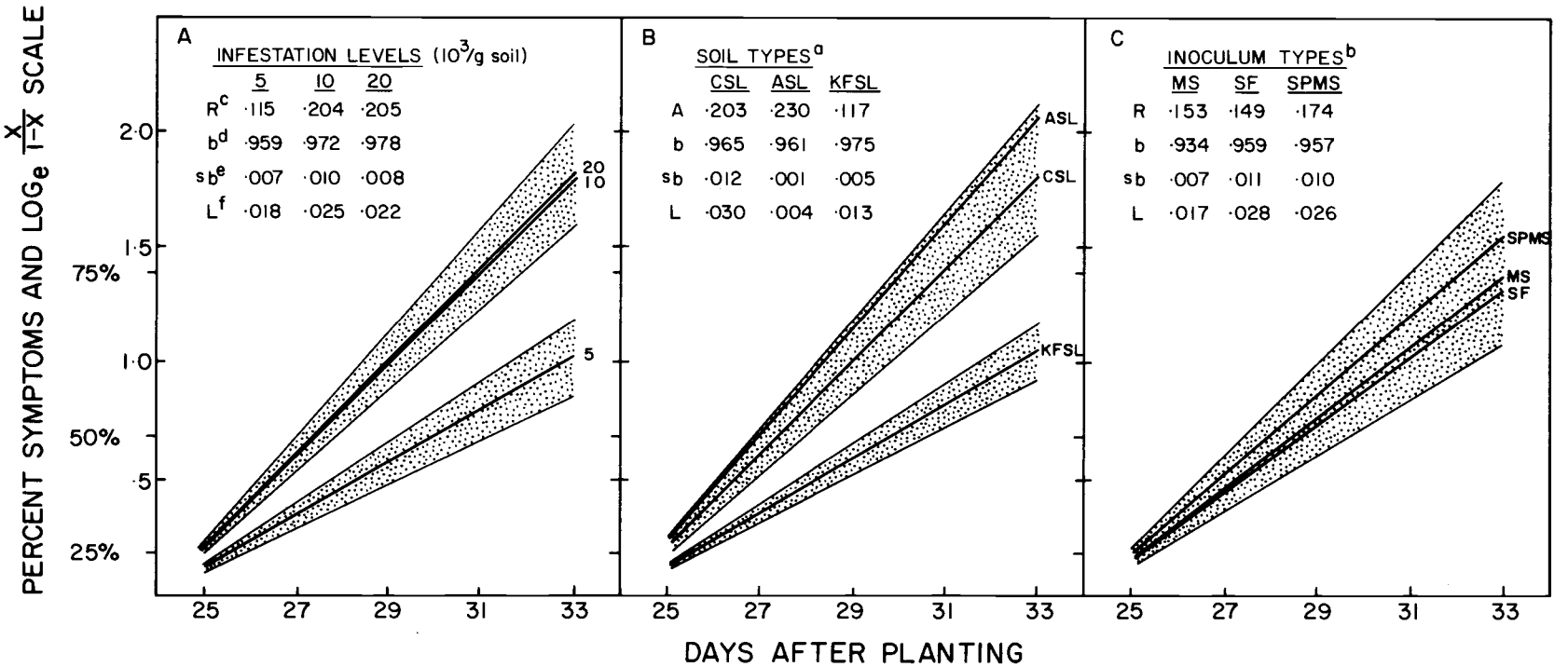
d. Correlation coefficient b =

$$\frac{\Sigma xy - \frac{\Sigma x \Sigma y}{n}}{\sqrt{\left(\Sigma(x^2) - \frac{(\Sigma x)^2}{n}\right) - \left(\Sigma(y^2) - \frac{(\Sigma y)^2}{n}\right)}}$$

e. Variance s_b =

$$\sqrt{\frac{[\Sigma(x^2)\,\Sigma(y^2)] - (\Sigma xy)^2}{(n-2)(\Sigma x^2)^2}}$$

f. Fiducial limits $L = b \pm (t_{.05}\, s_b)$


A
INFESTATION LEVELS (10³/g soil)
5 10 20
R[c] ·115 ·204 ·205
b[d] ·959 ·972 ·978
sb[e] ·007 ·010 ·008
L[f] ·018 ·025 ·022
20
10
5
B
SOIL TYPES[a]
CSL ASL KFSL
A ·203 ·230 ·117
b ·965 ·961 ·975
sb ·012 ·001 ·005
L ·030 ·004 ·013
ASL
CSL
KFSL
C
INOCULUM TYPES[b]
MS SF SPMS
R ·153 ·149 ·174
b ·934 ·959 ·957
sb ·007 ·011 ·010
L ·017 ·028 ·026
SPMS
MS
SF
PERCENT SYMPTOMS AND LOGe X/(1-X) SCALE
2·0
1·5
1·0
·5
75%
50%
25%
25 27 29 31 33
DAYS AFTER PLANTING

considered alike. Where two or three regression lines are enclosed in a single stippled area, only the plus or minus limits of the most extreme L values are shown.

The two highest levels of V. dahliae soil infestation caused nearly identical rates of symptom development in eggplants (Figure 9). Initial infestation levels of 5,000 propagules/g soil resulted in a significantly lower symptom development rate. Although not indicated in Figure 9, the two higher infestation levels produced initial eggplant symptoms four days earlier than did the lowest infestation level. This may indicate that somewhere between initial rates of soil infestation of 5,000 propagules/g soil and 10,000 propagules/g soil a threshold was reached beyond which increased inoculum densities did not produce an increased rate of symptom development or disease severity.

The rates of symptom development of eggplants grown in infested ASL and CSL soils were statistically identical. The rate of symptom development of plants grown in the KFSL soils was significantly slower (Figure 9b).

No significant differences were shown between inoculum types as to the rate of symptom development (Figure 9c).

Root Assay for V. dahliae Infection Loci

After 41 days growth in the infested soils the plants were

removed from the pots and the soil washed from the roots. After washing and blotting, each plant's fresh weight was recorded (Table 12).

Each root system was excised, surface sterilized in 10% Clorox (sodium hypochlorite, 5.25%) for one minute, blotted, cut into 30 mm lengths and 0.1 g lots of the roots were covered with 25 ml molten ESA (41°C) in a sufficient number of petri plates to accommodate the entire root system. V. dahliae colonies arising from infection loci were recorded 27 days later (Table 12).

V. dahliae was isolated from the crowns and roots of all eggplants grown in the infested soils. Fresh plant weights increased with increased levels of inoculum; but the increases were not , in all cases, in excess of the weights of plants grown in the non-infested control soils (Table 12).

Fresh plant weights similar to those recorded for eggplants grown in V. dahliae infested CSL soils were recorded for plants grown in ASL soils (Table 12). Eggplants grown under all inoculum types and infestation levels in KFSL soils yielded fresh plant weights exceeding the control plants. Soil nitrogen levels and amounts of potential supplemental nitrogen added to the soils in the form of inoculum may be reflected in the fresh plant weight yields (Table 12).

The three soil types utilized in these experiments contained different levels of total nitrogen (Table 13). According to Bollen

Table 12. The influence of soil type, inoculum type and soil infestation level on wilt of eggplants grown in Verticillium dahliae infested soils for 41 days.

V. dahliae inoculum		Fresh plant weights (g)[a]			Infection loci/g roots			V. dahliae levels in soils at planting[b] (10^3/g soil)		
Types	Initial levels (10^3)	CSL[c]	ASL	KFSL	CSL	ASL	KFSL	CSL	ASL	KFSL
MS	5	3.6	5.3	5.9	30.4	31.7	13.7	11.8	12.9	3.5
	10	4.5	6.3	6.1	35.7	33.3	24.5	15.3	19.5	6.9
	20	5.3	8.1	6.7	38.5	31.2	27.3	27.1	29.8	8.1
SF	5	9.6	7.6	9.4	30.0	27.9	12.2	10.2	8.2	4.4
	10	10.0	8.0	12.3	30.2	30.7	19.0	15.1	17.3	6.9
	20	12.6	13.8	14.1	30.1	31.9	22.4	28.0	29.7	10.2
SPMS	5	10.8	8.8	6.9	15.6	14.8	25.4	-	-	-
	10	12.2	10.2	9.4	17.6	22.8	30.0	-	-	-
	20	12.5	10.3	10.1	22.5	28.5	35.3	-	-	-
Control		11.9	7.9	4.72	0	0	0	0	0	0

[a] The sum of 8 plants/figure.

[b] V. dahliae levels after 71 days incubation.

[c] Soil types: CSL = Chehalis silt loam, ASL = Ayre sandy loam, KFSL = Klamath fine sandy loam.

(1968) the order of nitrogen availability in these soils would normally be CSL > ASL > KFSL.

Table 13. Nitrogen levels in soils and in inoculum added to soils.

	Total N %	PPM N added to soils at initial rates of infestation		
		5[a]	10	20
Inoculum type				
MS	1.84	14	28	56
SF	1.49	57	114	228
SPMS	1.14	43	86	173
Soil type				
CSL	0.171	-	-	-
ASL	0.092	-	-	-
KFSL	0.080	-	-	-

[a]Propagules V. dahliae/g soil in thousands.

The soil nitrogen levels would be considered, so far as plant growth was concerned, adequate in CSL soils, limiting in ASL soils and deficient in KFSL soils (Table 2). The levels of total nitrogen added to soils in the form of inoculum was of the order SF > SPMS > MS and, of course, the levels of total nitrogen added would be increased with increasing levels of infestation.

After 71 days incubation and at the time of eggplant seedling transplant, the CSL and ASL soils infested with MS and SF inoculum maintained V. dahliae propagule levels in excess of the initially added levels (Table 12). The plants grown in these soils showed similar numbers of V. dahliae infection loci/g root. The V. dahliae populations in KFSL soils at the time of seedling transplant were below the initial rates of infestation. Plants grown in the KFSL soils initially infested with MS or SF inoculum at all levels of initial infestation reflected the levels of V. dahliae populations found after 71 days incubation.

The number of V. dahliae infection loci/g of roots of plants grown in all soils infested with SPMS inoculum increased with increasing infestation levels (Table 12). Comparing infection loci/g of roots of plants grown in the SPMS infested soils with those grown in MS and SF infested soils, it may be seen that a reversal in soil type effect had occurred in that those plants grown in the KFSL soils showed the greatest number of infection loci/g root. This agrees with previous results where SPMS inoculum breakdown and subsequent release of V. dahliae propagules into the soil was most rapid in KFSL soil. This would have the effect of increasing the numbers of potentially infective propagules that could come in contact with the roots.

DISCUSSION

The fluctuations of V. dahliae populations in soils are apparently due to germination and sporulation of microsclerotia. Twenty-two days after soil infestation with microsclerotia there was a substantial increase in the number of viable propagules (Table 4). Menzies and Griebel (1967) have presented evidence that microsclerotia germinate and sporulate when added to moist soils. Similar reports of an initial flush of V. dahliae growth following soil infestation have been presented by McKay (1926), Wilhelm (1954), Tolmsoff (1959), Martinson (1963), and Lacy (1965).

Population fluctuations in CSL soils appeared to be conditioned by a dominant soil factor which allowed the V. dahliae populations in CSL soils to fluctuate at all geographic locations, while those populations contained in ASL and KFSL soils fluctuated only at the Corvallis location (Figures 6 and 7).

Fluctuations of V. dahliae populations in all soils at the Corvallis location were similar in magnitude and frequency and maintained relatively high populations through most of the five year incubation period (Figure 6). Under the environmental conditions of the Corvallis location the microfloral populations extant in all soils may, at different times, have experienced varying environmental optima for increase or supression of individual species.

Thus, there would be fluctuating amounts of nutrients available. A certain portion of these nutrients would become available as carbon complexes and nitrogen to V. dahliae propagules and they, in turn, would be stimulated to germinate and grow. The conditions under which substrates may become available to V. dahliae and also whether there is a species specificity as to release of available carbon complexes for V. dahliae utilization are unknown factors.

At the Corvallis location, where all V. dahliae populations in all soils fluctuated similarly, the climate was apparently the determining factor in the intermittent availability of oxidizable carbonacious substrates to the V. dahliae populations. The climates of the Redmond and Klamath Falls locations modified the competition for and/or the availability of nutrients in the ASL and KFSL soils, but the dominant soil factor of the CSL soils was unaffected by climate.

The numbers of fungi in soils vary directly with organic matter content (Alexander, 1961). Green (1960) found that V. albo-atrum (MS) microsclerotia showed a far higher percent survival in muck soil than in mineral soil. CSL soil contains greater amounts of total nitrogen and organic matter than either ASL or KFSL soil (Table 2), and CSL soil would maintain a higher and more diverse microfloral population than either ASL or KFSL soil (Bollen, 1968). The diverse microflora of CSL soils may contain various genera which are adpative to the different environmental conditions encountered at

the Redmond and Klamath Falls locations. Thus, if these microorganisms were active in these environments and responsive to environmental changes, there would be a similar nutrient turnover as postulated for the soils incubated at the Corvallis location.

V. dahliae populations in the ASL and KFSL soils had declined considerably within 71 days after soil infestation at the Redmond and Klamath Falls locations (Figure 6). These populations thereafter maintained fairly stable but increasing populations through the five year incubation.

After five years incubation of MS infested soils, all V. dahliae populations had increased in levels following their apparent lows at 71 or 256 days (Table 4, Figures 6 and 7). Menzies and Griebel (1967) found that under certain conditions V. dahliae forms new propagules in the soil, many of which die in time, but their evidence shows that a number of propagules are produced which are resistant to the deleterious influences of the soil environment. Powelson (1966) observed that V. dahliae conidia which were imbedded in sucrose amended silica gel blocks and buried in soil for 30 days either germinated and formed secondary conidia or developed thickened, lightly pigmented conidial cell walls, or developed into microsclerotia. Powelson and Pyott (1966) subsequently concluded that, while nitrogen was necessary for V. dahliae conidial germination, available carbon substrates rather than nitrogen were the major

limiting factor for V. dahliae germination and growth in soils.

The high population levels maintained through five years incubation in all soils at all locations indicate that V. dahliae is capable of long-term survival in fallow soils (Table 5, Figures 6 and 7). Population levels were influenced by soil type and location.

An analysis of stem piece inoculum breakdown after 20 months incubation in the three soils showed that stem piece breakdown in CSL soils was far less than in either ASL or KFSL soils (Tables 6, 7 and 8). The order of stem piece breakdown as to soils was KFSL > ASL > CSL. This order of decomposition is reflected by the population levels shown in Figure 7. V. dahliae counts were directly proportional to tissue breakdown and the consequent propagule release into the soils (Table 6 and 7).

An eggplant bioassay made after five years incubation of SPMS infested soils showed that both soil type and geographic location effected the inoculum potentials of the V. dahliae populations. V. dahliae populations in KFSL soils at all locations and in all soils at the Klamath Falls location showed the greatest inoculum potentials (Table 9).

Two salient points emerge from the SPMS inoculum studies: (1) V. dahliae infested potato stem pieces decompose and release imbedded Verticillium propagules into soils at different rates in different soil types, and (2) climate moderates the rate of

decomposition of the infested potato stem pieces. Therefore, when V. dahliae infected potato debris is incorporated into different soil types there occurs a differential rate of propagule release and consequently a greater or lesser number of inoculum units for contact with roots and subsequent infection. With equivalent amounts of infected potato vine debris added to CSL soils at the Corvallis location and KFSL soils at the Klamath Falls location, threefold more propagules were released into the KFSL soil than the CSL soil. As a consequence, the incidence of Verticillium root infections would be greater in following host crops.

Dilution assays of laboratory incubated soils five days after infestation with MS inoculum showed a flush of population growth in all soils (Figure 9) similar to that shown in the field survival experiment (Table 4). The initial population increases were proportionate to initial infestation levels. As in the field survival experiment, the progression of V. dahliae survival in the laboratory incubated soils was moderated by soil type, both showing survival in the order of: CSL > ASL > KFSL.

The lowest levels of initial MS infestation showed the highest percent survival in all soils after 290 days incubation. These data are in agreement with those of Menzies and Griebel (1967) who found V. dahliae survival inversely proportional to initial soil infestation rates. Apparently, as the quantities of inocula are increased there

occurs an increased imbalance in the soil microflora which creates environmental conditions deleterious to V. dahliae microsclerotia and only those propagules most fitted for the specific environment survive.

Eggplants grown in the three laboratory incubated soils showed different fresh plant weights ranging from well below to far above the fresh plant weights of the controls (Table 12). A possible explanation of the differential plant weights may find its basis in two factors: (1) Disease potential of the pathogen as moderated by the effect of soil type on V. dahliae populations, and (2) available nitrogen added with the inoculum or in the soil before infestation.

The available nitrogen in either the soils or the inocula was not known but an explanation by way of nitrogen nutrition can be formulated on the indirect index of a comparison between control plants fresh weights and plants grown in soils artifically amended with known amounts of organic material containing known amounts of total nitrogen (Table 13).

The MS inoculum in CSL soils failed to supply sufficient nitrogen to overcome the disease potential of V. dahliae as measured by fresh plant weights of plants grown in infested soils compared to the fresh plant weight of the control plants. Only the MS inoculum rate of 20,000 propagules/g in the ASL soils supplied sufficient nitrogen to produce fresh plant weights in excess of the control plant weight.

All levels of MS infestation in KFSL soils supplied sufficient nitrogen to produce fresh plant weights in excess of the control. Similar effects were found with plants grown in soils infested with SF and SPMS inocula.

Eggplants grown in V. dahliae infested, laboratory incubated, CSL and ASL soils developed symptoms at a significantly more rapid rate than plants grown in KFSL soils (Figure 9). There were no significant differences between inoculum types on the rate of eggplant Verticillium wilt symptom expression. The infection loci/g root data, however, showed rather distinct differences in the amount of root infection between the plants grown in MS, SF and SPMS infested soils.

All of the eggplants were showing wilt symptoms when removed from the infested soils for analyses. Although all of the plants' roots were exposed to the infested soils for the same period of time, the factors of soil type and inoculum type and level moderated the numbers of V. dahliae infection loci/g root produced after 41 days growth in the infested soils. Wilt symptoms appeared first in plants growing in CSL and ASL soils, and symptoms were more severe than in the KFSL soil.

All of the infection loci/g data for eggplants growing in the MS and SF infested CSL and ASL soils exceeded the loci/g root of plants grown in KFSL soils, indicating that infection by a certain number

of V. dahliae propagules (a lower infection threshold), rather than the total infection loci/g root, is the determining factor in initial wilt symptom expression. Inoculum density threshold levels necessary for host symptom expression have been demonstrated by Wilhelm (1950) for tomatoes, Tolmsoff (1959) for potatoes, and Martinson (1963) for mint.

Root infection data reflected certain of the levels of soil infestation by V. dahliae. After 70 days incubation in the soils, V. dahliae populations in CSL and ASL soils were 135% to 280% above the initially added levels (Figure 8), whereas V. dahliae populations in KFSL soils had diminished to less than 70% of their initially added levels. The relative propagule levels were reflected in numbers of V. dahliae infection loci/g root where either MS or SF inoculum was added to KFSL soils (Table 12).

The infection loci/g root data for SPMS infested CSL and ASL soils compares favorably with the loci/g root data for plants grown in MS or SF infested KFSL soils (Table 12). The organic matter breakdown of stem pieces in these soils shows the progression as to soil type: KFSL > ASL > CSL (Table 6). Apparently the organic breakdown and subsequent release of V. dahliae propagules in SPMS infested KFSL soils accounts for the much higher infection loci/g root recorded in this soil compared to CSL and ASL soils.

The small differences in loci/g roots of plants grown in CSL

and ASL soils infested with either MS or SF inocula and KFSL soils infested with SPMS inoculum may indicate that an upper threshold level of infection loci/g root had been reached beyond which increased levels of soil infestation or more prolonged exposure of living roots to the infested soils would not increase the V. dahliae loci/g root.

V. dahliae microsclerotia produced in naturally infected potato stem tissue survive well (more than 5 years) under a wide range of climatic and soil conditions. The relative stability of inoculum densities may be due, in part, to periodic production of new resting propagules from the initial inoculum. Within certain limits, climate and soil type may modify the inoculum density and potential of V. dahliae.

BIBLIOGRAPHY

Alexander, M. 1961. Introduction to soil microbiology. New York, Wiley. 472p.

Baker, R. 1965. The dynamics of inoculum. In: the ecology of soil borne plant pathogens, ed. by K. F. Baker and W. C. Snyder. Berkeley, University of California. p. 395-403.

Berkeley, G. H., G. O. Madden and R. S. Willison. 1931. Verticillium wilts in Ontario. Scientific Agriculture 11:739-759.

Blank, L. M. and P. J. Leyendecker. 1951. Verticillium wilt spread to disease-free soil by infected cotton stalks. Plant Disease Reporter 35:10-11.

Brandt, W. H. 1964. Morphogenesis in Verticillium: effects of light and ultraviolet radiation on microsclerotia and melanin. Canadian Journal of Botany 42:1017-1023.

Brandt, W. H. and J. E. Reese. 1964. Morphogenesis in Verticillium: a self produced, diffusable, morphogenic factor. American Journal of Botany 51:922-927.

Bollen, W. B. 1968. Professor, Oregon State University. Department of Soil Microbiology. Personal communication. Corvallis, Oregon. May 20, 1968.

Burges, A. and E. Fenton. 1953. The effect of carbon dioxide on the growth of certain fungi. Transactions of the British Mycological Society 36:104-108.

Butler, F. C. 1953. Saprophytic behavior of some cereal-rot fungi. I. Saprophytic colonization of wheat straw. Annals of Applied Biology 40:284-297.

Corda, A. C. J. 1838. Icones fungorum. Vol. 2. Prague, J. G. Calre. 43p.

Davey, C. B. and G. C. Papavisas. 1963. Saprophytic activity of Rhizoctonia as affected by the carbon-nitrogen balance of certain organic soil amendments. Proceedings of the Soil Science Society of America 27:164-167.

Devaux, A. L. and W. E. Sackston. 1966. Taxonomy of Verticillium species causing wilt of horticultural crops in Quebec. Canadian Journal of Botany 44:803-811.

Dimond, A. E. and J. G. Horsfall. 1960. Inoculum and the diseased population. In: Plant pathology, an advanced treatise, ed. by J. G. Horsfall and A. E. Dimond. Vol. 3. New York, Academic. p. 1-22.

Emmatty, D. A. and R. J. Green. 1967. Germination of microsclerotia of Verticillium albo-atrum in soil. (Abstract) Phytopathology 57:810-811.

Engelhard, A. W. 1957. Host index of Verticillium albo-atrum Reinke and Berthold (including Verticillium dahliae Klebahn). Plant Disease Reporter Supplement 244:23-49.

Evans, G., S. Wilhelm and W. C. Snyder. 1967. Quantitative studies by plate counts of propagules of the Verticillium wilt fungus in cotton field soils. Phytopathology 57:1250-1255.

Garrett, S. D. 1950. Ecology of the root-inhabiting fungi. Biologic Review of the Cambridge Philosophical Society 25;220-254.

Garrett, S. D. 1956. Biology of root-infecting fungi. Cambridge, Cambridge University. 293p.

Garrett, S. D. 1963. Soil fungi and soil fertility. New York, MacMillan. 165p.

Garrett, S. D. 1965. Toward biological control of soil-borne plant pathogens. In: Ecology of soil-borne pathogens, ed. by K. F. Baker, and W. C. Snyder. Berkeley, University of California. p. 4-17.

Gordee, R. S. and C. L. Porter. 1961. Structure, germination, and physiology of microsclerotia of Verticillium albo-atrum. Mycologia: 53:171-182.

Green, R. J. 1951. Studies of the host range of the Verticillium wilt of Mentha piperita L. Science 113:207-208.

Green, R. J. 1958. "Deep plowing" for controlling Verticillium wilt of mint in muck soils. Phytopathology 48:575-577.

Green, R. J. 1960. The survival of Verticillium albo-atrum in muck soil. (Abstract) Phytopathology 50: 637.

Heale, J. B. and I. Isaac. 1963. Wilt of lucerne caused by species of Verticillium. Annals of Applied Biology 52: 439-451.

Hoffman, H. 1854. Spermatein bei einam Fadenpilze. Botanische Zeitung 12: 249-254.

Horner, C. E. 1954. Pathogenicity of Verticillium isolates to peppermint. Phytopathology 44: 239-242.

Horner, C. E. 1962. Oregon State University mint wilt research project. In: Proceedings of the thirteenth annual meeting of the Oregon Essential Oil Growers League, Corvallis, 1962. p. 6-16.

Horner, C. E. 1963. Oregon State University mint wilt research project. In: Proceedings of the Fourteenth Annual Meeting of the Oregon Essential Oil Growers League, Corvallis, Oregon. p. 6-18.

Isaac, I. 1946. Verticillium wilt of sainfoin. Annals of Applied Biology 33: 28-34.

Isaac, I. 1949. A comparative study of pathogenic isolates of Verticillium. Transactions of the British Mycological Society 32: 137-157.

Isaac, I. 1957. The effects of nitrogen supply upon the Verticillium wilt of Antirrhinum. Annals of Applied Biology 45: 512-515.

Isaac, I. 1967. Speciation in Verticillium. In: Annual review of phytopathology. Vol. 5. Palo Alto, Annual Reviews. p. 201-222.

Isaac, I. and G. D. MacGarvie. 1962. Germination of resting bodies in Verticillium species. Nature 195: 826-827.

Isaac, I. and G. M. MacGarvie. 1966. Dormancy and germination of resting structures of Verticillium spp. Transactions of the British Mycological Society 49: 669-678.

Klebahn, H. 1913. Beitrage zur Kenntnis der Fungi Imperfecti. 1. Eine Verticillium Krankheit auf Dahliem. Mycologishes

Centralblatt 3:49-66.

Lacy, M. L. 1965. Influence of host and non-host plants upon populations of Verticillium dahliae in soil. Ph.D. thesis. Corvallis, Oregon State University. 95 numb. leaves.

Little, T. M. and F. J. Hills. 1963. Experimental methods for extension workers. Davis, University of California, Agricultural Extension Service. 58p.

Lucas, R. L. 1955. A comparative study of Ophiobolus graminis and Fusarium culmorum in saprophytic colonization of wheat straw. Annals of Applied Biology 43:134-143.

Luck, J. V. 1953. Studies on the Verticillium wilt of Mentha piperita L. with special emphasis on the causal organism, V. albo-atrum R & B. Ph.D. thesis. Lafayette, Purdie University. 116 numb. leaves.

Ludbrook, W. V. 1933. Pathogenicity and evironal studies on Verticillium hadromycosis. Phytopathology 23:117-134.

Macer, R. C. F. 1961. Saprophytic colonization of wheat straw by Cercosporella herpotrichoides Fron and other fungi. Annals of Applied Biology 49:152-164.

McKay, M. B. 1921. Transmission of some wilt diseases in seed potatoes. Journal of Agricultural Research 21:821-848.

McKay, M. B. 1926b. Potato wilt and its control. Corvallis. 23p. (Oregon. Agricultural Experiment Station. Bulletin 221)

McKay, M. B. 1926a. Further studies of potato wilt caused by Verticillium albo-atrum. Journal of Agricultural Research 32: 437-470.

Martinson, C. A. 1963. Inoculum potential relationships of Rhizoctonia solani measured with soil microbiological sampling tubes. Phytopathology 53:634-638.

Martinson, C. A. 1964. Active survival of Verticillium dahliae in soil. Ph.D. thesis. Corvallis, Oregon State University. 143 numb. leaves.

Martinson, C. A. and C. E. Horner. 1962. Importance of nonhosts in maintaining the inoculum potential of Verticillium. (Abstract) Phytopathology 52: 742.

Menzies, J. D. and G. E. Griebel. 1967. Survival and saprophytic growth of Verticillium dahliae in uncropped soils. Phytopathology 57: 703-709.

Nadakavukaren, M. J. 1960. The effect of soil moisture and temperature on survival of Verticillium microsclerotia. Ph. D. thesis. Corvallis, Oregon State University. 64 numb. leaves.

Nadakavukaren, M. J. 1962. Fine structure of microsclerotia of Verticillium albo-atrum Reinke and Berth. Canadian Journal of Microbiology 9: 411-413.

Nadakavukaren, M. J. and C. E. Horner. 1959. An alcohol agar medium selective for determining Verticillium microsclerotia in soil. Phytopathology 49: 527-528.

Nees van Esenbeck. C. G. 1816. Das System der Pilze und Schwamme. Wurzburg, Stahlelschen Buchhandlung. 329p.

Nelson, P. E. and S. Wilhelm. 1958. Thermal death range of Verticillium albo-atrum. Phytopathology 48: 613-616.

Nelson, R. 1950. Verticillium wilt of peppermint. East Lansing. 259 p. (Michigan. Agricultural Experiment Station. Technical Bulletin no. 221)

Parker, K. G. 1959. Verticillium hadromycosis of deciduous tree fruits. The Plant Disease Reporter Supplement 255: 39-61.

Pethybridge, G. H. 1916. The Verticillium disease of the potato. Scientific Proceedings of the Royal Dublin Society 15: 63-92.

Powelson, R. L. 1966. Availability of diffusable nutrients for germination and growth of Verticillium dahliae in soils amended with oat and alfalfa residues. (Abstract) Phytopathology 56: 895.

Powelson, R. L. 1968. Associate Professor, Oregon State University. Department of Botany and Plant Pathology. Personal communication. Corvallis, Oregon. March 1968.

Powelson, R. L. and J. Pyott. 1966. A silica gel diffusion technique for determining fungal germination and growth responses to nutrients. (Note) Phytopathology 56:248-249.

Presley, J. T. 1941. Saltants from a monosporic culture of Verticillium albo-atrum. Phytopathology 31:1135-1139.

Presley, J. T. 1950. Verticillium wilt of cotton with particular emphasis on variation of the causal organism. Phytopathology 40:497-511.

Pyke, T. R. 1961. Studies on the cellular composition and germination of microsclerotia of Verticillium albo-atrum R & B. Ph. D. thesis. Lafayette, Purdue University. 78 numb. leaves.

Reinke, J. and G. Berthold. 1879. Die Zersetzung der Kartoffel durch Pilze. Universitat Gottingen, Untersuchungen Botanisches Laboratorium 1:1-100.

Robinson, D. B. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology, and variability of the pathogen. Ph. D. thesis. Madison, University of Wisconsin. 97 numb. leaves.

Robinson, D. B., R. H. Larson and J. C. Walker. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology and variability of the pathogen. Madison. 49p. (Wisconsin. Agricultural Experiment Station. Research Bulletin no. 202)

Rudolph, B. A. 1931. Verticillium hadromycosis. Hilgardia 5: 197-353.

Schreiber, L. R. and R. J. Green. 1962. Comparative survival of mycelium, conidia, and microsclerotia of Verticillium albo-atrum in mineral soil. Phytopathology 52:288-289.

Sewell, G. W. F. 1959. Direct observation of Verticillium albo-atrum in soil. Transactions of the British Mycological Society 42:312-321.

Sewell, G. W. F. and J. F. Wilson. 1958. Weed hosts of the "progressive" hop strain of Verticillium albo-atrum Reinke & Berth. In: Annual Report of the East Malling Research Station, 1957, Kent, England. p. 126-218.

Smith, H. C. 1965. The morphology of Verticillium albo-atrum, V. dahliae, and V. tricorpus. New Zealand Journal of Agricultural Research 8:450-478.

Snathhorst, W. C. 1965. Origin of new growth in dormant microsclerotial masses of Verticillium albo-atrum. Mycologia 57: 343-351.

Snedecor, G. W. 1948. Statistical methods. 4th ed. Ames, Iowa State College, 1955. 485p.

Tolmsoff, W. J. 1959. The influence of quantity of inoculum on the severity of Verticillium wilt. Ph. D. thesis. Corvallis, Oregon State University. 137 numb. leaves.

Van Beyma Thoe Kingma, F. H. 1940. Uber einige Formen von Verticillium dahliae kleb. Antonie van Leeuwenhoek Journal of Microbiology and Serology 6:34-47.

Van den Ende, G. 1958. Untersuchungen uber den pflanzenparasiten Verticillium albo-atrum Reinke and Berth. Acta Botanica Neerlandica 7:665-740.

Van der Meer, J. H. H. 1925. Verticillium wilt of herbaceous and woody plants. Mededelingen van de Landbouwhoogeschool te Wageningen 28:1-82.

van der Plank, J. E. 1963. Plant diseases: epidemics and control. New York, Academic Press. 349p.

Wilhelm, S. 1948. The effect of temperature on the taxonomic characters of Verticillium albo-atrum R & B. (Abstract) Phytopathology 38:919.

Wilhelm, S. 1950. Vertical distribution of Verticillium albo-atrum in soils. Phytopathology 40:368-376.

Wilhelm, S. 1951. Effect of various soil amendments on the inoculum potential of the Verticillium wilt fungus. Phytopathology 41:684-690.

Wilhelm, S. 1954. Aerial microsclerotia of Verticillium resulting from conidial anastomosis. Phytopathology 44:610.

Wilhelm, S. 1955. Longevity of the Verticillium wilt fungus in the laboratory and field. Phytopathology 45: 180-181.

Wilhelm, S. and H. C. Thomas. 1952. Solanum sarachoides an important weed host to Verticillium albo-atrum. (Abstract) Phytopathology 42: 519-520.

Wollenweber, H. W. 1929. Die wirtelpilz-welkakrankheit (Verticillose) von Ulme, Ahorn and Linde usw. Arbeiten aus der Biologischen Reichsanstalt fur Land-u. Forstwirtschaft 17: 293-299.

Young, R. A. and W. J. Tolmsoff. 1957. Cultural and chemical treatments for control of the early maturity disease of potatoes. (Abstract) Phytopathology 47: 38.