AN ABSTRACT OF THE THESIS OF

Eileen Marie Shufelt for the degree of Master of Science in Botany and Plant Pathology presented on July 24,1987.

Title: Evaluation of Assay Systems for the Determination of Susceptibility of the Hop (Humulus lupulus) to Verticillium Wilt Caused by Verticillium dahliae

Abstract approved: Redacted for privacy

Dr. Robert G. Linderman

A rapid and reliable assay is needed to evaluate hop resistance to Verticillium wilt caused by Verticillium dahliae. Assays used in the past are laborious, require long incubation periods, and usually produce mild symptoms which are difficult to evaluate and are often not consistent. A study comparing several methods for evaluating resistance of hops to Verticillium wilt was conducted. Results from isolate pathogenicity / host range tests showed the isolates used in these experiments to differ in their pathogenicity on each host tested. More severe symptoms generally occurred with an increase in inoculum concentration.

In the first assay method, cuttings of resistant (R) cultivars Yakima Cluster and Bullion, moderately resistant (MR) Willamette, susceptible (S) Fuggle and Columbia, and three native North American hop types were grown in non-sterile field soil artificially infested with microsclerotia

of _V_. _dahliae_. After seven months, plants of the susceptible cultivars were very stunted and chlorotic. Cultivars reported to be resistant were less stunted. Three native North American hop clones resembled the resistant hop cultivars in visual symptom expression. Although vascular discoloration was observed, _Verticillium_ was not recovered on three isolation media, but an attempt to isolate the fungus by a sterile filter paper method was successful. Analysis of the infested soil by the wet sieve method showed a decreased number of microsclerotia by the end of the trial which may have resulted in the low recovery. In general, root weight increased with increased inoculum concentration among inoculated susceptible cultivars, and decreased for resistant cultivars. Bine dry weight varied significantly among cultivars and in relation to inoculum density, but there were no apparent trends among the cultivars.

In the second assay method, roots of the susceptible hop cultivar Hallertauer were inoculated by conidial root dip. Symptoms were mild, with symptomatic plants generally recovering from wilt. Differences in the ability of the isolates to induce symptoms of Verticillium wilt were apparent. There was no significant difference among root or bine dry weight of inoculated versus non-inoculated plants. The proportion of infected plants was significantly different among isolates. Marked symptom expression and recovery of the pathogen were positively correlated.

In the third method, standardized conidial suspensions were placed on detached leaves of a number of hop cultivars with different levels of field resistance to Verticillium wilt. Although this method has been used successfully elsewhere, no reaction was seen on the hop leaves in trials repeated numerous times.

In a fourth assay method, drops of V. dahliae culture filtrate containing presumed toxin were applied to detached leaves from hop cultivars differing in resistance to Verticillium wilt. Distinctive chlorotic spots were induced on leaves of susceptible cultivars Fuggle and Willamette, while little or no chlorosis was induced on leaves of resistant cultivars Bullion and Yakima Cluster. Boiling the filtrate for 1 min decreased the activity by almost 50%; autoclaving at 15 psi/15 min destroyed the activity.

The results of this study suggest that the application of culture filtrates to detached hop leaves is the best assay system of those compared, being both rapid and reliable, and useful to distinguish the relative susceptibility and resistance of hop cultivars to V. dahliae. This method correlated best with field observations on cultivar resistance to Verticillium wilt. Mechanical and physiological responses, however, may be equally important in determining overall resistance. For this reason, initial screening of new plant material or isolates should be done by comparing the results from conidial root dips and tests with the culture filtrate.

Evaluation of Assay Systems for the Determination of Susceptibility of the Hop (*Humulus lupulus*) to Verticillium Wilt Caused by *Verticillium dahliae*

by

Eileen Marie Shufelt

A THESIS

submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of
Master of Science

Completed July 24, 1987

Commencement June 1988

APPROVED:

Redacted for privacy

Professor of Botany and Plant Pathology in charge of major

Redacted for privacy

Head of Department of Botany and Plant Pathology

Redacted for privacy

Dean of Graduate School

Date thesis is presented July 24, 1987

Typed by Eileen Marie Shufelt for Eileen Marie Shufelt

ACKNOWLEDGEMENTS

I would like to thank the staff and students of the Horticultural Crops Research Lab for their friendship and support.

I am very appreciative of the guidance and encouragement received from Dr. Bob Linderman. His enthusiasm and unfailing confidence in my work was uplifting. My special thanks to Dr. Mary Powelson for her cheerful, encouraging words and valuable suggestions. Dr. Alfred Haunold provided much guidance on hop culture, and on many occasions supplied plant material.

I would like to especially recognize the support received from Dr. R. Hampton, Anheuser-Busch and the Hop Research Council.

I am grateful to my husband, Cary, for his encouragement and for the long hours spent discussing the trials and tribulations of working with _Verticillium_. This thesis is dedicated to Cary and to our daughter, Erin, who also has contributed in many ways to the completion of this study.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	7
Isolate Pathogenicity / Host Range	9
Microsclerotial Infestation of Soil	10
Conidial Root Dip Inoculation Method	13
Conidial Inoculation of Detached Leaves	14
Culture Filtrate (Toxin) on Detached Leaves	16
RESULTS	20
Isolate Pathogenicity / Host Range	20
Microsclerotial Infestation of Soil	21
Conidial Root Dip Inoculation Method	22
Conidial Inoculation of Detached Leaves	23
Culture Filtrate (Toxin) on Detached Leaves	23
DISCUSSION	26
LITERATURE CITED	54

LIST OF FIGURES

Figure		Page
1.	Scale used to evaluate reactions produced by the application of *Verticillium dahliae* culture filtrates to detached hop leaves: 0=no reaction; 1=mild chlorosis; 2=distinct chlorosis; 3=necrosis, with or without chlorosis.	42
2.	Recovery of *Verticillium dahliae* from susceptible hop cultivar Hallertauer inoculated by conidial root dip with C9, B, BV, X, M8 (hop), P and V (potato), V21 (maple), V6a (chrysanthemum), and Mt (mint). Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test.	44
3.	Reaction ratings to the application of ten-fold dilutions of culture filtrate from *Verticillium dahliae* isolate N (hop) on detached leaves of hop cultivars: Fuggle (S) (▪ ,———), Willamette (MR) (+ ,— - - —), and Bullion (R) (♦ ,- - -).	46

4. Reactions of detached leaves of hop cultivars: Fuggle (S) and Bullion (R) to the application of culture filtrates of *Verticillium dahliae* isolates C9, N, X, M8 (hop), P, V (potato), and Mt (mint). Filtrate, Control. Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test. 48

5. Reactions of detached leaves of hop cultivars: Fuggle (S), Willamette (MR), Bullion (R), and Yakima Cluster (R) to the application of culture filtrates of *Verticillium dahliae* isolates C9, N, and M8 (hop), and Mt (mint). Filtrate, Control. Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test. 50

6. Reactions of detached leaves of hop cultivar Fuggle (S) to application of autoclaved, boiled and unheated culture filtrates of *Verticillium dahliae* isolate N (hop). Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test. 52

LIST OF TABLES

Table		Page
1.	*Verticillium dahliae* isolates used in the evaluation of assay systems for determination of the susceptibility of hop germplasm to Verticillium wilt.	36
2.	Hop cultivars with various levels of resistance to Verticillium wilt and native North American hop clones of unknown resistance.	37
3.	Means of leaf symptom ratings and growth parameters of eggplant (*Solanum melogena* var. Black Beauty) inoculated by root dip with different isolates of *Verticillium dahliae*, at different conidial concentrations.	38
4.	Means of leaf symptom ratings and growth parameters of tomato (*Lycopersicon lycopersicum* var. Bonny Best) inoculated by root dip with different isolates of *Verticillium dahliae*, at different conidial concentrations.	39

5. Means of leaf symptom ratings and growth parameters of tomato (*Lycopersicon lycopersicum* var. Ace 55 VF) inoculated by root dip with different isolates of *Verticillium dahliae*, at different conidial concentrations. 40

6. Mean root and bine dry weights of susceptible (S), moderately resistant (MR) and resistant (R) hop cultivars grown in field soil infested with *Verticillium dahliae* microsclerotia. 41

Evaluation of Assay Systems for the Determination of Susceptibility of the Hop (*Humulus lupulis*) to Verticillium Wilt Caused by *Verticillium dahliae*

INTRODUCTION

The first authentic account of a fungus causing a vascular disease of plants was reported by Reinke and Berthold in 1879 (41). The fungus, *Verticillium albo-atrum*, was described as producing dark mycelia or sclerotia. In 1913, Klebahn (30) isolated from *Dahlia* a fungus which formed true microsclerotia by lateral budding from a single hyphal strand and melanizing of the cell walls to form a multicellular resting structure. Because *V.albo-atrum* forms only dark resting mycelium from hyphal cells with thick, melanized walls, Klebahn believed the isolate from Dahlia was sufficiently different from *V. albo-atrum* to be a separate species. After years of controversy over the taxonomy of these species, it has been suggested that *V. albo-atrum* and *V. dahliae* are valid names for the dark mycelium and microsclerotial forms, respectively (43). *Verticillium* species can be distinguished by morphological traits and physiological traits such as optimum growth temperatures (22,23).

The commercial hops industry in the Pacific Northwest began during the period from 1849-1869 (13). Verticillium wilt of hops in Oregon was first reported in 1956 (15).

Prior to 1962, all outbreaks were caused by _V. dahliae_, but an outbreak caused by _V. albo-atrum_ occurred in 1962 (15). These species of _Verticillium_ cause a gradual decline in hop productivity. Growers have observed that perennial crowns die if the disease is present for several years (15). _Verticillium_ penetrates the hop root and passes upward in the bine in the vascular system. Symptoms first appear in late July or early August on the lowest leaves of the bine. Upper leaves become progressively symptomatic nearer to harvest time. Leaf symptoms consist of chlorotic patches and irregular necrotic areas between major veins, and leaves eventually fall from the bine. The most characteristic symptom is a streaking or brown discoloration of the vascular tissue. Occasionally there is a swelling of affected bines from the crown upwards to approximately 1.2 meters (28).

The hop industries in Great Britain and Germany have suffered severe economic losses due to Verticillium wilt caused by _V.albo-atrum_ and _V. dahliae_. In the Bavarian district of Germany, Verticillium wilt of hops was responsible for a change from the profitable high quality, but susceptible, varieties to the less profitable resistant ones (54). The hop industry in the United States has not suffered the severe losses reported in Germany and Great Britain. Nugget, a newly released resistant hop cultivar in the U.S.A., however, showed symptoms of Verticillium wilt

caused by V. dahliae four years after its release. The Verticillium isolates in Oregon apparently are less virulent than those in Great Britain, but wilt in Great Britain was considered to be a minor problem for 10 years until the progressive form was discovered and severe losses occurred thereafter (15).

In the past, assays to determine the relative pathogenicity of Verticillium species or susceptibility of cultivars have been laborious and required long incubation periods. The variations in symptom expression seen on plants grown in infested soil, the most widely used assay, have been attributed to variations in soil temperature (1,48). Furthermore, this method has not yielded consistently reliable results (1). Root dips into conidial suspensions have been used to determine the susceptibility of a number of crops to wilt diseases (1,31,42,52). As with soil infestation, however, results from root dips are variable due to the influence of soil temperature (7,10,24). Thus, a rapid and uniform inoculation method is needed to enable a large number of plants of various cultivars or genetic lines to be screened.

As a possible alternative to root inoculation, Thanassoulopoulos (51, as referenced in 21), suggested using leaves for evaluation of resistance to V. albo-atrum. Leaf inoculations have been used to rapidly screen potato (20,21) and tomato (40) for resistance to Verticillium wilt. When

droplets of V. albo-atrum spore suspensions were applied to young detached hop leaves, necrotic spots of different intensities occurred in certain Verticillium -hop combinations (3). Similar results have been reported with some V. dahliae-inoculated hop leaves (R. Harris, personal communication).

A correlation between stem invasion and severity of leaf symptoms has been suggested by Keyworth (27). He suggests that leaf symptoms, chlorosis and necrosis without a loss of turgor, are due to a toxin rather than to plugging of xylem vessels. Bewley (2) was the first to report that toxins may be produced in liquid culture by isolates of V. albo-atrum and V. dahliae. Years later, Green (8) found proteinaceous and polysaccharide fractions in the filtrate of V. albo-atrum cultures. McLeod (32) has used culture filtrates from pathogenic strains of V. dahliae to screen large numbers of tobacco seedlings for resistance to Verticillium wilt. Since these early experiments, others have provided evidence for and have used toxic substances in the culture filtrate from Verticillium species to screen plants for Verticillium wilt susceptibility (6,32,33). A number of researchers have used the culture filtrate in detached leaf assays (11,34,35,36,45).

Toxic fractions in the culture filtrate of Verticillium spp. have been characterized (26,34,35,36,38,49,50). Keen and Long (26) have isolated a protein-lipopolysaccharide

complex from a _V_. _albo-atrum_ cotton isolate. Although they provide evidence for the possible involvement of the complex in Verticillium wilt of cotton (25), they were unable to provide conclusive evidence that the toxic moiety was present in diseased tissue. Nachmias et al. (36) have characterized a low molecular weight phytotoxin from a protein-lipopolysaccharide complex produced by a potato isolate of _V_. _dahliae_ in liquid culture. Their data suggest that a virulent isolate of the fungus produces at least two different phytotoxic materials in culture, and these compounds are not produced or are produced in altered form by a non-virulent mutant. Susceptibility of potato cultivars to Verticillium wilt and their sensitivity to the toxic molecule were positively correlated (34,36), Serological studies suggest that a substance present in _V_. _dahliae_ infected potato tissue is similar to a substance produced by the fungus _in-vitro_ (34,36).

Verticillium filtrates have been used to evaluate resistance in crops other than potato. _V_. _albo-atrum_ culture filtrate has been used in screening hop susceptibility to Verticillium wilt (Connell, S.A., personal communication). A positive correlation between susceptibility to the pathogen and to the toxin was found.

In-vitro methods using a conidial suspension or a culture filtrate on detached hop leaves could prove to be rapid and reliable assay systems to determine hop cultivar

susceptibility to wilt caused by _V. dahliae_.

The purpose of this investigation was to compare the rapidity and reliability of several assay systems in the evaluation of resistance of hop germplasm to _V. dahliae_. Four assay systems were compared by inoculating several cultivars of hops that have reported differences in _Verticillium_ susceptibility (based on field observations): (1) inoculation of young cuttings by planting in soil infested with microsclerotia, (2) dipping roots in conidial suspensions, (3) application of conidia to detached leaves, and (4) application of culture filtrate to detached leaves.

MATERIALS AND METHODS

Isolations and culture maintenance

An estimation of the inoculum level in the soil required for Verticillium wilt symptom expression in hop was made by sampling hop yard soil in the St. Paul and Buena Vista growing areas of Oregon for Verticillium dahliae with the Anderson air sampler (5) and wet sieve (19) methods.

V. dahliae isolates used in this study were isolated from hop, potato, mint, maple, and chrysanthemum (Table 1). Isolations from hop were made from diseased cultivar Willamette bines on selective lactose agar (SLA) prepared to a final volume of 1 l of sterile distilled water (dw) by adding 1 g yeast extract, 10 g lactose, 15 g agar and autoclaving (15 psi/15 min); to the cooled (45 C) medium were added 1 g pentachloronitrobenzene (PCNB), 0.5 g oxgall, 1 g $NaB_4O_7 \cdot 10 H_2O$, and 0.3 g streptomycin sulfate. Isolates were purified by single spore transfer and stored on lactose yeast agar (LYA) prepared to a final volume of 1 l of dw by adding 10 g lactose, 1 g yeast extract, and 15 g agar followed by autoclaving; when cooled (45 C), 100 ppm streptomycin sulfate were added. The exception was isolate C9, which was stored by adding a conidial suspension to test tubes of air-steam pasteurized (60 C/30 min) peat-loam-sand mix (1-1-1 v:v:v) which had been moistened with sterile dw and allowed to dry at room temperature. Stock agar slant cultures were kept at 16 C in the dark, and were used to

inoculate plates of LYA or PDA (Difco) when inoculum was needed. Plates were incubated at room temperature in the dark for approximately 10 days before being used.

Plant material

Rhizomes from hop cultivars (Table 2) with various levels of resistance to Verticillium wilt (as determined by field observations) were taken from a hop breeding yard in Corvallis, Oregon with no known history of Verticillium wilt and planted in an air-steam pasteurized (60 C/30 min) soil mix of equal volumes of peat, loam and sand. Dolomite lime (30 g/38 l soil) and osmocote 14-14-14 (120 g/38 l soil) were added. Native North American hop rhizomes, collected from the mid-western United States, were grown in the same manner. From November to January these contained dormant plants were put outdoors. Supplemental light (high pressure sodium vapor, intensity of 300 μ einsteins m^2) was supplied for 16 hours in the greenhouse from September to November and from January to March. Plants were fertilized weekly to drain-through with Peter's fertilizer (20-20-20) at 5 ml/3.8 l.

Young plants were obtained by rooting softwood cuttings taken from greenhouse plants. The basal portion of the cuttings was dipped for 3 seconds into a mixture of boric acid and IBA (1000 ppm each when mixed). Cuttings were inserted in trays of sterile quartz sand or in sterile

vermiculite and misted until roots formed, usually in 4-6 weeks.

ISOLATE PATHOGENICITY / HOST RANGE

Several V. dahliae isolates were grown on LYA plates for 10 days to produce conidial inoculum for pathogenicity tests: P (from potato), V (from potato), Mt (from mint), and C9, X, M8, N (from hop). Conidia were removed into 10 ml sterile dw by gently rubbing the agar surface of each plate with a glass rod. The concentrations were adjusted to 10^2, 10^4, 10^6 conidia/ml with a hemacytometer.

Nine plants each of eggplant (Solanum melogena var. Black Beauty) and tomato (Lycopersicon lycopersicum var. Bonny Best and Ace 55 VF) and seven plants of pepper (Capsicum annum var. Early Bountiful) were grown from seed and inoculated at the second true-leaf stage by wounding the roots with a razor blade and dipping them in the three concentrations of conidial suspensions for 1 hr. Following inoculation, plants were potted into Terra Lite Soil Mix and Conditioner (Western formula). Treatments were randomized in a greenhouse where air temperatures averaged 25.6 C, and soil temperatures ranged from 14-23 C. Plants were watered daily, and fertilized with Peter's fertilizer (20:20:20) 2x/wk.

Eggplants were harvested 5 weeks after inoculation, peppers and tomatoes after 9 weeks. At harvest plant height was recorded and plant symptoms of leaf chlorosis and

necrosis were rated, using the Horsfall-Barratt grading system (18) where 0=0% chlorosis/necrosis; 1=1%; 2=5%; 3=10%; 4=25%; 5=50%; 6=75%; 7=90%; 8=95%; and 9=99%. Portions of the lower stems were plated on reduced lactose yeast agar (RLYA) prepared to a final volume of 1 l of dw by adding 1 g lactose, and 0.1 g yeast extract and autoclaving, and 100 ppm streptomycin sulfate after cooling to 45 C. The remaining portions of stems and roots were air dried separately for 48 hours at 75 C and weighed.

MICROSCLEROTIAL INFESTATION OF SOIL

Inoculum

An isolate (C9) from a diseased 'Willamette' bine was stored in sterile soil, and used in this experiment. It produced both abundant conidia and microsclerotia when the soil was plated on LYA. After approximately 10 days, the conidia were washed from these plates with sterile dw and the conidial suspensions used to inoculate jars of sterile oat straw. The latter were covered mason jars containing 15 g oat straw and 50 ml dw which had been autoclaved for 45 min at 15 psi. The jars were cooled for one day before each was inoculated with 5 ml conidial suspension (10^6 conidia/ml). The jars were shaken and incubated at room temperature in the dark. After three weeks, microsclerotia (ms) were visible on the straw which was then air dried for 7 days and milled to a fine powder. The number of ms/g and

their viability were determined by counting the number of microsclerotia in 0.1 g of straw, and by mixing 0.1 g straw with 10 ml sterile dw, and plating serial dilutions on SLA. Jars of concentrated inoculum were prepared by adding the milled straw to a mixture of field soil, from a hop yard known to be free of Verticillium, and pasteurized sand (1:1) (3.3 g and 6.6 g straw inoculum/l soil mix to be used for the 50 and 100 ms/g treatments, respectively). These jars of concentrated inoculum were incubated for 2 weeks at room temperature in the dark and then were incorporated into a mixture of the same non-treated hop yard soil and pasteurized sand to give final concentrations of 50 and 100 ms/g (1 l of concentrated inoculum to 38.7 l of soil). Control soil consisted of the above mix with no addition of straw inoculum.

Plant material and inoculation procedure

In late April, rooted cuttings of resistant (R) cultivars Yakima Cluster and Bullion, moderately resistant (MR) Willamette, susceptible (S) Fuggle and Columbia, and three native North American hop clones (#38, #47, BC16) of unknown susceptibility were rooted as previously described and planted in 9.2 cm x 15.2 cm band pots (Anderson MFG Co., Tigard, OR) filled with soil prepared as described above. Each treatment consisted of seven plants and was replicated three times. The treatments were randomized and placed on a greenhouse bench where the temperature ranged from 12-34 C

during the course of the experiment. The average soil temperature was 20 C. Plants were watered twice daily during the summer months and once daily thereafter; Rapid Grow fertilizer (23-19-17) was applied at the rate of 5 ml/3.8 l approximately every week.

Evaluation of disease reaction

Plants were examined visually bimonthly for symptoms and then were harvested in October after 6 months growth. Bines and roots were washed and air dried separately at 75 C for 48 hrs and weighed. Air-dried soil samples were analyzed by the wet-sieve method (19).

In a preliminary harvest, portions of the crowns of three 'Columbia' plants from the 100 ms/g treatment and from the control treatment were homogenized and plated, or surface disinfested and plated on SLA, LYA, ethanol streptomycin agar (ESA)(37), and water agar (WA). Crown tissue was divided into 2 cm sections and soaked in 10% bleach for 10 minutes, followed by three sterile water washes. In the homogenization method, 0.5 g tissue in 10 ml sterile dw was homogenized in a Virtis blender at a setting of 60 for 1 minute. Blades were washed with 95% ethanol between sections and treatments. The macerate was serially diluted and plated. Crown tissue that was not homogenized, but surface sterilized, was aseptically plated to LYA, SLA, pectate agar (19), and water agar (Difco, 15 g/l). The presence of vascular browning was noted at the

time of plating. Because no Verticillium was detected on the plates inoculated with macerated tissue, the remainder of the plant samples were assayed by the tissue plating method.

CONIDIAL ROOT DIP INOCULATION METHOD

Inoculum preparation and plant material

The V. dahliae isolates to be used as inoculum grown on LYA in the dark for 12 days were: C9, B, X, BV, N, and M8 (hop); V6a (chrysanthemum); P and V (potato); Mt (mint); and V21 (maple). These isolates were used to inoculate rooted Hallertauer (S) hop cuttings prepared as previously described and rooted in BR-8 synthetic soil blocks (Famco Inc., WI).

Inoculation procedure

Root tips were excised with a razor blade and submerged in a suspension of 10^6 conidia/ml for 2 hrs. Viability of conidia was determined by plating serial dilutions on LYA.

Following inoculation, plants were potted in a pasteurized (60 C/30 min) mixture of equal parts of peat-loam-sand and placed in a greenhouse where temperatures ranged from 20-30 C for the 6 month duration of the experiment. Soil temperatures averaged 22 C. Supplemental light (high pressure sodium vapor lamps, intensity of 300 μ einsteins m^{-2}) was supplied for the duration of the experiment; plants were fertilized with Peter's (20-20-20)

fertilizer (5 ml/3.8 l) to drain-through approximately every week.

Evaluation of disease reaction

During the course of the experiment plants were examined for visual symptoms of Verticillium wilt. Six months after inoculation, the plants became chlorotic and began to wilt, at which time the bines and roots were washed and dried separately for 48 hrs at 75 C and weighed. Crowns were surface disinfested as previously described, and sections were aseptically removed and placed on sterile moist filter paper in petri plates and on plates of RLYA. After 3-7 days incubation in the dark at room temperature, the plates were examined for V. dahliae.

CONIDIAL INOCULATION OF DETACHED LEAVES

Inoculum preparation and plant material

Conidia of V. dahliae isolates C2, C9, C31, C35, X and SJ from diseased 'Willamette' bines were used to inoculate detached leaves from hop cultivars with variable levels of resistance to Verticillium wilt. To determine if substrate and conidial age were important factors, inoculum was on grown plates of LYA and PDA, in the dark at room temperature for 7 days or 15 days. Conidial suspensions in sterile dw were prepared at concentrations of 10^2 - 10^9 conidia/ml. Detached leaves from hop cultivars Yakima

Cluster (R), Bullion (R), Cascade (R), Willamette (MR), Fuggle (S), Columbia (S), and Hallertauer (S) were used.

Inoculation procedure

Three separate drops (30 µl each) of a conidial suspension were placed on the lower surface of detached leaves. The second through the fifth leaves from the apex were used in preliminary experiments, and there appeared to be no significant difference in reaction due to leaf age. Prior to inoculation, leaves were either surface disinfested (0.05% sodium hypochlorite for 30 seconds followed by three sterile water washes) or nontreated; leaves were wounded with a glass rod, or with carborundum, or with a razor blade, or were not wounded. The following basal support media were tried in the petri plates: WA, streptomycin water agar, hydra gel, moist sterile vermiculite, and moist sterile filter paper. Since there was no apparent effect from different support media, most experiments were done on filter paper. Petri plates were placed into humidity chambers and incubated at 21 C with continuous light for 5-7 days and then evaluated using the following scale: 0=no chlorosis; 1=chlorotic lesions with or without trace necrotic flecks; 2=clearly defined necrotic flecks; 3=discrete necrotic lesions; 4=multiple, coalescing lesions; 5=entire leaf surface beneath droplet one solid lesion.

CULTURE FILTRATE (TOXIN) ON DETACHED LEAVES

Toxin preparation

V. dahliae culture filtrate was produced in liquid culture in flasks containing 100 ml of a glucose medium (36). The flasks were inoculated with PDA plugs (8 mm) of the following isolates: P and V (potato); C9, X, N, and M8 (hop); and Mt (mint). Flasks were incubated for 21 days at 26 C in the dark. Filtrate was collected by pouring the cultures through cheesecloth, and filtering the liquid through Whatman #1 followed by cellulose acetate (0.22 u) filter sterilization. Aliquots of the filtrate sampled before and after filter sterilization were plated on PDA to check for contamination and *Verticillium* spores. The filtrate was further prepared according to the methods of Nachmias et al. (36) and was concentrated under vacuum at 50 C, and treated with four volumes of acetone at -20 C overnight. The resulting precipitate was collected by centrifugation at 5000 rpm for 15 min at 5 C, dried under nitrogen, and resuspended in distilled water (1/50 the initial volume of the culture fluid), and centrifuged at 8000 rpm for 10 min. The supernatant was retained for testing.

Plant material and application of culture filtrate

Detached leaves from hop plants, grown under greenhouse conditions as previously described, were used in this study. The lower surface of five detached leaves of each of the hop

cultivars Fuggle (S), Willamette (MR), Bullion (R), and Yakima Cluster (R) were treated with three separate drops (30 μl each) of the culture filtrate or one of its dilutions. Control treatments were drops of noninoculated broth, treated in the same way as the filtrate, placed on the leaf opposite a treatment leaf. Leaves were placed on moist sterile filter paper in petri plates. Plates were incubated in humidity chambers under continuous light at 27 C. Filtrate from each isolate was tested four times: twice with freshly prepared filtrate and twice with frozen aliquots of the fresh preparations.

Evaluation of the reaction to the culture filtrate

Five days after the culture filtrate was applied to the detached leaves, the following scale was used to evaluate the degree of leaf chlorosis and/or necrosis: 0=no reaction; 1=mild chlorosis; 2=distinct chlorosis; and 3=necrosis, with or without chlorosis (Figure 1).

Experiments with the culture filtrate

In preliminary experiments, hop isolate N produced strong reactions of chlorosis and necrosis on detached leaves of 'Fuggle' (S), but not on 'Bullion' (R). To determine an effective concentration of the culture filtrate for use in distinguishing susceptible and resistant hop cultivars, a dilution series (10^{-1} - 10^{-3}) was made of the culture filtrate as previously described. The lower surface of five detached leaves of each of the hop cultivars Fuggle

(S), Willamette (MR) and Bullion (R) were treated with three separate drops (30 μl each) of the supernatant or one of its dilutions. Controls were done as previously described.

To determine if the relative susceptibility of hop cultivars to Verticillium wilt could be determined in a detached leaf assay using _V. dahliae_ culture filtrate, culture filtrate was prepared as previously described from the following isolates : C9, N, M8, X (hop); P and V (potato); and Mt (mint). Five detached leaves from each of the hop cultivars Fuggle (S), and Bullion (R), were treated with 3 separate drops of the prepared filtrate. Drop 1 was the resuspended acetone precipitate, drop 2 was a 1:3 dilution, and drop 3 was a 1:4 dilution. Controls were done as previously described. Leaves were incubated as previously described, and the degree of chlorosis-necrosis rated after 5 days. In the same manner, culture filtrates from hop isolates C9, N, and M8, and mint isolate Mt were applied to the lower leaf surface of detached leaves of hop cultivars Fuggle (S), Willamette (MR), Bullion (R), and Yakima Cluster (R).

To determine the heat stability of the toxic moiety, samples of the resuspended acetone precipitate from the culture filtrate of isolate N and noninoculated broth were boiled (1 min), or autoclaved (15 psi/15 min) and placed on detached leaves of 'Fuggle' as described above.

STATISTICAL METHODS

Symptom expression (leaf chlorosis and/or necrosis), plant height, shoot and stem dry weights, and reaction ratings on leaves treated with the toxin were analyzed by a multi-factor analysis of variance. The means were separated by Fisher's protected L.S.D.

RESULTS

ISOLATE PATHOGENICITY / HOST RANGE

Pathogenicity of isolates of _V_. _dahliae_ from various hosts was compared in a root dip assay on eggplant, two cultivars of tomato and on pepper. The isolates differed in their ability to induce symptoms of Verticillium wilt within a host tested.

The severest symptoms on eggplant were induced by hop isolates X, and M8, and potato isolate P. Symptoms of leaf chlorosis and necrosis were most severe at the highest conidial concentrations (Table 3). Plant height, shoot and root dry weights decreased with increased inoculum concentration. Percent recovery ranged from 85%-100% for all the isolates except 'V' which was only 19%. There appeared to be a correlation between symptoms, growth parameters and recovery of _V_. _dahliae._

Reactions of the tomato cultivars, using the root dip inoculation, were less distinctive than those on the inoculated eggplant (Tables 4 & 5). The hop isolates induced leaf symptoms on cultivar Bonny Best (S), but they were less severe than those induced by the same isolate on eggplant. The isolates had no effect on the height or shoot dry weights of Bonny Best; nor was there any apparent increase in severity with increased inoculum concentration. No leaf symptoms of Verticillium wilt were seen on cultivar Ace 55 VF (R), nor did treatments have any effect on the

shoot and root dry weights. Recovery of *V. dahliae* from both cultivars was less than 10%.

Pepper plants inoculated by the root dip method displayed no symptoms of Verticillium wilt, and the fungus was never isolated from the plants.

MICROSCLEROTIAL INFESTATION OF SOIL

Hops planted into field soil infested with *V. dahliae* showed symptoms of chlorosis and wilt, however, symptomatic plants generally recovered from wilt. A few plants had leaves with necrotic margins. Only occasionally was the tiger stripe pattern of interveinal chlorosis and necrosis, typical of the progressive form of Verticillium wilt of hops in Great Britain observed (47). 'Columbia' (S) was visibly stunted in all inoculation treatments, whereas 'Fuggle' (S) and 'Willamette' (MR) were stunted only in the treatment with 100 ms/g soil. The resistant cultivars, Bullion and Yakima Cluster, were not visibly stunted at any inoculum level. The native North American hop clones #38 and #47 were stunted in the treatment with 100 ms/g soil; BC16 was not visibly stunted at any inoculum level.

Visual assessments of decreased growth were substantiated in a statistical analysis of the bine and root dry weights. Columbia (S) and Willamette (MR) both had significantly less bine dry weight where an increased inoculum concentration was used. Bullion (R) had a

significantly higher bine dry weight with an increase in inoculum concentration (Table 6). These results were seen to a lesser degree with the other cultivars. Although results were not consistent, there appeared to be a trend of decreasing root weight with increased inoculum concentration. 'Columbia', however, (S) had an increased root dry weight. Inoculum concentration appeared to have no significant effect on the severity of reaction on the native North American hop clones.

CONIDIAL ROOT DIP INOCULATION METHOD

Symptoms induced by dipping roots of the susceptible hop cultivar Hallertauer in a conidial suspension were mild and infrequent throughout most of the six month experiment. At harvest, however, a few plants inoculated with each of the isolate groups, except V, and V6a, showed chlorosis, necrosis, or severe wilt. The most severe symptoms were on plants inoculated with isolates P, Mt and B. Isolates V and V6a, which produced few microsclerotia or conidia in culture, were not readily recovered from infected tissue, and they induced only mild stunting.

There was no significant difference among root or bine dry weights of inoculated versus non-inoculated plants in the conidial root dip assay, but the proportion of infected plants was significantly different between isolates

(Figure 2). There appeared to be a correlation between marked symptom expression and recovery of the pathogen.

CONIDIAL INOCULATION OF DETACHED LEAVES

The application of drops of a conidial suspension to detached hop leaves produced mild chlorosis around infection sites, but this response was inconsistent. Most often no reaction occurred. Treatment of the tissue prior to inoculation (wounded or nonwounded), the age of inoculum, and the medium used to produce the conidia, all had no effect. Chlorosis occurred with higher conidial concentrations, but it frequently was obscured by the presence of mycelium. The younger, more terminal leaves were more sensitive than older leaves. Tiny necrotic flecks occasionally occurred on the susceptible and resistant leaves, but these flecks did not develop further and there was no chlorosis.

CULTURE FILTRATE (TOXIN) ON DETACHED LEAVES

The application of ten-fold dilutions of the culture filtrate from hop isolate N on detached leaves of hop cultivars with variable levels of field resistance to Verticillium wilt, produced significantly different reactions at all concentrations (Figure 3). Responses by the moderate and resistant cultivars, however, were not significantly different at any concentration, although there

was an apparent trend for the resistant cultivar to have lower disease reaction ratings than the moderately resistant cultivar at all concentrations. Ten-fold dilutions decreased the activity of the filtrate on all the cultivars. The differences among the cultivars became less significant with increased dilution. The nondiluted filtrate provided the best distinction among cultivars.

It was necessary to compare the means of reaction ratings for every dilution-treatment in this dilution experiment because a small sample size was used. A few of the leaves did not respond to the treatments, possibly due to some physiological differences among the leaves. These results suggest that groups of leaves should be considered as replications, and the means of the groups used in statistical analysis.

When the culture filtrates of a number of V. dahliae isolates were placed on detached hop leaves from hop cultivars with variable levels of field resistance, there were significant differences among isolates (Figures 4 & 5). The strongest reactions were induced on the most susceptible cultivar, Fuggle. A decrease in reaction severity with any single isolate correlated positively with increased resistance, with the mildest reactions occurring on the most resistant cultivar, Yakima Cluster (Figure 5).

Differences in reactions induced by culture filtrate from various isolates were apparent. While there was no

significant difference in reactions from filtrate treatments on 'Bullion' (R), reactions from isolates N and C9 (hop), and P (potato) were significantly different from the rest of the isolates on 'Fuggle' (S) (Figure 4). There was also a significant difference between the reactions produced by the hop isolates and the mint isolate on 'Willamette'(MR) and 'Yakima Cluster'(R) (Figure 5). There were no differences in the reactions produced by the filtrate, regardless of the degree of dilution. On most cultivars, the application of noninoculated medium induced background reactions, largely from contaminating microorganisms that grew on the extracted medium as a substrate. The background reactions were greatest on 'Fuggle' (S).

When autoclaved, boiled or untreated culture filtrate from isolate N (hop) was applied to detached leaves of hop cultivar Fuggle the results showed that the filtrate was sensitive to heat. The unheated filtrate produced strong reactions on the leaves, while boiling decreased the activity of the filtrate by almost 50%, and autoclaving eliminated the activity (Figure 6).

DISCUSSION

Results of the pathogenicity / host range experiments suggest that evaluating resistance of the hop to Verticillium wilt may be complicated by the fact that a number of pathogenic isolates of V. dahliae exist in the Pacific Northwest. Results from the microsclerotial and conidial inoculations of hop confirm the observations of C. Horner (16,17) that 1) hop genotypes vary in their response to different isolates of the fungus, 2) the interaction of any hop genotype and a V. dahliae isolate is extremely variable from plant to plant, and 3) the strains of V. dahliae now in the hop yards of the Pacific Northwest are not very pathogenic on hop, although under certain environmental conditions, symptoms will be expressed. Although the strains do not rapidly kill hops, they may cause a gradual decline in productivity.

Recovery of V. dahliae from inoculated plants was difficult. The best medium for isolation was RLYA. V. dahliae produces both conidia and microsclerotia on this medium in 7-10 days. Due to a high frequency of secondary organisms, a duplicate plate of sterile moist filter paper is recommended for isolation from hop. Other researchers have had similar problems in recovering Verticillium from hops inoculated in the greenhouse (Dr. H. Melouk, personal communication).

The general irregularity of symptom expression on hops which have been root-inoculated with V. dahliae makes assessment of susceptibility and resistance difficult in the greenhouse. A delay in symptom expression in hops after root-inoculation has been reported by others (44). Uniform disease expression is desirable when studying disease-resistance. Furthermore, a lack of visual symptoms does not necessarily indicate resistance (53). Other factors, such as temperature, may influence the biochemical and physiological responses that in turn affect whether a plant exhibits a susceptible or resistant reaction. Environmental conditions in the greenhouse were monitored, and, for the most part, remained within the limits (20-30 C) required (43,53) for infection by V. dahliae. There were, however, days when air temperatures exceeded the optimum of 22.5 C, and this may have had an adverse affect on the pathogen.

Following successful inoculation with Verticillium, a decrease in root dry weight was usually observed. The exception was 'Columbia', which exhibited an increase in root dry weight. Verticillium is most able to infect young roots, especially in the area were there is little suberinization of the endodermis. It is possible that a delayed or slow suberinization would offer less resistance to invasion through the endodermis and into the vascular tissue. Movement of V. albo-atrum in hop is related to the rate of suberinization in the cell walls of the endodermis,

the endodermis of a tolerant plant is more suberinized (46). The endodermis in 'Columbia' roots may be colonized heavily due to a slow rate of suberinization.

It was difficult to isolate Verticillium from hop plants planted into soil infested with microsclerotia. Verticillium was not recovered from homogenized tissue, and plating on media was largely unsuccessful. The best recovery was from surface disinfested tissue placed on sterile, moist filter paper. Due to the low percentage of recovery, it is questionable if adequate infection was achieved with this method of inoculation. Results confirm the observation (14) that infection by planting into soil infested with microsclerotia may be an inadequate testing procedure.

Soil analysis indicated good viability of the microsclerotia at the start of the experiment. Approximately 50% decrease in inoculum was observed by harvest. Although the inoculum density was low at harvest, analysis of field soil, from yards where Verticillium was isolated from diseased plant tissue (unpublished results), showed that a very low inoculum level was detectable in the soil, yet infection and symptom expression occurred.

The microsclerotial experiment was very labor intensive due to the climbing growth habit of the hops. The experiment required a long incubation period, and symptom expression was mild and irregular. The mild symptom

expression is usually seen in the greenhouse at a time when it is difficult to distinguish normal plant senescence from Verticillium wilt. In the field, plants usually show the most noticeable symptoms at or near harvest. The greenhouse plants, however, rarely produce cones. This physiological difference may influence, and even obscure, disease expression in the greenhouse (4). Inoculation by soil infestation is not recommended for evaluating hop resistance to Verticillium wilt.

Inoculating wounded hop roots by dipping them into conidial suspensions increased infection, symptom expression, and recovery over that from plants infected by planting in soil infested with microsclerotia. A greater distinction between isolate virulence based upon symptom expression, may have been found if an inoculum dilution series had been used. Including moderately resistant and resistant cultivars, and allowing for a longer incubation in the plant may have resulted in significant differences in bine and root dry weights.

Using synthetic rooting blocks facilitated rooting, but made it a slow and tedious process to recover roots for dry weight determination.

The conidial root dip inoculation method is recommended for whole plant analysis of hop resistance and susceptibility to Verticillium wilt.

The chlorotic response of detached hop leaves to conidial suspensions applied as droplets was too mild and inconsistent to be of use in an assay system. The occasional appearance of tiny necrotic flecks was not useful in distinguishing cultivars due to its inconsistent and non-selective appearance on both susceptible and resistant hop cultivars. This is in contrast to results reported on potato leaves, where a hypersensitive reaction was seen on resistant leaves (21). Blake et al. (3) have also reported necrotic spots of different intensities on certain V. albo-atrum -hop combinations when conidial suspensions were applied as droplets to the lower surface of detached leaves. R. Harris (personal communication) also reported differential reactions due to the application of conidial suspensions of certain V. dahliae isolates to detached leaves of a number of hop cultivars with reported variable levels of resistance. Using the same procedures his results could not be confirmed, however.

The application of crude V. dahliae culture filtrates to detached hop leaves appears to be, of the assay methods tested, the most reliable and rapid means of determining the relative susceptibility and resistance of hop cultivars to this pathogen. In preliminary studies, when acetone precipitation was not done, results were inconsistent. When the filtrate was dialyzed (Spectraphor 1) against distilled water for 10 hr in the cold, activity was decreased or lost.

Filtration through cellulose nitrate filters also resulted in a loss of activity. The temperature at which the filtrate cultures were incubated was critical, if the temperature was not maintained within 5-10 degrees of 27 C, activity was lost. This may be expected because favorable temperature ranges for secondary metabolism are usually narrower than that for growth (9).

A consistent and significant difference in the reactions produced by the filtrate was found on the leaves of the most susceptible and most resistant hop cultivars tested. The relative order of resistance, as determined by years of field observation, was confirmed when detached leaves from four cultivars with variable levels of resistance were treated with the filtrate. A specificity of isolates for various hop cultivars and for other crops was suggested by the differential reactions produced on detached leaves by any one of a number of isolates.

The similar reactions to filtrates and noninoculated medium seen on some of the leaves tested, may be an artifact of the bioassay method, ie: a non-specific response to large molecular weight compounds (macromolecules) in the medium. It is possible, as suggested by Hodgson et al. (12), that some of the large molecular weight molecules accumulate in cells and interfere with cellular activity. These molecules might be more effective than smaller ones in blocking movement of solutions through membranes. Such accumulation

and interference with cellular activity may be responsible for the appearance of chlorosis on the control leaves. The susceptible hop cultivars, however, produced stronger reactions to the control treatment than did the resistant cultivars. The possibility of indigenous leaf microflora, growing in the extracted, noninoculated medium, causing the mild reactions on the control leaves cannot be dismissed.

The phytotoxic portion of _V. dahliae_ culture filtrates has been characterized as a large molecular weight protein-lipopolysaccharide (PLP) (25,26,36). The toxic activity and specificity produced by the PLP is due mostly to a low molecular weight polypeptide fraction (3000 daltons) which dissociates from the PLP under non-reducing conditions (34). Dialyzing and purifying the filtrate and noninoculated medium control would eliminate responses which were not due to the toxin because the non-specific sensitivity to the macromolecule(s) would not be observed. Others, however, have reported success in evaluating susceptibility and resistance to _V. dahliae_ with crude culture filtrates such as that used in these studies (11,36).

Autoclaving the culture filtrate resulted in decreased activity. This confirms the results of McLeod and Smith (32). Others, however, have reported no inactivation of the filtrate when it was autoclaved for 10 min (25) or boiled (25,36).

The effect of the culture filtrate on detached leaves of eggplant, tomato (resistant and susceptible varieties), and potato (unpublished results) did not correlate well with the results of the pathogenicity / host range study. A conidial root dip inoculation of eggplant resulted in high disease severity; the toxin, however, failed to elicit a reaction on detached eggplant leaves. The lack of response to the toxin may be due to the inability of the filtrate to sufficiently enter the leaf. Susceptible and resistant tomato cultivars could only be distinguished by their reaction to the toxin when the filtrate was diluted 10^{-1} and 10^{-2} (data not reported). Potato leaf tissue responded equally to the noninoculated medium control and culture filtrate from hop isolates, but the response to the culture filtrate from potato isolates was greater than the response to the control. Application of culture filtrate from a potato isolate reported to produce a toxic filtrate was obtained from A. Nachmias (Gilat Experiment Station, Negev, Israel) and applied to detached leaves of hop, tomato, potato, and eggplant (unpublished). The eggplant did not respond to the toxin; the potato responded equally to control and filtrate; and the tomato and hop leaves responded as they did to the application of culture filtrate from hop isolate N. In these experiments the culture filtrate produced the mildest reactions on the resistant hop cultivar Bullion. There is an inconsistency in the

correlation of the reaction of some crops when inoculated with the fungus and when culture filtrate is applied to detached leaves. This discrepancy may be due to different mechanisms of resistance in crops, or to the method of application of toxin.

Conclusions and recommendations

A rapid and reliable assay is needed because whole plant assays are laborious, require long incubation periods, and usually produce mild symptoms which are difficult to evaluate and are often not repeatable. The application of culture filtrates to detached hop leaves is both rapid and reliable and may be used to distinguish the relative susceptibility and resistance of hop cultivars to *V. dahliae*. This method correlated best with field observations on cultivar resistance to Verticillium wilt. In addition, culture filtrate assays may be useful in evaluating European isolates on American hop germplasm without having to bring the isolates to the United States.

Mechanical and physiological responses may be equally important in determining overall resistance. Keyworth (29) suggests that toxins may lower stem resistance. The toxin's effect might depend upon the extent of fungal growth in the vascular tissue of the roots. Stems of cultivars with different levels of resistance might be equally influenced by equal amounts of toxin (29), with the amount of toxin being determined by the amount of fungus in the xylem, which

is in turn determined by the relative resistance of the root and pathogenicity of the isolate. For this reason, until the mechanisms of resistance are better understood and experimental results are more conclusive, an initial screening of new plant material or isolates should be performed by using a combination of tests. This initial screening might include rooting hops in synthetic rooting blocks, inoculating them by wounding the roots and dipping into a conidial suspension, and planting them outside in a traditional hop yard testing block. Planting outside would be less labor intensive because the plants could be trained up strings rather than up plant stakes, and the plants would not need to be transplanted. Environmental effects could also be taken into account, with results being collected over a couple of seasons. While the plants are being tested in the yard, screening with culture filtrate could be done to give an indication of the physiological resistance. Other researchers have also suggested this combination of tests (11,39). Pegg (39) suggested that the study of systemic vascular wilts be approached by examining simple experimental systems, such as plant parts or cell free systems, to obtain clear results, and by studying whole plants with the hope that the results would correlate.

Table 1. *Verticillium dahliae* isolates used in the evaluation of assay systems for determination of the susceptibility of hop germplasm to Verticillium wilt.

ISOLATE	SOURCE HOST	SOURCE PERSON
C9	Hop	E. Shufelt[1]
X	Hop	E. Shufelt[1]
M8	Hop	E. Shufelt[1]
N	Hop	E. Shufelt[1]
B	Hop	E. Shufelt[1]
BV	Hop	E. Shufelt[1]
C2	Hop	E. Shufelt[1]
C31	Hop	E. Shufelt[1]
C35	Hop	E. Shufelt[1]
SJ	Hop	E. Shufelt[1]
P	Potato	J. Orlowsky[1]
V	Potato	M. Powelson[1]
Mt	Mint	J. Orlowsky[1]
V6a	Chrysanthemum	A. McCain[2]
V21	Norway Maple	C. Shufelt[1]

1. Oregon State University, Corvallis, Oregon
2. University of California, Berkeley, California

Table 2. Hop cultivars with various levels of resistance to Verticillium wilt, and native North American hop clones of unknown resistance.

CULTIVAR	LEVEL OF RESISTANCE
Hallertauer	Susceptible (S)
Columbia	Susceptible (S)
Fuggle	Susceptible (S)
Willamette	Moderately Resistant (MR)
Bullion	Resistant (R)
Cascade	Resistant (R)
Yakima Cluster	Resistant (R)
#38	Unknown
#47	Unknown
BC16	Unknown

Table 3. Means of leaf symptom ratings and growth parameters of eggplant (Solanum melogena var. Black Beauty) inoculated by root dip with different isolates of Verticillium dahliae, at different conidial concentrations.

Isolate[1,2] and concentration	Leaf[3] symptoms	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)
C9				
10^2	2.8bc[4]	7.8bcdef	12.5ef	11.3bc
10^4	4.0bcde	6.6bc	11.4bcd	11.1ab
10^6	3.1bc	5.4ab	11.1abc	10.9a
N				
10^2	3.3bc	9.0def	12.6ef	11.5bcd
10^4	4.3bcde	7.3bcde	11.8bcde	11.1ab
10^6	3.4bc	5.9b	11.5bcd	10.9a
X				
10^2	3.3bc	9.4f	13.0fg	11.7cde
10^4	7.2gh	5.9b	10.2a	11.2abc
10^6	8.3h	3.8a	10.9ab	10.8a
M8				
10^2	3.8bcd	9.5f	12.8ef	11.9de
10^4	6.4fg	7.0bcd	11.6bcd	11.2abc
10^6	5.1def	6.2bc	11.5bcd	11.0ab
P				
10^2	5.6fg	8.2cdef	12.6ef	11.4bcd
10^4	5.3def	7.9bcdef	12.1cdef	11.2abc
10^6	6.9gh	6.3bc	11.4bcd	10.9a
V				
10^2	3.1bc	9.3ef	13.0fg	12.0e
10^4	4.4cde	6.7bc	12.1cdef	11.4bcd
10^6	2.7b	7.0bcd	11.8bcde	11.2abc
Mt				
10^2	2.9bc	9.5f	12.7ef	11.5bcd
10^4	4.3bcde	8.2cdef	12.6ef	11.4bcd
10^6	4.0bcde	7.5bcdef	12.3def	11.2abc
CTRL	0a	11.6h	13.9g	12.0e

1. Hop: C9, N, X, M8; potato: P, V; Mint: Mt
2. Conidia/ml
3. Leaf symptoms: chlorosis and necrosis as evaluated by Horsfall-Barratt Scale (see text).
4. Values within a column not followed by the same letter are significantly different, P<0.01 (Fisher's protected L.S.D. test).

Table 4. Means of leaf symptom ratings and growth parameters of tomato (Lycopersicon lycopersicum var. Bonny Best) inoculated by root dip with different isolates of Verticillium dahliae, at different conidial concentrations.

Isolate[1,2] and concentration	Leaf[3] symptoms	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)
C9				
10^2	0.6abc[4]	52.2a	22.0a	13.0ab
10^4	0.7abc	59.1a	20.2a	13.3b
10^6	0.3ab	56.3a	19.8a	12.9ab
N				
10^2	0.7abc	53.7a	20.0a	13.3ab
10^4	1.3c	50.0a	21.2a	12.8ab
10^6	0.7abc	52.4a	20.7a	12.9ab
X				
10^2	0.3ab	48.8a	21.1a	13.6cd
10^4	0.2ab	51.8a	20.9a	13.3b
10^6	0.6abc	50.1a	22.0a	13.8d
M8				
10^2	0.3ab	48.4a	21.5a	13.2ab
10^4	0.8bc	54.2a	20.6a	13.0ab
10^6	0.3ab	55.4a	21.9a	13.1ab
P				
10^2	2.1d	51.5a	20.5a	12.9ab
10^4	1.2c	55.4a	20.9a	13.2ab
10^6	0.3ab	54.3a	21.3a	13.0ab
V				
10^2	0.0a	52.7a	22.7a	13.3b
10^4	0.1ab	56.3a	22.9a	13.1ab
10^6	0.0a	54.1a	23.9a	13.3b
Mt				
10^2	0.1ab	52.7a	22.5a	13.2ab
10^4	0.3ab	50.9a	22.2a	13.0ab
10^6	0.0a	56.4a	22.7a	13.1ab
CTRL	0.0a	49.8a	22.5a	13.3b

1. Hop: C9, N, X, M8; potato: P, V; mint: Mt
2. Conidia/ml
3. Leaf symptoms: chlorosis and necrosis as evaluated by Horsfall-Barratt Scale (see text).
4. Values within a column not followed by the same letter are significantly different, $P<0.05$ (Fisher's protected L.S.D. test).

Table 5. Means of leaf symptom ratings and growth parameters of tomato (Lycopersicon lycopersicum var. Ace 55 VF) inoculated by root dip with different isolates of Verticillium dahliae, at different conidial concentrations.

Isolate[1,2] and concentration	Leaf[3] symptoms	Plant height (cm)	Shoot dry weight (g)	Root dry weight (g)
C9				
10^2	0	46.1a	25.1a	12.7a
10^4	0	49.4a	25.1a	12.8a
10^6	0	43.7a	25.6a	12.5a
N				
10^2	0	41.7a	23.3a	12.8a
10^4	0	40.8a	24.4a	13.2a
10^6	0	46.5a	23.2a	13.1a
X				
10^2	0	41.8a	23.3a	12.9a
10^4	0	43.3a	23.3a	13.2a
10^6	0	42.4a	24.2a	12.8a
M8				
10^2	0	45.1a	24.4a	13.1a
10^4	0	46.8a	24.4a	13.0a
10^6	0	48.2a	24.8a	12.8a
P				
10^2	0	49.2a	25.1a	12.5a
10^4	0	47.2a	26.2a	12.6a
10^6	0	46.4a	26.2a	12.6a
V				
10^2	0	45.6a	26.1a	12.7a
10^4	0	41.1a	24.3a	12.9a
10^6	0	41.8a	25.7a	13.2a
Mt				
10^2	0	46.0a	24.4a	13.2a
10^4	0	50.9a	26.0a	12.4a
10^6	0	45.2a	25.8a	12.5a
CTRL	0	47.8a	25.8a	12.8a

1. Hop: C9, N, X, M8; potato: P, V; mint: Mt
2. Conidia/ml
3. Leaf symptoms: chlorosis and necrosis as evaluated by Horsfall-Barratt Scale (see text).
4. Values within a column are not significantly different, $P>0.05$ (Fisher's protected L.S.D. test).

Table 6. Mean root and bine dry weights of susceptible (S), moderately resistant (MR) and resistant (R) hop cultivars grown in field soil infested with Verticillium dahliae microsclerotia.

Cultivars	Inoculum density (ms/g soil)	Mean root dry weight[1] (g)	Mean bine dry weight[1] (g)
Columbia (S)	0	1.11a	0.46b
	50	1.33ab	0.35ab
	100	1.59b	0.30a
Fuggle (S)	0	1.22a	0.46ab
	50	1.36a	0.40a
	100	1.36a	0.50b
Willamette (MR)	0	1.29a	0.35b
	50	1.28a	0.21a
	100	1.27a	0.26a
Bullion (R)	0	1.32b	0.21a
	50	1.10ab	0.44b
	100	0.95a	0.41b
Yakima Cluster (R)	0	1.58b	0.53a
	50	1.03a	0.37a
	100	1.01a	0.42a
BC 16	0	0.85a	0.32a
	50	0.87a	0.31a
	100	0.91a	0.25a
# 47	0	0.76a	0.48b
	50	0.65a	0.35a
	100	0.72a	0.40a
#38	0	0.80a	0.32a
	50	0.97a	0.32a
	100	0.78a	0.24a

1. Values within a column not followed by the same letter are significantly different, at $P<0.05$ according to Fisher's protected L.S.D. test.

Fig. 1. Scale used to evaluate reactions produced by the application of *Verticillium dahliae* culture filtrates to detached hop leaves: 0=no reaction; 1=mild chlorosis; 2=distinct chlorosis; 3=necrosis, with or without chlorosis.

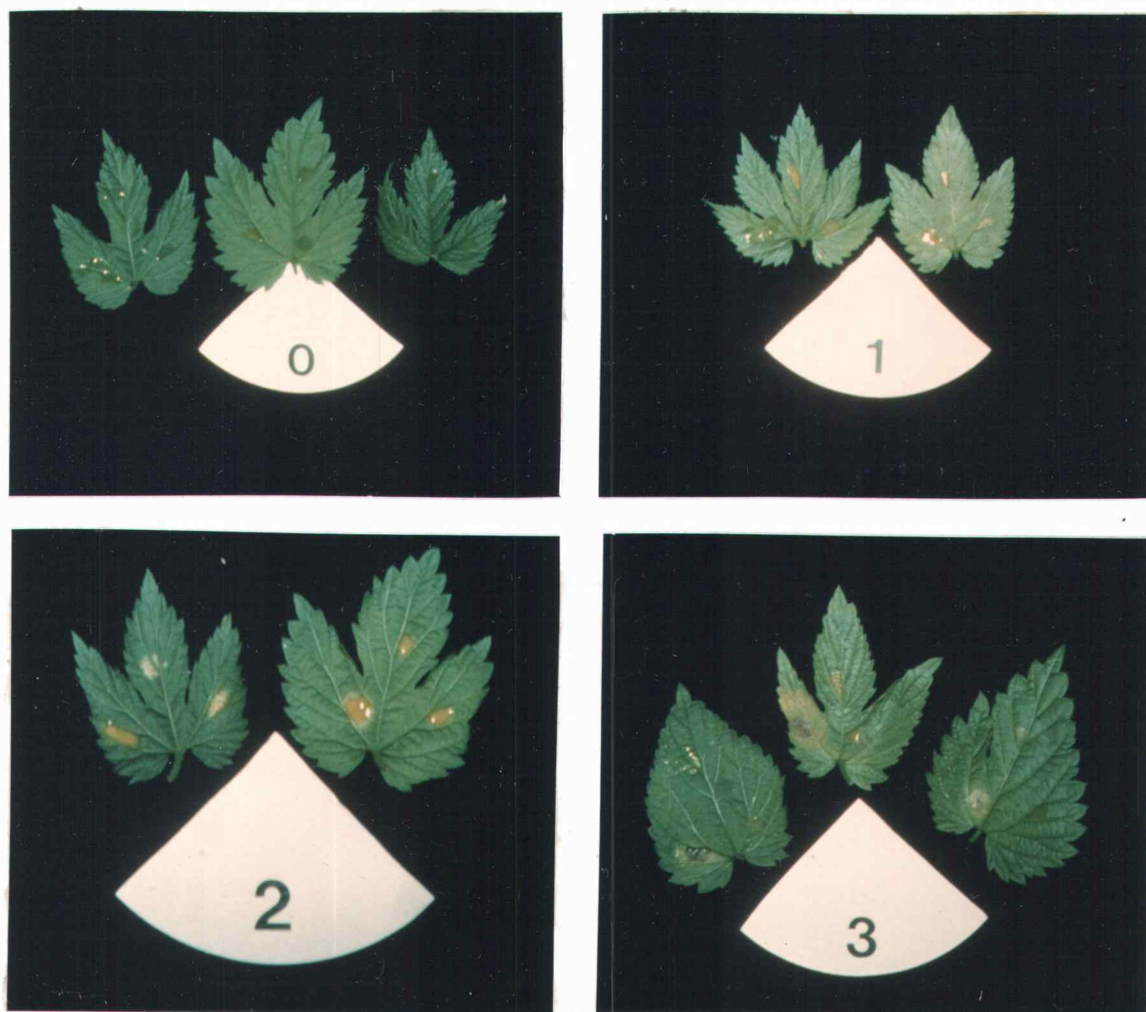

Fig. 1

Fig. 2. Recovery of <u>Verticillium</u> <u>dahliae</u> from susceptible hop cultivar Hallertauer inoculated by conidial root dip with C9, B, BV, X, M8 (hop), P and V (potato), V21 (maple), V6a (chrysanthemum, and Mt (mint). Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test.

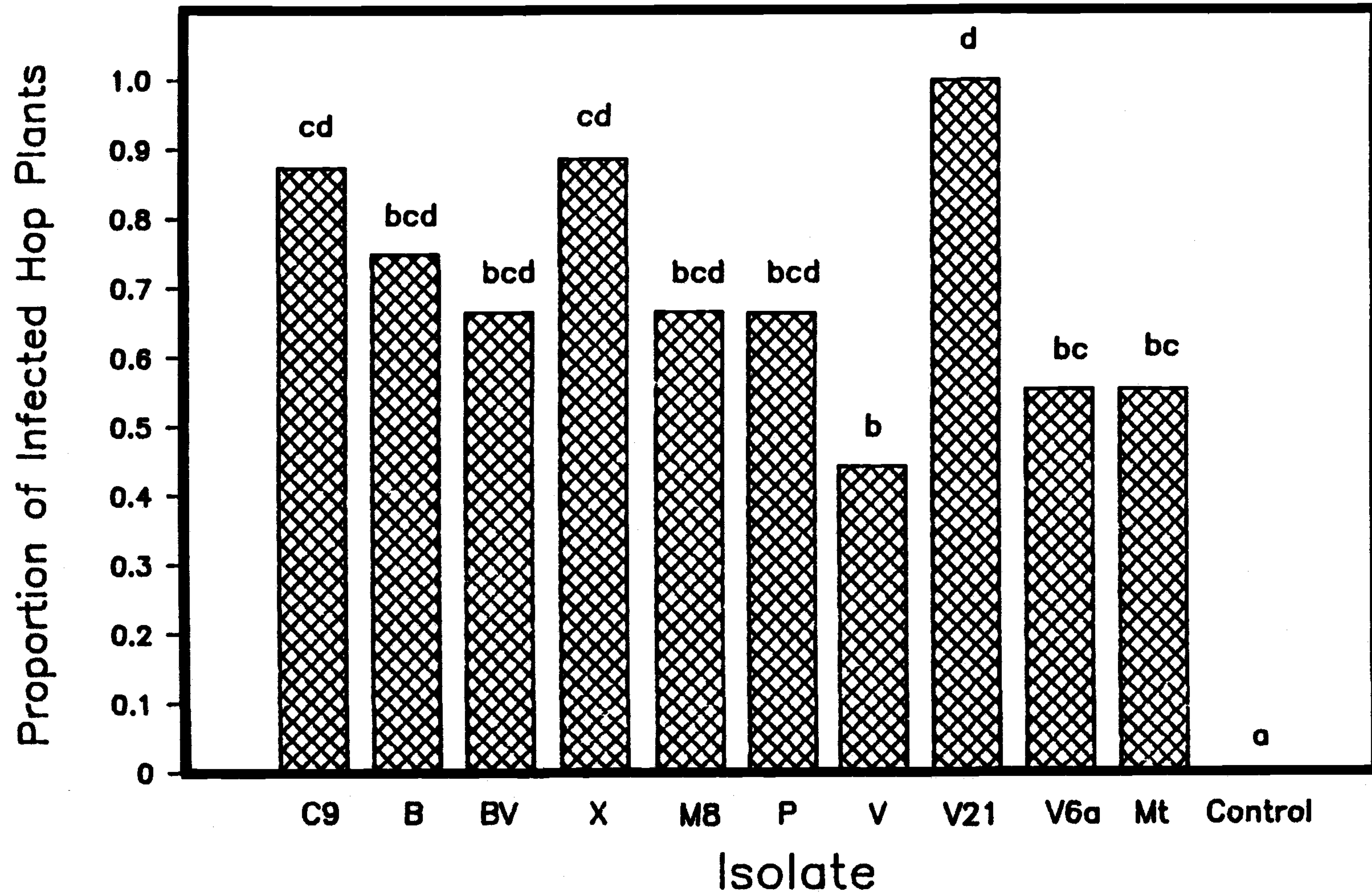

Fig. 2

Fig. 3. Reaction ratings to the application of ten-fold dilutions of culture filtrate from *Verticillium dahliae* isolate N (hop) on detached leaves of hop cultivars: Fuggle (S) (▪ ,———), Willamette (MR) (+ ,— — — —), and Bullion (R) (♦ ,— — —).

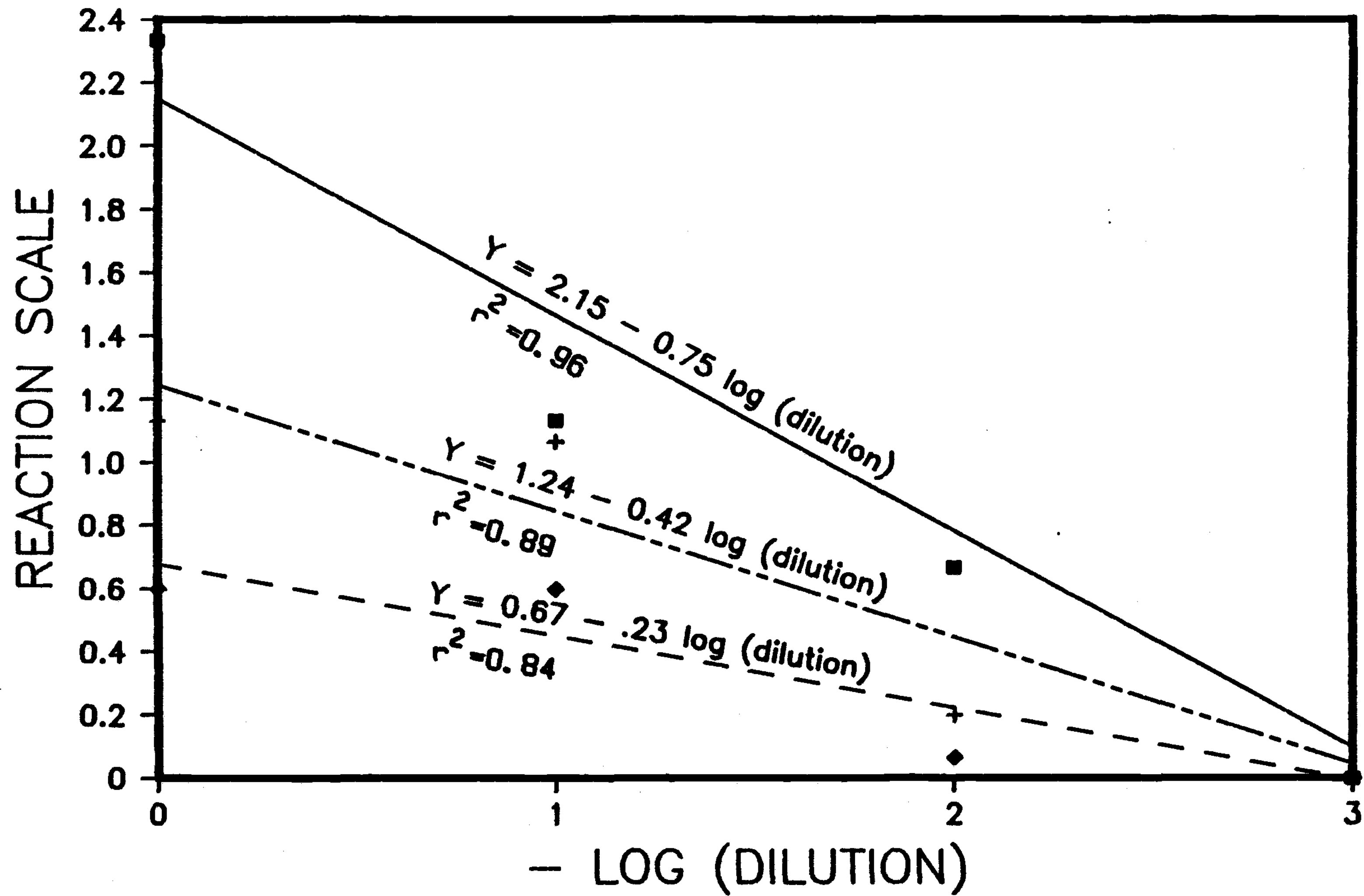

Fig. 3

Fig. 4. Reactions of detached leaves of hop cultivars: Fuggle (S) and Bullion (R) to the application of culture filtrates of Verticillium dahliae isolates C9, N, X, M8 (hop), P, V (potato), and Mt (mint). ☒ Filtrate, ◪ Control. Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test.

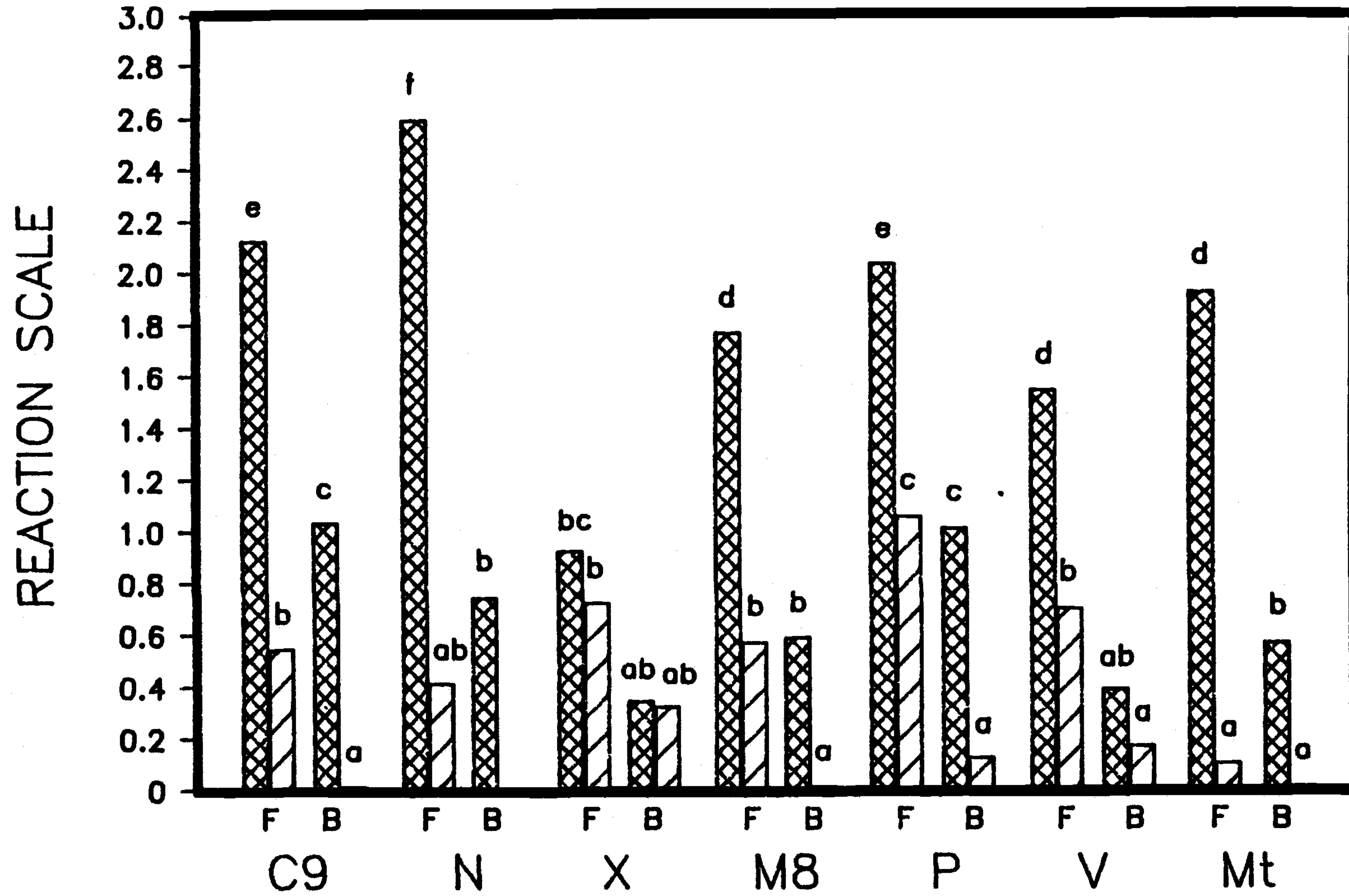

Fig. 4

Fig. 5. Reactions of detached leaves of hop cultivars: Fuggle (S), Willamette (MR), Bullion (R), and Yakima Cluster (R) to the application of culture filtrates of *Verticillium dahliae* isolates C9, N, and M8 (hop), and Mt (mint).

⊠ Filtrate, ▧ Control. Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test.

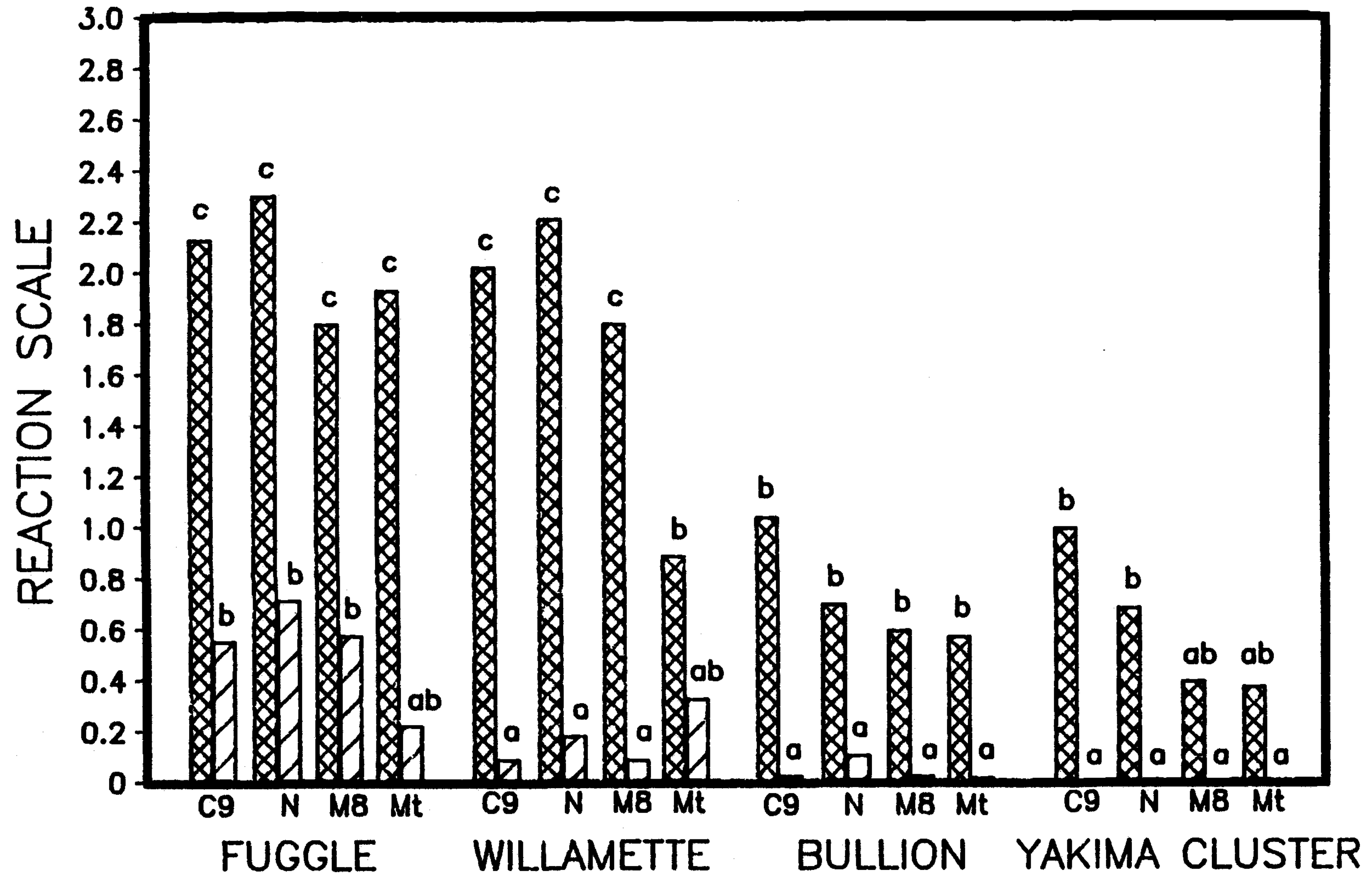

Fig. 5

Fig. 6. Reactions of detached leaves of hop cultivar Fuggle (S) to application of autoclaved, boiled and unheated culture filtrates of *Verticillium dahliae* isolate N (hop). Bars followed by the same letter(s) are not significantly different at $P<0.05$ according to Fisher's protected L.S.D. test.

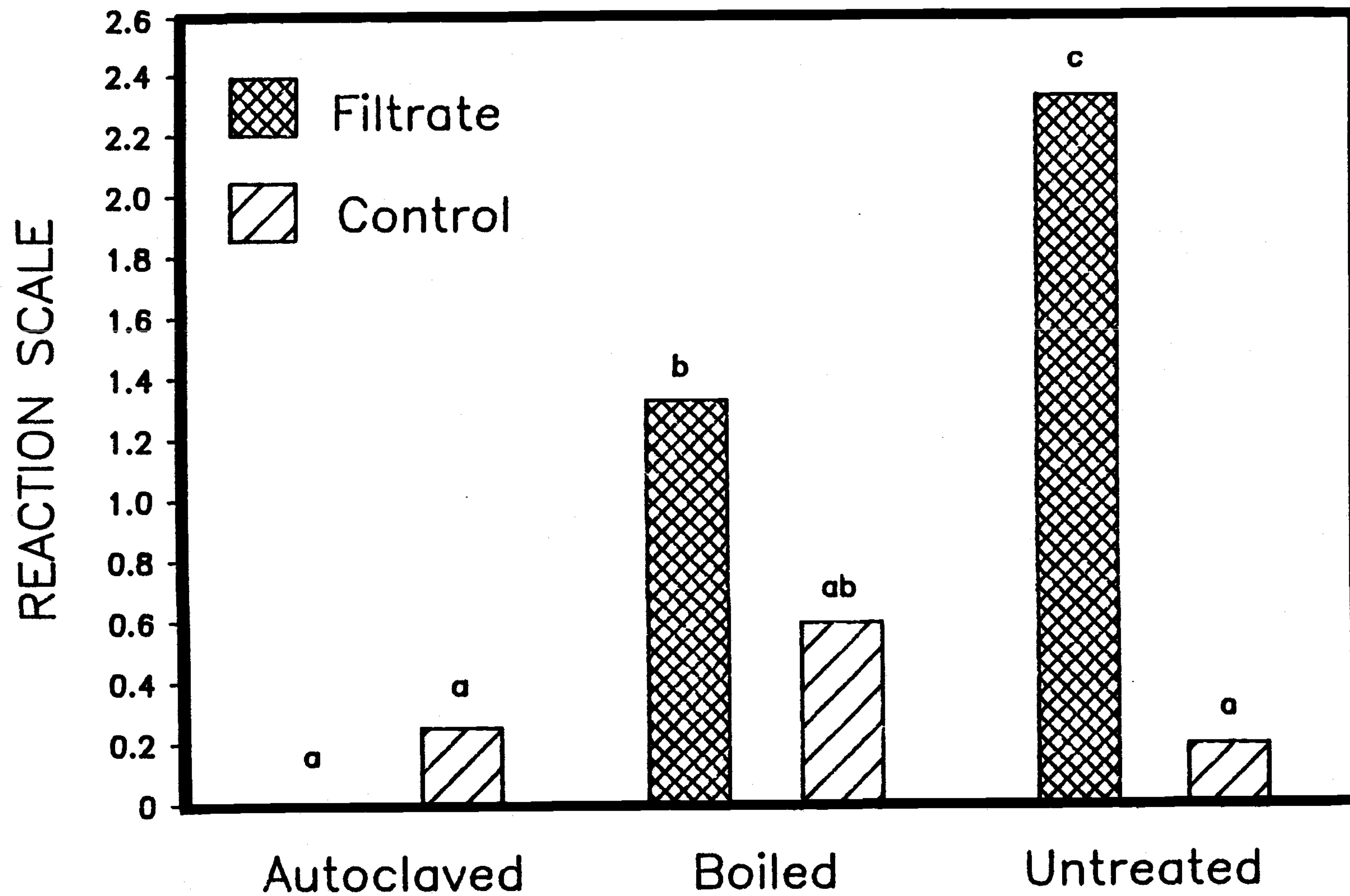

Fig. 6

LITERATURE CITED

1. Berry, S.Z. and C.A. Thomas. 1961. Influences of soil temperature, isolates, and methods of inoculation on resistance of mint to Verticillium wilt. Phytopathology 51:169-174.

2. Bewley, W.F. 1922. Sleepy disease of tomato. Annals of Applied Biology 9:116-134.

3. Blake, P.S.,L.D. Hunter, M.K. Davies, and P.W. Talboys.1982. Hop resistance to Verticillium wilt. East Malling Reports. East Malling Research Station. Nr. Maidstone, Kent.

4. Busch, L. V., and Schooley, H.D. 1970. Environmental influence on symptom expression in Verticillium wilt chrysanthemum. Canadian Journal of Botany 48:1939-1941.

5. Butterfield, E.J., and J.E. DeVay. 1977. Reassessment of soil assays for _Verticillium_ _dahliae_. Phytopathology 67:1073-1078.

6. Cronshaw, D.K. and Pegg, G. F. 1976. Ethylene as a toxin synergist in Verticillium wilt of tomato. Physiological Plant Pathology 9:33-44.

7. Edgington, L.V., and J.C. Walker. 1957. Influence of soil and air temperature on Verticillium wilt of tomato. Phytopathology 47:594-598.

8. Green, Ralph J.,Jr. 1954. A preliminary investigation of toxins produced in-vitro by Verticillium albo-atrum. Phytopathology 44:433-437.

9. Griffin, D. H. 1981. Fungal Physiology. John Wiley and Sons, New York. p.383.

10. Halisky, P.M., R.H. Garber, and W.C. Schnathorst. 1959. Influence of soil temperature on Verticillium hadromycosis of cotton in California. Plant Disease Reporter 43:584-587.

11. Hartman, C.L., and G.A. Secor. 1985. Comparative response of potato leaves, tuber tissue and stem callus to culture filtrates of Verticillium spp. Phytopathology 75:1377 (Abstr.).

12. Hodgson, R., W.H. Peterson, and A.J. Reiker. 1949. The toxicity of polysaccharides and other large molecules to tomato cuttings. Phytopathology 39:47-62.

13. Hop Production. Agriculture Information Bulletin No. 240. Ag. Research Service U.S. Dept. of Agriculture 1961.

14. Horner, C.E. 1962. Annual report hop research, U.S.D.A.,p.33.

15. Horner, C.E. 1965. Verticillium wilt of hops in Oregon. Plant Disease Reporter 49:538-540.

16. Horner, C.E. 1966. Annual report hop research, U.S.D.A., p.66.

17. Horner, C.E. 1969. Annual report hop research, U.S.D.A., p.74.

18. Horsfall, J.G., and R.W. Barratt. 1945. An improved grading system for measuring plant diseases. Phytopathology 35:655.

19. Huisman, O. C., and L.J. Ashworth, Jr. 1974. Quantitative assessment of *Verticillium albo-atrum* in

field soils: procedural and substrate improvements. Phytopathology 64:1043-1044.

20. Hung, K.S.,and N. J. Whitney. 1976. Leaf inoculation as a rapid means of screening potatoes for resistance to Verticillium wilt. International Verticillium Symposium, U.C. Berkeley, (Abstr.)

21. Hung, K.S., and N.J. Whitney. 1982. Reaction of two potato cultivars to leaf infection by Verticillium albo-atrum. Canadian Journal of Botany 60:554-556.

22. Issac I. 1949. A comparative study of pathogenic isolates of Verticillium. Brit. mycol. Soc. Trans. 32:137-157.

23. Isaac, I. 1953. A further comparative study of pathogenic isolates of Verticillium: V. nubilum Pethybr. and V. tricorpus sp. nov. Brit. mycol Soc. Trans. 36:180-195.

24. Jones, J.P., P. Crill, and R.B. Volin. 1975. Effect of light duration on Verticillium wilt of tomato. Phytopathology 65:647-648.

25. Keen, N.T., M. Long, and D.C. Erwin. 1972. Possible involvement of a pathogen-produced protein-lipopolysaccharide complex in Verticillium wilt of cotton. Physiological Plant Pathology 2:317-331.

26. Keen, N.T., and M. Long. 1972. Isolation of a protein-lipopolysaccharide complex from *Verticillium albo-atrum*. Physiological Plant Pathology 2:307-315.

27. Keyworth, W. G. 1950. Inoculation of plants with vascular pathogens. Nature 166:955.

28. Keyworth, W.G. 1942. Verticillium wilt of the hop (*Humulus lupulus*). Annals of Applied Biology 29:346-357.

29. Keyworth, W.G. 1953. Verticillium wilt of the hop. VI The relative roles of root and stem in the determination of wilt severity. Annals of Applied Biology 40:344-361.

30. Klebahn, H. 1913. Beitrage zur kenntnis der fungi Imperfecti. I. Eine *Verticillium*-Krankheit auf Dahlien. Mycologisches Zentralblatt. 3:49-66.

31. Mathre, D.E., D.C. Erwin, A.O. Palus, And A.V. Ravenscroft. 1966. Comparison of the virulence of isolates of Verticillium albo-atrum from several cotton growing regions in the United States, Mexico and Peru. Plant Disease Reporter 50:930-933.

32. McLeod, A.G., and H.C. Smith. 1961. Verticillium wilt of tobacco III. The effect of culture filtrates of Verticillium dahliae Kleb. on tobacco. New Zealand Journal of Agricultural Research 4:123-128.

33. Michail, S.H. and A.J.H. Carr. 1966. Use of culture filtrates as a rapid technique for screening lucerene for resistance to Verticillium albo-atrum. Trans. Brit. mycol. Soc. 49:133-138.

34. Nachmias, A., and V. Buchner and Y. Burstein. 1985. Biological and immunochemical characterization of a low molecular weight phytotoxin isolated from a protein-lipopolysaccharide complex produced by a potato isolate of Verticillium dahliae Kleb. Physiological Plant Pathology 26:43-55.

35. Nachmias, A., V. Buchner, L. Tsror, Y. Burstein, and N. Keen. 1987. Differential phytotoxicity of peptides from culture fluids of Verticillium dahliae Races 1 and 2

and their relationship to pathogenicity of the fungi on tomato. Phytopathology 77:506-510.

36. Nachmias, A., and V. Buchner, and J. Krikun. 1982. Comparison of protein-lipopolysaccharide complexes produced by pathogenic and non-pathogenic strains of Verticillium dahliae Kleb. from potato. Physiological Plant Pathology 20:213-221.

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

38. Otroshchenko, O.S., A.S. Sadykov, S.Z. Mukhamedzhanov, S.L. Komarevtsev, and N.N. Stepanichenko. 1974. Cytopathogenic metabolite of the fungus Verticillium dahliae. Khimiya Prirodnykh Soedinenii 6:779-780.

39. Pegg, G. F. 1981. Biochemistry and physiology of pathogenesis. In Fungal Wilt Disease of Plants (M.E. Mace, A.A. Bell, and C.H. Beckman, Eds.) Academic Press, New York. pp.640.

40. Provvidenti, R., and W. T. Schroeder. 1959. Foliage infection of tomato and eggplant by Verticillium. Plant Disease Reporter 43:821-826.

41. Reinke, J. and G. Berthold. 1879. Die zersetzung der kartoffel durch pilze. Universitat Gottingen, Untersuchungen Botanisches Laboratoruim. 1:1-100.

42. Schnathorst, W.C., and D.E. Mathre. 1966. Host range and differentiation of a severe form of Verticillium albo-atrum in cotton. Phytopathology 56:1155-1161.

43. Schnathorst, W.C. 1973. Nomenclature and physiology of Verticillium species, with emphasis on the V. albo-atrum versus V. dahliae controversy. In: Verticillium wilt of cotton. Proceedings of a work conference national cotton pathology research laboratory. College Station, Texas. ARS-S-19.

44. Sewell, G.W.F., and Wilson, J.F. 1964. Occurrence and dispersal of Verticillium conidia in xylem sap of the hop (Humulus lupulus L.). Nature (London) 204:901.

45. Stoddart, J.L. and A.J.H. Carr. 1966. Properties of wilt toxins produced by Verticillium albo-atrum Reinke & Berth. Annals of Applied Biology 58:81-92.

46. Talboys, P.W. 1958. Some mechanisms contributing to Verticillium-resistance in the hop root. Trans. Brit. mycol. Soc. 41:227-241.

47. Talboys, P.W. 1958. Association of tylosis and hyperplasia of the xylem with vascular invasion of the hop by Verticillium albo-atrum. Trans. Brit. mycol. Soc. 41:249-260.

48. Talboys, P.W., and J.F. Wilson. 1970. Effects of temperature and rainfall on the incidence of wilt (Verticillium albo-atrum) in hops. Annals of Applied Biology 66:51-58.

49. Ten, L.N., Tyshchenko, A.A., Stepanichenko, N.N., Gusakova, S.D., Mukhamedzhanov, S.Z., Otroshchenko, O.S., and Kas'Yanenko, A.G. 1977. Metabolites of the pathogenic fungus Verticillium dahliae. VII. Pentaketide metabolites and neutral lipids of virulent and avirulent strains. Khimiya Prirodnykh Soedinenii 5:632-635..

50. Ten, L.N., S.J. Mukhimedshanov, O.S. Otroshchenko. 1981. Structure of the peptide moiety of Verticillin. Khimiya Priordnykh Soedinenii. 4:476-479.

51. Thanassoulopoulos, C.C. 1967. Aspects on infection of Verticillium wilt of potatoes. Ph.D. thesis, Michigan State Univer., Ann Arbor.

52. Wellman, Frederick L. 1939. A technique for studying host resistance and pathogenicity in tomato Fusarium wilt. Phytopathology 29:945-956.

53. Wiles, A.B. 1973. Methods of inoculation and techniques for evaluation. In: Verticillium wilt of cotton. Proceedings of a work conference. ARS-S-19. pp. 134.

54. Zinkernagel, V. 1981. Some aspects concerning the susceptibility and tolerance of hop varieties against Verticillium wilt. In Pathobiology of Verticillium species. Ed. Matteo Cirulli. The Mediterranean Phytopathological Union. Bari, Italy. pp. 134.