AN ABSTRACT OF THE THESIS OF

Ingrid E. Berlanger for the degree of Master of Science in Botany and Plant
Pathology presented on November 22, 1999. Title: Effect of a Broccoli Green
Manure, Soil Solarization, and Isolates of *Verticillium dahliae* on Verticillium Wilt
of Agronomic and Nursery Crops.

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Green manures, soil solarization, and long rotations with crops not

susceptible to Verticillium wilt are among the disease management tactics currently

under investigation as alternatives to chemical fumigation of soil. The effect of a

broccoli green manure on soil microsclerotial populations of three isolates of

Verticillium dahliae and on Verticillium wilt of peppermint, potato, and red maple

was evaluated in a field study. Compared to the fallow control, soil populations of

V. dahliae declined by at least 30% ($P=0.0405$) following the incorporation of a

broccoli green manure (3.87-4.63 kg/m^2). Disease severity of potato was reduced

by up to 40%, ($P=0.0001$); however, disease severity of peppermint was not

affected by the broccoli green manure treatment. No symptoms of Verticillium wilt

were observed in red maple. Potato tuber yield was up to 38% greater following the

broccoli green manure compared to the fallow treatment ($P=0.2484$).

The effects of a broccoli green manure and of soil solarization, individually

and in combination, on soil populations of *V. dahliae* and on Verticillium wilt of

royal purple smokebush and amur maple were examined in a field study. Following

incorporation of the broccoli green manure (2.65 kg/m^2) and 2 mo of soil

solarization, soil populations of *V. dahliae* were 40% less (*P*=0.0377) in plots that

received the broccoli green manure treatment compared to fallowed plots, however,

the solarization treatment did not affect soil populations on any sampling date.

Disease severity of smokebush soon after symptom onset was 35% less (*P*=0.0264)

in plots which were solarized compared to nonsolarized plots; however, the

broccoli green manure treatment did not affect disease severity.

Aggressiveness of three isolates of *V. dahliae* on potato, eggplant, and

peppermint were evaluated in three field studies. An isolate recovered from potato

was more aggressive on potato than was an isolate recovered from maple

(*P*=0.0329) and more aggressive on eggplant than were isolates obtained from

maple and mint (*P*=0.0001). Mint and potato isolates were more aggressive on the

host from which they were isolated than the mint isolate was to potato and vice

versa. Inoculum density of the mint and potato isolates as a predictor of disease

severity was significant only in the host from which the isolate was recovered.

Effect of a Broccoli Green Manure,
Soil Solarization, and Isolates of
Verticillium dahliae on Verticillium Wilt of
Agronomic and Nursery Crops

by

Ingrid E. Berlanger

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CONTRIBUTION OF AUTHORS

Dr. Mary Powelson and Dr. Ken Johnson assisted in the experimental design, data analysis and interpretation, and writing of each manuscript. Dr. Jack Pinkerton and Kelly Ivors were involved in the study design and data collection of Chapter 3: Influence of a broccoli green manure and soil solarization on Verticillium wilt of smokebush and amur maple.

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INTRODUCTION

Control of Verticillium wilt, which is caused by the soilborne fungus, *Verticillium dahliae*, is problematic for growers of many crops including potatoes, eggplant, cauliflower, peppermint, red maple, and green ash. Within the species *V. dahliae*, isolates of the pathogen can vary in host range and relative aggressiveness on specific hosts. Host range has been shown to correlate with vegetative compatibility groups (VCG) that exist within *V. dahliae*. All isolates of *V. dahliae* produce long term survival structures, called microsclerotia, which can persist in the soil for several years. Chemical soil fumigation is an effective method of destroying the microsclerotia and suppressing disease, however, concerns about the toxicity of soil fumigants and future curtailments of their production have prompted researchers and growers to search for alternative disease management strategies.

Green manures, soil solarization, and long crop rotations with non-susceptible plant species are alternative strategies for suppression of Verticillium wilt that may play a larger role in disease management programs of the future. A green manure is any plant matter which is incorporated into the soil while it is still green. During the process of soil solarization, a transparent plastic film is placed over moist soil for several weeks during hot and sunny weather. The goal of both green manuring and soil solarization is to quickly reduce the number of

microsclerotia in the soil in order to suppress disease in subsequent crops. A third strategy, long rotations to non-susceptible crops, relies on the natural decline of *V. dahliae* microsclerotia in the soil over time. In the presence of susceptible crops, the number of microsclerotia in the soil quickly increases, however, when non-susceptible crops are planted, the number decreases slowly over several seasons. Knowledge of the VCGs of the *V. dahliae* isolates that make up the fungal population infesting a particular field and the relative aggressiveness of those isolates on various crops can contribute to the choice of non-susceptible rotation crops. In previous studies, various green manures, soil solarization, and long rotations have been shown to suppress fungal vascular wilt diseases in crops including cauliflower, potato, eggplant, and cabbage.

The objectives of my research are (i) to investigate whether a broccoli green manure is an effective strategy for suppression of Verticillium wilt of several crops which are economically important in the Williamette Valley of Oregon (peppermint, potato, and nursery commodities); (ii) to determine whether the suppression of Verticillium wilt using a broccoli green manure can be enhanced by utilizing soil solarization; and (iii) to assess the aggressiveness of isolates of *V. dahliae* obtained from potato, maple, and mint on potato, eggplant, and peppermint. The ultimate goal of this research is to decrease the dependence of growers on chemical soil fumigation by developing effective alternative disease management strategies.

CHAPTER 1

Literature Review

DISEASE SIGNIFICANCE

Verticillium wilt, caused by the soilborne fungus, *Verticillium dahliae*,

affects a wide range of dicotyledonous plant species, including herbaceous annuals

and perennials as well as many woody perennials (Powelson and Rowe, 1993). In

Oregon, Verticillium wilt of peppermint, potato, and nursery commodities causes

significant losses in yield or quality annually. Peppermint hay weight and oil yields

are decreased in fields infested with *V. dahliae* (Crowe et al., 2000). Currently,

there are few non-infested fields in peppermint production areas (Crowe, personal

communication). In the potato industry, yield losses of up to 30% have been

attributed to Verticillium wilt (Cappaert et al., 1992; Rowe et al., 1987).

Verticillium-infected nursery commodities, such as maple trees and smokebush,

undergo premature leaf discoloration, defoliation and death (Harris, 1998) and are

undesirable in home and public landscapes. Annual losses of up to 85% have been

attributed to Verticillium wilt in the nursery industry (Bedwell and Childs, 1938).

This disease is problematic to growers of an increasing range of crops. Symptoms

of Verticillium wilt were recently observed for the first time in green ash trees in

Oregon (Heffer, 1996) and in cauliflower in California (Koike et al., 1994).

PATHOGEN BIOLOGY

V. dahliae persists in the soil as microsclerotia, which can survive dormant in the soil for many years (Schnathorst, 1981) even in fallow fields. Triggered by the plant root exudates of both susceptible and non-susceptible plant species (Evans and Gleeson, 1973), these microsclerotia germinate. The resulting hyphae penetrate the plant near the root cap or in the region of elongation. The hyphae grow through the cortex and the endodermis toward the vascular tissue and may penetrate xylem vessel elements in susceptible plant species. Once the fungus reaches the xylem, it sporulates, producing conidia that move through the xylem during transpiration. Fungal colonization of the xylem in stems and petioles can block water transport to distal portions of the plant, resulting in wilting and foliar chlorosis and necrosis. Fungal metabolites, enzymes, and growth regulating compounds also damage host cells and plug the xylem, exacerbating symptoms of disease (Pegg, 1981). The first symptoms are often chlorosis of one or more of the lower leaves, followed by wilting and premature foliar senescence, caused by a reduction in the availability of water to the leaves (Harrison, 1971). Over time, foliar symptoms expand progressively up the plant and may result in stunted plants and/or premature death of the plant. Often, the symptoms occur on only one side of the leaf or the plant, suggesting blockage in only some of the vascular bundles within a stem petiole. Vascular browning is commonly observed in severely affected plants. Net photosynthesis is reduced following premature defoliation and can lead to reduced yields (Rowe et al., 1987). Microsclerotia begin to form on or within dying tissues

and are returned to the soil when the plant tissues decompose, leading to a buildup

of fungal inoculum (Schnathorst, 1981). Spread of the pathogen occurs by

movement of infected clonal stock and infested plant debris and soil (Rowe et al.,

1987).

CHEMICAL SOIL FUMIGATION

Chemical fumigation is commonly used to sanitize soil infested with

microsclerotia of *V. dahliae* (Harris, 1990; Wilhelm and Paulus, 1980). Methyl

bromide-chloropicrin and metam sodium are effective soil fumigants which have

been in use worldwide for years. However, the production of methyl bromide, a

volatile soil fumigant listed as an ozone-depleting compound in the Montreal

Protocol of 1993, will be halted in developed countries in the year 2010

(Anonymous, 1995). The regulatory potential also exists to curtail use of other soil

fumigants in the near future (Powelson and Rowe, 1993). Regulatory concerns

about use of soil fumigants include detrimental effects on soil fertility, water

quality, and human and animal health. These concerns, as well as the high cost of

fumigants, have prompted investigation into alternative disease management

strategies (Anonymous, 1995; Powelson and Rowe, 1993).

ALTERNATIVE STRATEGIES

Several non-chemical strategies for disease control are already utilized by many growers, often in combination with chemical soil fumigation. Verticillium wilt-resistant or tolerant potato and maple cultivars are available to growers (Corsini and Pavek, 1996; Townsend and Hock, 1973). Physical removal of plant debris by raking or propane flaming of crop residues immediately following harvest, a practice commonly utilized by growers of peppermint, prevents the accumulation of additional fungal inoculum in the soil (McIntyre and Horner, 1973). Managing soil fertility and limiting the application of irrigation water, especially prior to tuber initiation, can reduce disease severity in potatoes (Cappaert et al., 1992, 1994). In addition to these tactics, Verticillium wilt has been suppressed in infested soils by incorporating green manure crops such as broccoli (Subbarao and Hubbard, 1996) or sudangrass (Davis et al., 1996; Davis, Huisman et al. 1994) and by soil solarization (Katan, 1980). The success of these nonchemical management strategies for controlling Verticillium wilt provides evidence that agricultural production without chemical soil fumigants may be a viable option in the future.

GREEN MANURES

Green manures are plants which are plowed down while still green and incorporated into the soil. As the plant tissue decomposes, it can affect pathogen populations. Numerous plant species have been used as green manures for disease suppression with varying degrees of success including several legumes, vegetables, and cereal crops.

In several studies on sudangrass as a green manure, conflicting results have been reported. Foliar senescence in Idaho potato, due to Verticillium wilt, was significantly less after either a sudangrass or a corn green manure compared to a fallow treatment (Davis et al., 1996). In contrast, in the Columbia Basin of Washington, the severity of Verticillium wilt of potato was not suppressed with a sudangrass green manure compared to clean fallow soil (Cappaert and Powelson, 1997). These conflicting results may be due, in part, to environmental or soil type differences between the two locations (Parks, 1998).

Other researchers have also successfully reduced disease with sudangrass and other green manures. The experiments of Dillard (1985) demonstrated that planting to a vetch/rye, rye, or sudangrass green manure between lettuce crops significantly reduced the incidence of lettuce drop in California. Easton and Nagle (1987) examined the effect of both green pea and sudangrass as green manures. Although severity of Verticillium wilt symptoms in potato was not affected, vascular colonization by *V. dahliae* was decreased and tuber yield and quality increased in soil amended with both green manures.

Several of the *Brassica* species also have been used as green manure crops. Aphanomyces root rot of peas decreased and yield increased after two consecutive seasons of a white mustard green manure (Muehlchen et al., 1990). Soil populations of the pathogen, *Aphanomyces euteiches*, also were reduced. Chan and Close (1987) observed that Aphanomyces root rot of peas was less severe in soil amended with cabbage, kale, or rape than in nonamended soil, and that oospore survival declined as the time of exposure to decomposing *Brassica* tissue increased.

Subbarao et al. (1999) measured the effect of a broccoli green manure on *V. dahliae* soil populations and on cauliflower vigor. Soil populations declined and cauliflower plant height, numbers of marketable heads, and head weights increased in green manure treated soil. Furthermore, the green manure treatment was as effective or more than fumigation with chloropicrin or metam sodium.

Green manuring has additional benefits besides disease control. Following the incorporation of both a broccoli green manure and a broccoli green manure plus a spent mushroom compost, Sances and Ingham (1997) reported that weeds were less problematic and strawberry fruit yields were higher than in nontreated plots. Populations of root-knot nematodes also have been suppressed using rapeseed as a green manure treatment (Mojtahedi et al., 1991, 1993).

AMOUNT OF GREEN MANURE BIOMASS

The effectiveness of a green manure may be a function of the amount of plant biomass incorporated, either within one season or throughout numerous successive seasons. V. *dahliae* soil populations were reduced more following two successive broccoli crops than following only one (Xiao et al., 1998). Ramirez-Villapudua and Munnecke (1988) found *Fusarium oxysporum* soil populations declined to lower levels as the amount of cabbage green manure incorporated increased. Similarly, populations of root-knot nematodes decreased in proportion to the amount of rapeseed green manure biomass incorporated (Mojtahedi et al., 1993), and sclerotia of *Sclerotium rolfsii* decreased in proportion to the amount of alfalfa meal added (Johnson, 1953).

DRY VS. FRESH GREEN MANURES

Ramirez-Villapudua and Munnecke (1988) explored the effect of drying a green manure before incorporation on disease suppression potential. A fresh cabbage green manure required 10 days longer to achieve the same decrease in soil populations of *Fusarium oxysporum* as dried cabbage. The delay was attributed to the longer decomposition time of and slower glucosinolate production by fresh plant material. In contrast to these findings, Subbarao and Hubbard (1996) found that fresh broccoli residue was more effective than dry broccoli residue in

suppressing Verticillium wilt of cauliflower. They hypothesize that different volatile compounds are produced by dry vs. fresh plant material as they decompose (Fenwick et al., 1983). They also suggest that the increased energy consumption and cost associated with drying large quantities of plant material would make a fresh green manure the better choice.

MECHANISMS OF DISEASE SUPPRESSION

The mechanism by which a green manure reduces disease is unresolved. Proposed mechanisms include increased soil fertility, biological control, and natural soil fumigation. Incorporating plant material into the soil increases both the soil fertility and the soil microbial community. Balanced levels of nutrients including nitrogen and phosphorous are beneficial to plants (Davis et al., 1994; Pennypacker, 1989). Soil organic matter content and nutrient availability is increased after plant residues are added, often creating a more favorable environment for many soil microorganisms (Alexander, 1961). Populations of soil microorganisms, especially bacteria, increase rapidly in response to the newly available nutrient source (Alexander, 1961). Soils amended with a green manure generally have higher population counts of fungi, VAM, and bacteria and higher total microbial activity than chemically fumigated or nonamended soils (Cappaert and Powelson, 1997; Kirchner et al., 1993; Parks, 1998; Sances and Ingham, 1997). Many of these microorganisms, including *Bacillus, Pseudomonas, Flavobacterium,*

and *Gluconobacter* species, are antagonistic to *V. dahliae* and other pathogens in vitro (Azad et al., 1985; Baker and Cook, 1974).

Living plant material also stimulates antagonistic soil microbial activity. Potato cultivars resistant to Verticillium wilt have higher bacterial population counts than less resistant cultivars; plant root exudates from resistant cultivars are thought to stimulate the proliferation of bacteria antagonistic to *V. dahliae* (Azad et al., 1985).

In addition to increasing the fertility and biological activity of the soil, a green manure may work as a natural soil fumigant. For example, as the plant tissues of many species within the Cruciferae family decompose, sulfur-containing compounds are released (Kjaer, 1976). These glucosinolates may be further broken down by the enzyme, myrosinase. Fungistatic or fungicidal compounds such as allyl isothiocyanate are produced. Allyl isothiocyanate is structurally similar to and comparably as fungitoxic as methyl isothiocyanate, an ingredient in some chemical soil fumigants (Brown et al., 1991; Brown and Morra, 1997). Cabbage, broccoli, and cauliflower produce many of the same volatile compounds (Buttery et al., 1976).

Different Cruciferous species vary in their disease suppressiveness. Vaughn et al. (1993) found that black mustard and Indian mustard produced allyl isothiocyanate which inhibited growth of *Helminthosporium solani*, the cause of potato silver scurf, as well as methyl isothiocyanate, while rape produced 3-butenyl isothiocyanate which did not inhibit growth. In other studies, cabbage, kale,

mustard, and turnip reduced pea root rot more than did Brussels sprouts and radish (Papavizas, 1966; Papavizas and Lewis, 1971).

Mayton et al. (1996) found that the fungicidal effect of the leaf tissues of various *Brassica* species was correlated with the concentration of allyl isothiocyanate emitted from the tissue. All species with concentrations greater than 0.10 mg/g of leaf tissue were suppressive to the pathogen, *Fusarium sambucinum*. Similarly, suppression of the plant pathogenic nematode, *Meloidogyne chitwoodi*, by a rapeseed (*Brassica napus*) green manure increased as the age and the concentration of glucosinolates in the tissues of the rapeseed increased (Mojtahedi et al., 1993). While high concentrations of glucosinolates in a green manure may be desirable for disease suppression, feeding large amounts of plant material containing high glucosinolate concentrations to non-ruminant animals can be toxic (Fenwick et al., 1983). Selective breeding for oilseed rape with low glucosinolate concentrations is underway. However, an oilseed rape cultivar with low glucosinolate concentrations had more extensive symptoms of dark leaf spot (*Alternaria brassicae*) than did another cultivar with a high concentration (Doughty et al., 1991). This suggests a potential involvement of the volatile compounds contained in Cruciferous species in resistance to infection by pathogenic microorganisms.

SOIL SOLARIZATION

Another alternative to chemical soil fumigation for disease control is soil solarization. Soil temperatures can be elevated by covering moist soil with transparent plastic sheets for several weeks during dry and sunny weather. Solarization reduces soil populations of many microorganisms, including *Fusarium oxysporum* f. sp. *vasinfectum* and *V. dahliae*, by up to 100% (Davis and Sorensen, 1986; Katan et al., 1976, 1983; Pullman et al., 1981).

Ashworth and Gaona (1982) evaluated the effect of soil heating by clear polyethylene mulching in established pistachio nut groves. *V. dahliae* soil populations were reduced by high temperatures to depths of 40 cm after 6 wk. In a second experiment, the effectiveness of polyethylene sheets when placed with gaps of varying size between them was compared to overlapping polyethylene sheets. Soil populations were reduced most when sheets overlapped. They did not observe any effect of tarping on tree growth. Fusarium wilt in cotton and Verticillium wilt in eggplant and tomato have also been significantly reduced following soil solarization (Katan et al., 1976, 1983). Yields of eggplant, potato, cotton, and other crops often increase following soil solarization in soils infested with *F. oxysporum* or *V. dahliae* (Katan, 1980; Katan et al., 1983). The effectiveness of soil solarization is decreased in areas where frequent summer rains and cloud cover prevent high soil temperatures (Chellemi et al., 1994); however, in regions of dry and warm summers such as the Williamette Valley of Oregon, soil solarization may be an effective disease management strategy.

TREATMENT COMBINATIONS

The effectiveness of a broccoli green manure treatment may be enhanced when combined with other cultural control practices. Verticillium wilt of cauliflower following a broccoli green manure treatment combined with a deficit irrigation regime was significantly lower than the green manure treatment with a moderate or excessive irrigation regime (Xiao et al., 1998).

Broccoli green manure and soil solarization treatments individually have been shown to suppress disease. Pathogen populations and disease severity may be less in soils treated with a green manure accompanied by soil solarization than in soils with only a single treatment. Environmental conditions such as prolonged cloud cover and rainfall sometimes prevent tarped soils from reaching temperatures optimal for pathogen population reductions. Significant pathogen population declines may still be achieved at lower soil temperatures if a green manure is first incorporated (Keinath, 1996). Conversely, when the amount of broccoli biomass incorporated into soil is less than optimal for disease suppression, due to inadequate cultural or environmental conditions, better disease suppression may be achieved when the soil is also solarized.

Some studies, however, have found that the combination of a green manure and soil solarization did not decrease disease more than solarization alone. Coelho et al. (1999) reported that cabbage amendment did not add to the effectiveness of the soil solarization treatment; however, they hypothesize that this lack of effect

was due to insufficient amounts or to the uneven incorporation and decomposition of the cabbage biomass.

Gamliel and Stapelton (1993) found that soil populations of *Pythium ultimum* and *Sclerotium rolfsii* decreased more in cabbage green manure amended soils when soil temperatures were raised, either by solarization or in a water bath, than in nonheated soils. Analyses of the volatile compounds evolved from each soil treatment showed that soils which were amended with cabbage after soil heating contained isothiocyanates and other sulfur-containing compounds, whereas nonheated cabbage amended soils did not. A wider range and higher concentrations of volatile compounds were produced as temperatures of the solarized soils increased. Higher temperatures increased the vapor pressure of volatile compounds released during cabbage green manure decomposition, resulting in higher concentrations of phytotoxic compounds in the soil. Microbial activity, however, was less in heated than in nonheated soil, indicating that solar and direct heating may have destroyed beneficial microorganisms along with pathogens.

Incorporation of dry cabbage residue, followed by soil solarization, decreased *Fusarium oxysporum* f. sp. *conglutinans* population size and cabbage yellows disease incidence better than cabbage or soil solarization alone (Ramirez-Villapudua and Munnecke, 1987). The cabbage green manure treatment alone did not decrease disease severity compared to the nontreated controls, suggesting that tarping may be valuable not only in raising soil temperatures, but also in preventing the loss of volatile fungicidal compounds produced by decomposing green manures.

In contrast, some experiments with green manures followed by soil solarization found no differences between tarped and nontarped treatments. Subbarao et al. (1999) found that *V. dahliae* soil populations decreased in broccoli amended soil, but that tarping did not have an additional effect, indicating that trapping the volatile compounds produced by broccoli decomposition did not increase the effectiveness of the broccoli green manure treatment. Soil temperatures were not increased by tarping the soil, therefore no conclusions can be drawn from this study on the effect of soil solarization on the efficacy of a green manure.

Keinath (1996) observed a greater decrease in gummy stem blight of watermelon and a greater increase in plant size and fruit yield when infested soil was amended with cabbage and solarized than when soil was rotated to wheat and soybean for two seasons or continuously cropped to watermelon. This study did not examine cabbage green manure and soil solarization separately.

An additional benefit of soil solarization is often weed control (Katan et al, 1976; Katan, 1980; Ramirez-Villapudua and Munnecke, 1987). The effect of soil solarization may last for multiple seasons; severity of Fusarium wilt in cotton was consistently less in solarized than nonsolarized field plots for 2 yr following one season of solarization (Katan et al., 1983).

VEGETATIVE COMPATIBILITY GROUPS

Within the species, *Verticillium dahliae,* isolates with varying morphology and pathogenicity have been identified (Okoli et al., 1993, 1994). Isolates have been classified into four or five vegetative compatibility groups (VCG), some of which may further be divided into two subgroups (Bhat and Subbarao, 2000; Joaquim and Rowe, 1991). A VCG consists of isolates which have the ability to anastomose and form heterokaryons with each other (Joaquim and Rowe, 1990). The exchange of genetic information between fungal isolates is blocked by vegetative incompatibility. In this way, vegetative compatibility grouping helps to maintain genetic diversity within the species (Barbara et al., 1998).

Isolates within each VCG are generally associated with a related group of plant hosts and may not be as pathogenic to plant species outside this range. For example, VCG 2 isolates are very pathogenic to peppermint, but may be less pathogenic to other species (Green, 1951). Joaquim and Rowe (1991) assigned 187 *V. dahliae* isolates into one of three VCGs using nitrate-nonutilizing (*nit*) mutants. In their study, isolates obtained from maple, mint, and potato were assigned to VCG 1, 2, and 4, respectively. Furthermore, VCG 4 was split into two sub-divisions based on the severity of the disease on potato. Isolates of VCG 4A caused extensive chlorosis and necrosis and stunting of potato and could kill plants within 42 days, whereas isolates of VCG 4B caused milder symptoms, usually only limited chlorosis without associated stunting or death.

CROSS PATHOGENICITY

Isolates from different VCG groups affect some hosts more severely than others. Subbarao et al. (1995) tested the pathogenicity of nine isolates from other hosts on cauliflower and of two isolates from cauliflower on 15 plant species. While finding no absolute host specificity in any isolate tested, disease severity was less when hosts were infected with isolates belonging to a different VCG than that of their own corresponding isolate. An isolate from cabbage, a Cruciferous species, was more aggressive on cauliflower and other crucifers, except broccoli and Brussels sprouts, than isolates from tomato, potato, strawberry, watermelon, pepper, cotton, or artichoke.

Since *V. dahliae* isolates belonging to separate VCG groups may have differential virulence and may therefore have diverse genetic codes (Bhat and Subbarao, 2000), the question of whether they also respond differently to control strategies, such as green manures, warrants study. A broccoli green manure has been shown to be effective at suppressing Verticillium wilt of cauliflower caused by a cauliflower isolate (Subbarao et al., 1999), but further research is needed to determine whether the treatment is equally effective for control of disease in peppermint, potato, and red maple caused by each of their respective isolates. Knowing which isolates affect which host(s) is also valuable information for growers looking to avoid planting species susceptible to the *V. dahliae* isolates which were left behind in the soil by previous infected crops.

INOCULUM DENSITY

As soil populations of *V. dahliae* increase, disease severity increases (Nnudo and Harrison, 1979). Some studies have reported that there is a minimum number of *V. dahliae* microsclerotia required to cause significant yield and quality losses, below which no significant losses occur. Nnudo and Harrison (1979) found this minimum to be 17.5-23 propagules/g of soil for potato in Colorado. The minimum number, however, may vary depending upon the isolate, host, and environment. It is possible that isolates originating from one host may cause more or less disease in a different host, even at the same inoculum density, and that equal amounts of different isolates may cause different levels of disease, even in their host of origin.

OBJECTIVES

The primary objectives of these studies were to (i) assess the effect of a broccoli green manure on Verticillium wilt of agronomic and nursery plant species (peppermint, potato, red maple) and determine whether the effect is greater with higher amounts of broccoli green manure biomass; (ii) evaluate the efficacy of a green manure coupled with soil solarization on Verticillium wilt of woody perennial nursery plant species (royal purple smokebush and amur maple); and (iii) determine the aggressiveness of *V. dahliae* isolates obtained from potato, maple,

and mint on potato, eggplant, and peppermint. This information will help to clarify whether a broccoli green manure is an effective disease management option for a range of plant species, whether its effectiveness can be enhanced by increasing the amount of green manure biomass or by coupling it with soil solarization, and whether Verticillium wilt can be avoided using crop rotation with species resistant to all or some isolates of *Verticillium dahliae*.

CHAPTER 2

Influence of a Broccoli Green Manure on Verticillium Wilt of Peppermint, Potato, and Red Maple

Ingrid E. Berlanger, Mary L. Powelson, and Kenneth B. Johnson

ABSTRACT

Efficacy of a broccoli green manure on reducing soil microsclerotial

populations of *Verticillium dahliae*, on root and stem colonization by *V. dahliae*,

and on severity of foliar symptoms of Verticillium wilt of peppermint, potato, and

red maple was evaluated in a field study conducted in Corvallis, Oregon in 1997-98

and in 1998-99. Approximately 4.2 kg/m^2 of chopped broccoli stems and leaves

(2% weight of broccoli: total weight of broccoli plus soil) was incorporated into

soil artificially infested with *V. dahliae*. Broccoli green manure amendment led to

reductions of up to 82% ($P \leq 0.10$) in measurable numbers of *V. dahliae*

microsclerotia compared to fallow controls. Colonization of potato roots by *V.*

dahliae was 88% ($P=0.0316$) and 75% ($P=0.2371$) less in broccoli-amended

compared to fallowed plots in 1997-98 and 1998-99, respectively; colonization of

peppermint and red maple roots and colonization of stem tissue of peppermint and

potato were not affected by the broccoli green manure treatment. Disease severity

of potatoes was 40% ($P=0.0001$) and 13% ($P=0.3788$) less and tuber yield was

10% ($P=0.5387$) and 38% ($P=0.2484$) higher in broccoli-amended plots in 1997-98

and 1998-99, respectively. Disease severity of peppermint was not affected by the

broccoli green manure treatment. No symptoms of Verticillium wilt were observed

in red maple.

The effect of increasing amounts of broccoli green manure biomass on soil

populations of *V. dahliae* was evaluated. Field soil infested with microsclerotia of

V. dahliae was amended with either 0, 0.28, 0.56, 1.12, or 2.24 kg broccoli stems

and leaves per L of soil (0, 2, 4, 8, or 16%) and incubated in a growth chamber at

$24 \pm 2°C$ for 19 days. The experiment was conducted twice. Soil populations of *V. dahliae* decreased more when larger amounts of broccoli biomass were added to the soil. The amount of broccoli green manure biomass incorporated was a significant predictor of measurable numbers of *V. dahliae* microsclerotia remaining in the soil (R^2=0.8646 and 0.7099).

INTRODUCTION

Verticillium wilt, caused by the soilborne fungus, *Verticillium dahliae*, leads to significant yield or quality losses in numerous plant species, including peppermint, potato, and maple. Current control strategies rely heavily on chemical soil fumigants such as methyl bromide, chloropicrin, and metam sodium to destroy microsclerotia, the long-term survival structure of *V. dahliae*. These compounds are effective, but are expensive and highly toxic (Gamliel et al., 1997; Harris, 1990). The production of methyl bromide, a volatile soil fumigant which contributes to ozone depletion, will be halted in developed countries in the year 2010 (Anonymous, 1995), and the use of other soil fumigants also may be curtailed (Powelson and Rowe, 1993). The development of non-chemical alternative disease management strategies, such as green manures, is vital to meeting the agricultural and horticultural needs of the future.

The incorporation of various green manure crops, such as sudangrass (Davis et al., 1996), white mustard (Muehlchen et al., 1990), and broccoli (Subbarao et al., 1999), has reduced the severity of plant diseases including Verticillium wilt. Using a broccoli green manure to control Verticillium wilt in cauliflower, *V. dahliae* soil populations have been reduced and cauliflower yield increased in California (Subbarao et al., 1999). Furthermore, the green manure treatment was equal to or more effective than fumigation with chloropicrin or metam sodium (Subbarao et al., 1999).

Research on broccoli as a green manure has been limited to suppression of cauliflower isolates of *V. dahliae* and Verticillium wilt of cauliflower. The purpose

of this study was to examine the efficacy of a broccoli green manure as a disease management strategy for Verticillium wilt for three host species, peppermint, potato, and red maple. Additionally, we wanted to identify the dose-response relationship between the amount of broccoli green manure biomass incorporated and *V. dahliae* soil populations. The effectiveness of a green manure in reducing soil populations has been shown to be a function of the amount of plant biomass incorporated, either within one season or throughout numerous successive seasons (Johnson, 1953; Mojtahedi et al., 1993; Ramirez-Villapudua and Munnecke, 1988; Xiao et al., 1998). A broccoli green manure treatment which is ineffective at suppressing disease at low amounts of biomass may be more effective if the amount of green manure biomass incorporated into the soil is increased.

MATERIALS AND METHODS

Inoculum production. *V. dahliae* isolates, recovered from symptomatic peppermint, potato, and red maple plants, were maintained on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). Single spore cultures of each isolate were grown on Bacto Czapek-Dox (Difco Laboratories, Detroit, MI) agar at room temperature (20-24°C) in the dark for 2 wk in the spring of 1997, 1998, and 1999. Each plate was flooded with 20 ml sterile distilled water, and the surface was rubbed gently with a glass stirring rod to release the conidia. Aliquots (1 ml) of the conidial suspension were transferred onto plates of *Verticillium* inoculum medium

(VIM) (Puhalla and Spieth, 1983) overlain with sterile, uncoated cellophane (Bio-Rad, Hercules, CA). After 3 wk of growth at 20-24°C in the dark, the microsclerotia-covered cellophane was removed and chopped in a blender with distilled water for 1 min. The suspension was poured through nested screens (1.18 and 0.5 mm mesh), rinsed with distilled water to remove the cellophane, and dried in white plastic weighing boats at room temperature for 1-2 wk. The resulting inoculum concentrate was then ground in a Wiley™ Mill (Arthur H. Thomas Co., Philadelphia, PA) with a #40 mesh screen and stored in plastic bags at room temperature in the dark for 3-10 wk.

A total of 0.01 g of inoculum concentrate of each isolate was mixed by hand with 9.99 g sterile sand; 0.1 g aliquots were then serially diluted with 9.9 g sterile sand. From the 10^{-2} and 10^{-3} dilutions, 0.17 g aliquots were distributed onto each of 10 petri plates containing Sorensen's NP-10 (Sorensen et al., 1991) plates using the Anderson air sampler (Andersen Samplers Inc., Atlanta, GA) technique (Butterfield and DeVay, 1977). Plates were incubated at room temperature in the dark for 2 wk. The surface of each plate was then washed with running water to remove adhering sand particles. Number of colony forming units of *V. dahliae* per gram of soil (CFU/g) was enumerated with the aid of a dissecting microscope. Inoculum for infestation of field plots was prepared by diluting the inoculum concentrate with pasteurized sand.

Field study. Field plots were established in 1997 and 1998 at the Department of Botany and Plant Pathology Research Farm, Corvallis, OR in a sandy loam soil.

The soil did not have a measurable population of *V. dahliae*. Three *V. dahliae* isolates were combined factorially with two green manures for a total of six treatments. Putative vegetative compatibility groups for the *V. dahliae* isolates obtained from maple, mint, and potato are VCG 1, VCG 2, and VCG 4, respectively (Joaquim and Rowe, 1991). In 1998, a non-infested control was added to the *V. dahliae* isolate treatment. The green manure treatments were broccoli green manure or weed free fallow. Treatments were arranged in a randomized complete block design and replicated five times.

Plots were 1.84 m x 1.84 m and separated by a 2.76 m wide border of grass (60% Chewings fine fescue; 40% Nui perennial ryegrass). Thirty plots were established in 1997 and 40 in 1998. Soil samples were collected with a hand trowel in the spring of each year prior to planting for determination of soil nutrient status. In the first year of each experiment, one sample, comprised of 20 randomly collected soil cores from the entire field, was collected. In the second year of each experiment, one sample was collected from the broccoli-amended plots and one from the fallowed plots. Each of these two samples was comprised of two soil cores from each plot belonging to that treatment. Soil nutrients (phosphorous, potassium, calcium, magnesium, manganese, sodium, zinc, percent organic matter, total Kjeldahl nitrogen, pH, and soluble salts) were determined by the Central Analytical Laboratory, Department of Crop and Soil Science, Oregon State University (Table A1). Fertilizer was applied according to the OSU Extension Service recommendations. The fertilizers N, P_2O_5, K_2O, and boron were broadcast by hand at rates of 112, 140, 120, and 11 kg/ha, respectively.

Inoculum of each *V. dahliae* isolate was applied by hand to the soil surface of each plot at a rate of 20 and 0 or 40 CFU/g field soil in late May 1997 and 1998, respectively. The inoculum was immediately incorporated into the soil to a depth of 15 cm using a rototiller. Broccoli seedlings (*Brassica oleracea* L. var. *italica* cv. Excelsior) (Northwest Transplants, Molalla, OR) were transplanted at a four-leaf stage in early June to half of the plots. Transplants were spaced 25 cm apart within rows, and rows were spaced 51 cm apart (28 plants/plot). The remaining plots were maintained as weed-free fallow.

Weeds in both the broccoli and fallow plots were controlled principally by hand weeding. In 1998, each plot also was sprayed with sethoxydim (Poast®, BASF Corporation, Research Triangle Park, NC) on 2 July at a rate of 1.75 L/ha using a backpack sprayer to control monocot weeds. Slug damage was controlled by sprinkling metaldehyde (Corry's Slug and Snail Death, E.M. Matson Jr. Co., Inc., Seattle, WA) around each broccoli plot 1 wk after planting. Plots were irrigated 2-3 times each week as necessary until broccoli heads were fully formed. Two weeks prior to incorporation of the broccoli green manure, urea (46-0-0) at 269 kg/ha was side-dressed by hand, and plots were watered immediately.

Broccoli heads were harvested by hand in 1997, but were not harvested in 1998, in order to increase the amount of biomass incorporated into the soil. In 1997, the amount of biomass incorporated was determined by weighing five plants selected at random in each plot. In 1998, biomass was determined by weighing all the plants in five randomly selected plots. The harvested plants were returned to each plot for incorporation. Plants were chopped into small pieces using a flail, and

immediately incorporated into the soil to a depth of 15 cm using a rototiller. The amount of broccoli biomass incorporated was 4.63 and 3.87 kg/m^2 in 1997 and 1998, respectively. Four kg/m^2 is equivalent to approximately 45 metric tonnes ha^{-1} (fresh weight) or 6 metric tonnes ha^{-1} (dry weight). After incorporation, all plots were irrigated twice a week for the next 3 wk. Soil temperatures were monitored using a CR21X data micrologger (Campbell Scientific, Logan, UT) for 17 days. One probe was buried 15 cm deep in each of four or two broccoli plots and four or two fallow plots in the 1997-98 and 1998-99 experiments, respectively (Table A2; Table A3).

In the year following the green manure treatment, each plot was planted to either peppermint (*Mentha piperita* L. cv. Black Mitcham) (Mint Condition, Milton-Freewater, OR) (24 rooted cuttings/plot), potato (*Solanum tuberosum* L. cv. Russet Norkotah) (eight 50-100 g single-eye seed pieces or single-drop tubers/plot), or red maple rooted cuttings (*Acer rubrum* L. cv. Red Sunset) (J. Frank Schmidt and Son, Co., Boring, OR) (eight 1 m tall seedlings/plot). Host species were planted to plots infested with the corresponding *V. dahliae* isolate (e.g. red maple was planted into plots infested with the maple isolate). In 1999, a second crop of broccoli, rather than peppermint, was planted in plots infested with the mint isolate in the 1998-99 experiment. The broccoli (4.01 kg/m^2) was incorporated into the soil in late July as described above. Peppermint will be planted in these plots in the spring of 2000. The planting schedule for each experiment is listed in Table 2.1.

Potatoes were sprayed three times during the season with chlorothalonil (Bravo 720, ISK Biosciences Corporation, Mentor, OH) at a rate of 1.2 (first

application) or 2.3 (second and third applications) L/ha using a backpack sprayer for control of late blight. Plots were treated once with sethoxydim for control of monocot weeds as described above.

Potatoes were grown for one season, whereas peppermint was grown for two and maple for one or two consecutive seasons beginning in 1998 and 1999, respectively.

Table 2.1. Broccoli green manure treatment and host species planting schedule in field plots established in 1997 (1997-98 experiment) and 1998 (1998-99 and 1999-2000 experiments) at the Botany and Plant Pathology Research Farm, Corvallis, OR.

Experiment	1997	1998	1999	2000
1997-98	Broccoli	Peppermint Potato Red maple	-----	-----
1998-99	-----	Broccoli	Potato Red maple	-----
1999-2000	-----	Broccoli	Broccoli	Peppermint

Soil populations of *V. dahliae* were assayed 1 mo after fungal infestation, 17 days post-broccoli incorporation, and at the time of planting the following year. Six soil cores, each 1.75 cm in diameter, were taken from the top 15 cm of each plot in an "N"-shaped sampling pattern using a hand-held soil corer. The soil cores were bulked to form one sample per plot. Samples were kept overnight at 5°C. Each soil sample was mixed thoroughly by hand and dried at room temperature for 3 wk. Dried soil samples were ground with a mortar and pestle and mixed. Using

an Anderson air sampler (Butterfield and DeVay, 1977), soil aliquots of 0.17 g

were plated onto each of 10 petri plates that contained Sorensen's NP-10 medium

(Sorensen et al., 1991). After incubation at room temperature in the dark for 3 wk,

adhering soil particles were washed off the agar surface under running tap water.

Plates were examined with the aid of a dissecting microscope. Number of colony

forming units of *V. dahliae* per gram of dry soil was expressed as CFU/g.

Two samples containing both soil and roots were taken with a metal bulb-

planter (2.5 cm in diameter x 10 cm long) from two plants in each plot of potato

and maple shortly before symptom onset and bulked. Four random cores were

taken from each peppermint plot and bulked. Samples were kept at 5°C overnight,

and then spread out on plastic trays. Roots were removed from the soil by hand,

washed thoroughly in running distilled water, and rinsed with a 1% Tergitol

solution to remove adhering soil particles. A total of 100-150 cm of root length per

plot was distributed among four petri plates containing Sorensen's NP-10 medium

(Sorensen et al., 1991), a modification of the technique described by Evans et al.

(1974). Plates were incubated at room temperature in the dark for 2 wk, and

colonies of *V. dahliae* were counted with the aid of a dissecting microscope.

Number of colony forming units of *V. dahliae* per length of root was expressed as

CFU/cm.

Five randomly selected symptomatic stems of peppermint and potato were

collected from each plot at symptom onset in July. Leaves were removed and stems

were surface sterilized in a 10% bleach solution for 30 sec. Stems were cut into thin

cross sections and placed onto Sorensen's NP-10 medium (Sorensen et al., 1991).

After 1 wk incubation at room temperature in the dark, segments were examined for microsclerotia of *V. dahliae* using a dissecting microscope.

In early August, the terminal 5 cm of 10 symptomatic stems per plot of peppermint and potato were collected. Leaves were stripped and stems were bulked to form one sample per plot. Stem samples were dried in paper bags at room temperature for 4 wk and ground in a Wiley™ Mill with a #40 mesh screen. Aliquots of dried peppermint (0.14 g) and potato (0.10 g) stems from each plot were distributed onto each of four petri plates that contained Sorensen's NP-10 medium (Sorensen et al., 1991) using an Anderson air sampler.

Plates were incubated at room temperature in the dark for 2 wk and washed under running tap water to remove adhering stem particles. Colony forming units of *V. dahliae* per unit weight of stem tissue were counted with the aid of a dissecting microscope and expressed as CFU/g dried stem.

Disease severity in potato was assessed on a scale of 0 to 100%, where 0%= no symptoms and 100%= total foliar senescence. In peppermint, the number of symptomatic stems (strikes) in each plot was counted. Disease assessments were made at regular intervals (6-7 days) beginning at symptom onset until mid- to late August.

Each year, all above ground plant material of peppermint and potato was harvested in mid-August. Plant material was placed in burlap sacks, dried at 100°C for 48 hr, and weighed. In early August, potato tubers were harvested by hand and weighed. In mid-September, the height of each maple tree was measured. A total of

20 leaves also was collected from the top of each tree in each plot in 1998 and were processed in an LI-3000 Leaf Area Meter to obtain the total leaf area for each tree.

Numerical counts of *V. dahliae* populations from soils, roots, and stems were transformed [ln (x+0.01)] prior to analysis. Area under the senescence progress curve (AUSPC) was computed for proportional assessments of symptom severity in plots of peppermint and potato (Shaner and Finney, 1977). Data on *V. dahliae* soil, root and stem populations, disease severity, and plant growth and yield were subjected to analysis of variance using a general linear model procedure (proc GLM of SAS version 6.12, SAS Inst., Cary, NC) to evaluate the significance of the broccoli green manure main effect. Significance of isolate and of the interaction between the broccoli green manure and isolate main effects was evaluated for the soil population data. Treatment means were separated by Fisher's protected least significant difference (LSD) test. Significant differences were accepted at $P \leq 0.05$.

Growth chamber study. Sandy loam field soil infested with a potato isolate of *V. dahliae* in May 1998 was collected from plots located at the Department of Botany and Plant Pathology Research Farm, Corvallis, OR in October 1998 and in March 1999. Soil was dried at room temperature for 3 wk, screened with a #10 mesh (mesh opening size = 2 mm) to remove rocks and debris, and ground with a mortar and pestle. In the greenhouse, this field soil was amended with 0, 2, 4, 8, or 16% broccoli biomass (weight of broccoli: total weight of broccoli plus soil; approximately 0, 0.28, 0.56, 1.12, or 2.24 kg/L soil). Broccoli was obtained from the Oregon State University Horticulture Research Farm, Corvallis, OR in mid-

November 1998 (cv. Excelsior) or purchased at local grocery stores in April 1999.

Stems and leaves of broccoli were chopped by hand into pieces no larger than 1 cm

in diameter and further processed in a Waring commercial blender at high speed for

5 sec. The broccoli and soil were mixed thoroughly. Square plastic pots (9 cm x 10

cm) were lined with paper towels, filled with 700 g of the broccoli-soil mixture,

and watered to soil moisture capacity. Pots were placed in a Russell Model UE650

Controlled Environment Chamber (Hoffman Manufacturing, Albany, OR) and

incubated at $24 \pm 2°$ C in the dark for 19 days. Treatments were arranged in a

completely random design and replicated eight times.

Soil from each pot was spread out on large paper sheets and dried at room

temperature for 2 wk. Dry soil was ground with a mortar and pestle. Using the

Anderson air sampler (Butterfield and DeVay, 1977), 0.17 g aliquots of dry soil

from each replication was distributed onto each of nine petri plates that contained

Sorensen's NP-10 medium (Sorensen et al., 1991). Plates were incubated at room

temperature in the dark for 3 wk. After incubation, soil was removed from the

plates under running tap water and *V. dahliae* colonies were counted with the aid of

a dissecting microscope. Number of colony forming units of *V. dahliae* per gram of

dry soil were expressed as CFU/g.

Mean numbers of *V. dahliae* colony forming units/g of soil (CFU/g) at each

level of broccoli green manure biomass were transformed [ln (x+0.01)] and

regressed on the percentage of broccoli biomass using a least squares regression

procedure (proc REG of SAS) to test for linear trends.

RESULTS

Field study. In both the 1997-98 and 1998-99 experiments, respectively,

infestation of plots with laboratory-grown microsclerotia of *V. dahliae* at rates of

20 and 40 CFU/g soil established soil pathogen populations that could be detected

with the Anderson sampler technique. One month after the fungal inoculum was

incorporated into the soil and prior to the incorporation of the broccoli green

manure, populations in the 1997-98 experiment averaged 1.09, 2.54, and 3.39

CFU/g of soil for mint, potato, and maple isolates, respectively, and in the 1998-99

experiment averaged 7.18, 14.15, and 15.64 CFU/g (Table 2.2). Across the three

isolates of *V. dahliae*, these counts were 83-95% and 61-82% lower in the 1997-98

and 1998-99 experiments, respectively, than was expected based on treated soil

volume calculations made at the time of infestation. In general, initial populations

were similar among plots that were to receive the broccoli green manure and those

that were to be fallowed. The exception to this was the mint isolate in the 1998-99

experiment, when the *V. dahliae* population size was 107% higher in plots that

were to receive the broccoli green manure compared to plots that were fallowed.

Three weeks after incorporation of the broccoli green manure, soil

populations of *V. dahliae* across all three isolates were 50-78% and 30-82% lower

in the 1997-98 and 1998-99 experiments, respectively, compared to the fallow

control. In the spring following the incorporation of the broccoli green manure, soil

populations in the 1997-98 experiment were not significantly different among

broccoli-amended and fallowed plots for any isolate, but soil populations in the

1998-99 experiment remained significantly less in the broccoli-amended compared

to the fallowed plots. Microsclerotia of *V. dahliae* were detected in none of the noninfested control plots in the 1998-99 experiment, with the exception of a single plot on one sampling date in which 0.53 CFU/g were counted.

Soil populations of the mint isolate of *V. dahliae* in plots amended with a broccoli green manure for two consecutive years (1998 and 1999) averaged 0.22 CFU/g soil, a 66% reduction compared to the 0.66 CFU/g in plots fallowed during the same time period; however, this difference was not significant (P=0.3344).

Number of *V. dahliae* microsclerotia across green manure treatments was significantly higher ($P \leq 0.10$) in plots infested with the maple or potato isolates compared to the mint isolate. The exception occurred on the 10 mo post-broccoli sampling date in the 1997-98 experiment when the mean number of microsclerotia was not significantly different among isolates (P=0.4699). The interaction between the green manure and isolate main effects was not significant at either post-broccoli incorporation sampling date in either the 1997-98 or 1998-99 experiments.

In the 1997-98 experiment, potato root colonization by *V. dahliae* was 88% less (P=0.0316) in the broccoli green manure compared to the fallow treatment (Table 2.3). While the 75% decrease in root colonization in the broccoli green manure compared to the fallow treatment in the 1998-99 experiment was not significant (P=0.2371), root colonization was 94% less (P=0.0010) in noninfested control plots compared to plots that were infested with *V. dahliae*, regardless of broccoli green manure treatment. Root colonization of peppermint was not affected by the green manure treatment. In red maple, no root colonization was observed in

the 1997-98 experiment and no treatment differences (P=0.1423) were observed in the 1998-99 experiment.

In the 1997-98 experiment, no significant treatment differences in colonization of the stem were observed in peppermint or potato (Table 2.3). In the 1998-99 experiment, stem colonization of potato was 99.7% less (P=0.0001) in plots not infested with *V. dahliae* compared to plots infested with pathogen; however, the 43% decrease in stem colonization in the broccoli green manure compared to the fallow treatment was not significant (P=0.8270).

Number of symptomatic peppermint stems in each plot across green manure treatments in the 1997-98 experiment quickly increased from 39.9 in mid-July to 84.1 in early August when nearly all stems were symptomatic in both broccoli-amended and fallowed plots; the broccoli green manure did not suppress disease regardless of date of disease assessment (Table 2.4). Disease severity of potatoes grown in broccoli-amended soil was 40% less (P=0.0001) than in fallowed soil in the 1997-98 experiment. While disease severity of potatoes was also 13% less in the broccoli green manure compared to the fallow treatment in the 1998-99 experiment, this difference was not significant (P=0.3788). No symptoms were observed in red maple trees in either experiment, and *V. dahliae* was not recovered in culture from these trees.

Table 2.2. Soil populations (CFU/g soil) of mint, potato, and maple isolates of *Verticillium dahliae* prior to and after the incorporation of a broccoli green manure.

Year	Isolate	Green manure	Sampling date		
			Pre-broccoli incorporation	Post-broccoli incorporation	
			1 mo pre-broccoli[a]	17 days post-broccoli[a]	10 mo post-broccoli[a]
1997					
	Mint	Fallow	0.97	0.50	1.06
		Broccoli	1.21	0.25	3.30
	Potato	Fallow	1.45	2.41 ***	3.30
		Broccoli	3.63	0.62	1.60
	Maple	Fallow	2.90	1.67 ***	1.91
		Broccoli	3.87	0.37	0.42
1998					
	Mint	Fallow	4.68 ***	1.60 *	6.70 ***
		Broccoli	9.68	0.43	1.21
	Potato	Fallow	15.11	9.68 **	7.91 *
		Broccoli	13.19	6.81	4.18
	Maple	Fallow	17.13	8.51 ***	11.98 ***
		Broccoli	14.15	1.49	4.95
	Noninfested	Fallow	0.00	0.00	0.00
		Broccoli	0.00	0.11	0.00

[a] Within each year and isolate, means within a column separated by

'*'   are significantly different at $P = 0.10$,

'**'   are significantly different at $P = 0.05$, and

'***'   are significantly different at $P = 0.01$.

Analysis of variance was based on ln CFU/g. Means shown represent nontransformed data.

Table 2.3. Effect of a broccoli green manure on root (CFU/cm) and stem (CFU/g) colonization of peppermint, potato, and red maple by *Verticillium dahliae* in soil infested with the corresponding isolate of *V. dahliae* (mint, potato, or maple).

Plant species	*V. dahliae* isolate	Green manure	Root colonization (CFU/cm)[a]		Stem colonization (CFU/g)[a]	
			1997-98	1998-99	1997-98	1998-99
Mint	Mint	Fallow	0.012A	-[b]	1520.7A	-[b]
Mint	Mint	Broccoli	0.012A	-[b]	1423.9A	-[b]
Potato	Potato	Fallow	0.042A	0.211A	842.7A	5642.0A
Potato	Potato	Broccoli	0.005B	0.053A	374.0A	3196.0A
Potato	Noninfested	Fallow	-[c]	0.013A	-[c]	20.0B
Potato	Noninfested	Broccoli	-[c]	0.003B	-[c]	3.3B
Red maple	Maple	Fallow	0.000A	0.000A	-[d]	-[d]
Red maple	Maple	Broccoli	0.000A	0.008A	-[d]	-[d]

[a] Means within the same species in each column followed by the same letter are not significantly different ($P \le 0.05$) according to Fisher's protected least significant differences (LSD) analysis. Analysis of variance was based on ln CFU/cm and ln CFU/g. Means shown represent nontransformed data.

[b] No peppermint planted in 1998-99 experiment.

[c] No noninfested control plots in 1997-98 experiment.

[d] Not tested in red maple.

Table 2.4. Effect of a broccoli green manure on area under the senescence progress curve (AUSPC) of peppermint and potato grown in soil infested with a corresponding isolate of *Verticillium dahliae* (mint or potato).

Plant species	*V. dahliae* isolate	Green manure	AUSPC[a]	
			1997-98	1998-99
Mint	Mint	Fallow	945.6A	-[b]
Mint	Mint	Broccoli	1147.2A	-[b]
Potato	Potato	Fallow	1036.7A	1947.6A
Potato	Potato	Broccoli	625.2B	1699.4A
Potato	Noninfested	Fallow	-[c]	484.0B
Potato	Noninfested	Broccoli	-[c]	619.1B

[a] Means in the same column within the same species followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis.
[b] Not planted in the 1998-99 experiment.
[c] No noninfested control plots in the 1997-98 experiment.

Although aerial biomass of peppermint was 11% less and aerial biomass of potato was 27% more in the broccoli green manure compared to the fallow treatment in the 1997-98 experiment, these differences were not significant (Table 2.5). Aerial biomass of potato in the 1998-99 experiment was not significantly affected by the broccoli green manure treatment; however, aerial biomass of potato in *V. dahliae*-infested soil was 14% less ($P=0.0001$) than in soil not infested with the pathogen.

Similarly, potato tuber yield was not affected significantly by the broccoli green manure treatment; however, it was 10 and 38% higher in the broccoli green manure than in fallow treatment in plots infested with *V. dahliae* in the 1997-98 and 1998-99 experiments, respectively, and 76% higher (P=0.0001) in control plots

not infested with *V. dahliae* than in plots not infested with the pathogen, regardless

of broccoli treatment, in the 1998-99 experiment (Table 2.6).

Table 2.5. Effect of a broccoli green manure on aerial biomass (g) of peppermint
and potato grown in soil infested with a corresponding isolate of *Verticillium
dahliae* (mint or potato).

Plant species	*V. dahliae* isolate	Green manure	Aerial biomass[a]	
			1997-98	1998-99
Mint	Mint	Fallow	754.0A	-[b]
Mint	Mint	Broccoli	669.6A	-[b]
Potato	Potato	Fallow	130.1A	114.8A
Potato	Potato	Broccoli	165.8A	98.6A
Potato	Noninfested	Fallow	-[c]	242.3B
Potato	Noninfested	Broccoli	-[c]	177.0B

[a] Means in the same column within the same species followed by the same letter are
not significantly different ($P \le 0.05$) according to Fisher's protected least
significant differences (LSD) analysis. Analysis of variance was based on ln g.
Means shown represent nontransformed data.
[b] Not planted in the 1998-99 experiment.
[c] No noninfested control plots in the 1997-98 experiment.

For both experiments, height of red maple trees was not significantly

different among treatments (Table 2.7). Also, in the 1997-98 experiment, red maple

leaf area was not affected by the broccoli green manure treatment.

Table 2.6. Effect of a broccoli green manure on yield (kg) of potatoes grown in soil infested with a potato isolate of *Verticillium dahliae*.

V. dahliae	Green manure	1997-98[a]	1998-99[a]
+	Fallow	4.78A	2.89A
+	Broccoli	5.24A	3.99A
-	Fallow	-[b]	6.37B
-	Broccoli	-[b]	5.74B

[a] Means within each year followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis.
[b] No noninfested control plots in the 1997-98 experiment.

Table 2.7. Effect of a broccoli green manure on height (cm) and leaf area (cm^2) of red maple trees grown in soil infested with a maple isolate of *Verticillium dahliae*.

Green manure	Height[a]		Leaf area[a]
	1997-98	1998-99	1997-98
Fallow	124.3A	121.0A	1099.1A
Broccoli	116.6A	129.0A	1005.1A

[a] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis.

Growth chamber study. Recovery of *V. dahliae* microsclerotia from soil after broccoli green manure incorporation was affected significantly by the amount of broccoli biomass both times the experiment was conducted. The amount of broccoli green manure biomass incorporated was a significant predictor of measurable soil populations of *V. dahliae* ($R^2_1 = 0.8646$; $R^2_2 = 0.7099$) (Fig. 2.1). At broccoli biomass levels of 2 or 4% in the first experiment and 2% in the second experiment, soil populations after broccoli green manure amendment were not significantly

different from the nonamended control treatment. Incorporation of 8% broccoli

biomass reduced the measurable soil populations of *V. dahliae* by 54-94%,

compared to the nonamended control treatment; 16% broccoli biomass reduced the

measurable soil populations by 94-98%, compared to the nonamended control

treatment. As the amount of broccoli biomass incorporated into the soil was

increased 1.7-2.3-fold, the measurable soil populations of *V. dahliae* declined by

90%.
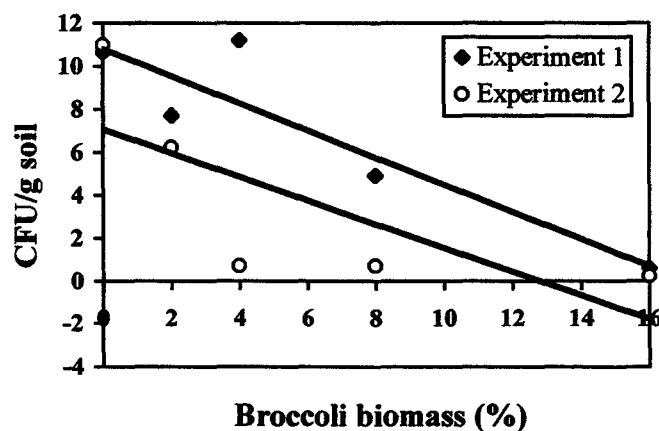
Figure 2.1. Effect of amount of broccoli green manure biomass on soil populations
of *Verticillium dahliae* in soil amended with either 0, 2, 4, 8, or 16% broccoli
biomass.[a]

[a] Soil was amended in pots and incubated at $25\pm2°C$ for 19 days. The experiment
was conducted twice; the replications are referred to as Experiments 1 and 2,
respectively. Analysis of variance was based on ln CFU/g. Means plotted represent
nontransformed data.

$y_1 = -0.1790x+2.6667$; $R^2_1 = 0.8646$

$y_2 = -0.2303x+1.7826$; $R^2_2 = 0.7099$

DISCUSSION

A broccoli green manure reduced the number of *V. dahliae* microsclerotia in the soil within 3 wk of incorporation. The effectiveness of a green manure increased as the amount of green manure biomass was increased. In the 1998-99 experiment, soil populations were still significantly less in broccoli-amended than in fallowed plots 10 mo after broccoli incorporation. In the 1997-98 experiment, however, 10 mo after broccoli incorporation soil populations of *V. dahliae* were low and not affected by the green manure treatment. Soil samples for this sampling date were collected in early spring for each experiment and soil population counts may have been low simply because of the time of year. Joaquim et al. (1988) also found that soil populations were low in early spring and peaked in mid to late summer. The interactions between green manure and isolate were not significant after incorporation of the broccoli, indicating that suppression of the *V. dahliae* microsclerotia was similar across isolates.

Amendment of *V. dahliae*-infested soil with a broccoli green manure suppressed severity of foliar symptoms in potato. This finding supports the hypothesis that a broccoli green manure is an effective disease management strategy in susceptible crops other than cauliflower (Subbarao, 1996; Subbarao et al., 1999; Xiao et al., 1998). Green manuring of broccoli did not reduce disease severity in peppermint even though soil populations of the mint isolate of *V. dahliae* were reduced; however, peppermint may be susceptible to lower inoculum densities than other plant species (Crowe et al., Greece; Nnudo and Harrison,

1979). When broccoli was green manured for two consecutive years in plots infested with the mint isolate, soil populations were again less in broccoli-amended compared to fallowed plots. Peppermint will be planted into each of these plots in the spring of 2000 to determine whether two consecutive years of a broccoli green manure will result in a significant reduction in Verticillium wilt severity. The effect of the broccoli green manure on Verticillium wilt of red maple is not yet known, since no disease symptoms were observed. The red maples will be monitored for symptoms of disease again during the summer of 2000. In a woody perennial such as maple, disease symptoms often do not develop for several years (Harris, 1998). Symptoms however, may not have been expressed because the red maple cultivar is not very susceptible to the *V. dahliae*. Red maple seedlings from different half-sib families, where the male parents are unknown, exhibit different levels of susceptibility to *V. dahliae* (Townsend and Hock, 1973), suggesting that the rooted cuttings used in this study were among the less susceptible. While Townsend and Hock (1973) reported disease symptom expression in red maple within several months, the quick expression of disease may be due to the inoculation technique used; seedlings were wounded and inoculated directly with a suspension of *V. dahliae* microsclerotia and conidia.

Root and stem colonization of potato was less following a broccoli green manure treatment compared to the fallow control treatment. Because both number of root and extent of vascular infections within the plant tissue were less numerous, less disease developed. While aerial biomass was not significantly higher in the broccoli green manure treatment, which had lower levels of root and vascular

colonization, tuber yield was higher nonetheless. Tuber yield may be reduced when aerial biomass and the resulting photosynthetic capacity of the plant are reduced (Gent et al., 1995).

Field plots were infested at rates of 20 or 40 CFU/g soil in the 1997-98 and 1998-99 experiments, respectively, but soil assays indicated that there were up to 90% fewer microsclerotia of *V. dahliae* soon after infestation. These observations were consistent with the findings of Menzies and Griebel (1967), who found that while soil microsclerotial populations of *V. dahliae* increased temporarily following the time of fungal infestation, populations then decreased naturally to final populations which were lower than initial populations. A mathematical equation to describe the decline in *V. dahliae* microsclerotial inoculum density in the soil over time was developed by Mol et al. (1996), however, soil populations in our study declined more quickly and did not fit the model. Evans et al. (1966) observed that *V. dahliae* microsclerotia which are embedded in plant residues in the soil remain viable longer than microsclerotia which are free in the soil. In our study, microsclerotia were incorporated into the soil free of plant residues. The loss of a portion of the microsclerotial inoculum may be due to competition or parasitism by indigenous antagonistic soil microorganisms (Katznelson, 1940). Soil populations of *V. dahliae* remained low throughout both seasons of both the 1997-98 and 1998-99 experiments, however, disease symptoms were severe in peppermint and potato. While it is possible that many of the *V. dahliae* microsclerotia died in the soil soon after being incorporated, microsclerotial growth on the culture medium may simply have been inhibited by other soil

microorganisms (Menzies and Griebel, 1967). It is also possible that the culture medium (Sorensen's NP-10) was not optimal for microsclerotial growth. However, soil populations of the mint isolate were also tested by plating infested soil onto a second culture medium, Modified Soil Extract Agar (MSEA) (Harris et al., 1993; Huisman and Ashworth, 1974), but the recovery rate was not increased. A study by Termorshuizen et al. (1998) reported that recovery of *V. dahliae* microsclerotia is generally less than 40% of the inoculum density calculated at the time of infestation and that the recovery rate varied among 12 researchers testing subsamples of the same soils. Evans et al. (1974) showed that plant roots can be utilized as traps to estimate the numbers of *V. dahliae* microsclerotia in soils infested with low inoculum densities, however, there were few treatment differences in root colonization in our study.

The lack of a significant effect of the broccoli green manure treatment on disease severity of potato in the 1998-99 experiment may have been due to the high numbers of microsclerotia of *V. dahliae* remaining in the soil. Immediately following the incorporation of the broccoli green manure, soil populations of the potato isolate declined 74 and 30% in the 1997-98 and 1998-99 experiments, respectively; however, the number of *V. dahliae* microsclerotia remaining in the soil was higher in the 1998-99 experiment (6.81 CFU/g) than in the 1997-98 experiment (0.62 CFU/g). While soil populations declined following the broccoli green manure in both experiments, the numbers of microsclerotia remaining in the soil in the 1998-99 experiment in both green manure treatments were high enough to cause severe disease symptoms in potato. The fungal inoculum densities used to

infest the soils in both experiments were higher than generally occur in naturally infested soils. Papavizas and Lewis (1971) reported a decrease in the effectiveness of cruciferous green manures at suppressing disease in soils infested with high populations of the pathogen, *Aphanomyces euteiches*. We used high inoculum densities to increase the likelihood that disease would develop within a single growing season. Field plots were infested at a rate of 20 and 40 CFU/g of soil in the 1997-98 and 1998-99 experiments, respectively. The measurable soil populations of *V. dahliae* were low throughout the 1997-98 experiment. Since the number of *V. dahliae* microsclerotia remaining in the soil appeared to be low, we assumed that symptom expression would be too low to measure treatment effects. In order to be certain that disease symptoms would be expressed in the 1998-99 experiment, we doubled the rate of fungal infestation. Two months later, we observed that, while soil populations of *V. dahliae* appeared to be very low in the 1997-98 experiment, disease symptoms were in fact severe in potato and peppermint. This indicates that the disease threshold levels of *V. dahliae* soil populations in some plant species may be close to the levels at which *V. dahliae* can be detected in the soil. Furthermore, in some species, the number of microsclerotia required to cause significant levels of disease is lower than in other species; in peppermint, minimum inoculum densities of 0.1 CFU/g soil (Crowe et al., 2000) compared to 17.5-23 CFU/g in potato (Nnudo and Harrison, 1979) have been shown to cause significant Verticillium wilt increases or yield losses.

The inconsistent effectiveness of the broccoli green manure across years and across *V. dahliae* isolates in the field study may be attributed to the low amount

of broccoli biomass incorporated into the soil. Supporting this hypothesis, the broccoli green manure treatment was increasingly effective at reducing measurable soil populations of *V. dahliae* as the amount of green manure biomass incorporated was increased. Similarly, in a study with a rapeseed green manure for suppression of the Columbia root-knot nematode, Mojtahedi et al. (1993) found that the effectiveness of the green manure increased with the amount of green manure biomass added to the soil. Significant suppression was reported at a biomass as low as 1.9% and increased as the amount of biomass increased to 5.9%. This may mean that a broccoli green manure which is ineffective as a disease management strategy at low amounts of green manure biomass may in fact be quite effective at higher amounts of green manure biomass. While the amount of broccoli biomass incorporated into the plots in our field study was approximately 2% (weight of broccoli: total weight of soil plus broccoli) or 45 metric tonnes/ha, other studies have found that in commercial fields in California, an average of 8% broccoli stem and leaf residue remain in the field following harvest of the heads (Subbarao and Hubbard, 1996). This difference in plant growth between our study in Oregon and studies in California may be due to differences in climate or in cultural practices. Chan and Close (1987) and Papavizas and Lewis (1971) found significant decreases in Aphanomyces root rot of peas following the incorporation of 0.5% (dry weight) Cruciferous green manures, which is equivalent to approximately 4.5% fresh biomass, more than twice the amount incorporated in our study. In a study by Mojtahedi et al. (1993), the amounts of rapeseed green manure biomass incorporated into the soil were 20 or 44 tonnes/ha, less or approximately equal to

the amounts of broccoli green manure biomass incorporated in our study. While the initial soil inoculum density was high (40-70 CFU/g soil) in a study by Davis et al. (1996), the amounts of fresh green manure biomass were also high (approximately 60-100 metric tonnes/ha) and severity of Verticillium wilt in potato was significantly decreased. Root colonization of strawberry by four fungal pathogens was not reduced by a broccoli green manure treatment at a rate of 51 metric tonnes/ha (Sances and Ingham, 1997). Since the amount of green manure biomass incorporated into the soil varied across studies, it is difficult to determine why the effectiveness of the green manure at suppressing disease varied.

While the broccoli green manure treatment did not satisfactorily control Verticillium wilt in the three plant species tested in this study, further experimentation with green manures may result in better disease control. Two approaches may be to increase the amount of green manure biomass or the number of consecutive years of treatment and to use various green manure crops besides broccoli.

CHAPTER 3

Influence of a Broccoli Green Manure and Soil Solarization on Verticillium Wilt of Smokebush and Amur Maple

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Jack Pinkerton, and Kelly Ivors

ABSTRACT

Green manures and soil solarization are two disease management strategies currently under investigation as alternatives to chemical soil fumigation. The effects of a broccoli green manure and of soil solarization on soil populations of *Verticillium dahliae* and on Verticillium wilt of royal purple smokebush and amur maple were evaluated in a field study conducted at a commercial ornamental plant nursery located on the northern edge of Oregon's Williamette Valley. Plots of broccoli and fallow controls were established in May 1998. After the broccoli matured, the crop was flailed down and incorporated (2.65 kg/m^2) into soil naturally infested with microsclerotia of *V. dahliae*. After incorporation, the plots were irrigated and a transparent polyethylene film was placed over the soil for 2 mo. Three weeks after incorporation of the broccoli green manure, measurable soil populations of *V. dahliae* were 50% less (P=0.0031) in broccoli-amended than in fallow plots. After 2 mo of soil solarization, soil populations remained 40% lower (P=0.0377) in broccoli-amended than in fallowed plots; however, there was no additional effect of soil solarization. Disease severity of smokebush soon after symptom onset was 35% less (P=0.0264) in plots which were solarized; however, the broccoli green manure treatment did not affect disease severity. No treatment differences were observed in smokebush 1 mo later or in amur maple on either disease assessment date. Because the broccoli green manure treatment did not suppress Verticillium wilt, it is unknown whether the effect of a broccoli green manure is enhanced by soil solarization. The low amount of broccoli biomass

incorporated into the soil may have contributed to the lack of effectiveness of the green manure treatment.

INTRODUCTION

Verticillium wilt, caused by the soilborne fungus, *Verticillium dahliae*, results in significant losses in nursery commodities (Bedwell and Childs, 1938). *V. dahliae* produces microsclerotia, which are infective propagules that persist in the soil for many years. Current control strategies rely heavily on chemical soil fumigants such as methyl bromide, chloropicrin, and metam sodium to destroy microsclerotia of *V. dahliae*. These compounds are effective, but many are expensive and highly toxic (Gamliel et al., 1997; Harris, 1990). The production of methyl bromide, a volatile soil fumigant which contributes to ozone depletion, will be halted in developed countries in the year 2010 (Anonymous, 1995), and the use of other soil fumigants also may be curtailed (Powelson and Rowe, 1993). The development of non-chemical alternative disease management strategies, including green manures and soil solarization, is vital to meeting the agricultural and horticultural needs of the future.

The incorporation of various green manure crops, such as sudangrass (Davis et al., 1996), white mustard (Muehlchen et al., 1990), and broccoli (Subbarao et al., 1999), has reduced the severity of several diseases including Verticillium wilt. Using a broccoli green manure to control Verticillium wilt in cauliflower, *V. dahliae* soil populations have been reduced and cauliflower yield increased in California (Subbarao et al., 1999). Furthermore, the green manure treatment was as or more effective than fumigation with chloropicrin or metam sodium (Subbarao et al., 1999).

During the process of soil solarization, soil temperatures are elevated by covering moist soil with transparent plastic sheets for several weeks during dry and sunny weather. Soil temperatures can reach 50°C under the plastic sheets, hot enough to reduce soil populations of many microorganisms, including *Fusarium oxysporum* f. sp. *vasinfectum* and *V. dahliae*, by up to 100% (Ashworth and Gaona, 1982; Davis and Sorensen, 1986; Katan et al., 1976, 1983; Pullman et al., 1981). Fusarium wilt in cotton and Verticillium wilt in eggplant and tomato have been reduced significantly following soil solarization (Katan et al., 1976, 1983). Yields of eggplant, potato, cotton, and other crops often increase following soil solarization in soils infested with *F. oxysporum* or *V. dahliae* (Katan, 1980; Katan et al., 1983). The effectiveness of soil solarization is decreased in areas where frequent summer rains and cloud cover prevent high soil temperatures (Chellemi et al., 1994), however, in regions of dry and warm summers such as the Williamette Valley of Oregon, soil solarization may be an effective disease management strategy.

Potentially, the incorporation of a green manure, in combination with a second treatment such as soil solarization, could reduce Verticillium wilt severity more than either of these management practices by themselves. Significant pathogen population declines may be achieved at lower soil temperatures when a green manure is first incorporated (Keinath, 1996) or at lower amounts of green manure biomass when the soil is also solarized. If cultural or environmental conditions are not optimal for either green manure biomass production or soil

temperature elevation, a combination of two control strategies may result in enhanced disease suppression.

In previous studies, conflicting results have been observed when green manures were combined with soil solarization (Ramirez-Villapudua and Munnecke, 1987; Subbarao et al., 1999). Incorporation of dry cabbage residue, followed by soil solarization, decreased *Fusarium oxysporum* f. sp. *conglutinans* population counts and cabbage yellows disease incidence better than cabbage or soil solarization alone (Ramirez-Villapudua and Munnecke, 1987). The cabbage green manure treatment alone did not decrease disease severity compared to the nontreated control, suggesting that tarping may be valuable not only in raising soil temperatures, but also in preventing the loss of volatile fungicidal compounds produced by decomposing green manures. In contrast, Subbarao et al. (1999) found that tarping the soil did not enhance the ameliorating effects of a broccoli green manure.

The following study is designed to evaluate the effect of a one year broccoli green manure treatment followed immediately by soil solarization on soil populations of *V. dahliae* and on Verticillium wilt of two susceptible nursery commodities, smokebush and amur maple, in a soil naturally infested by *V. dahliae*.

MATERIALS AND METHODS

Plot establishment. In 1998, field plots were established in a sandy loam soil at a commercial ornamental plant nursery located on the northern edge of Oregon's Williamette Valley. The field was divided into 6.1 m x 9.1 m plots with a 1.5 m fallow border along the width of each plot. Twenty plots with V. *dahliae* populations ranging from 1.5-21.9 CFU/g of soil (Pinkerton and Ivors, unpublished data) were selected and assigned to treatments in the following factorial arrangement: nontreated fallow control, broccoli green manure, soil solarization, and broccoli green manure followed by soil solarization. Treatments were arranged in a completely random design and replicated five times.

The broccoli plots were fertilized with N, P_2O_5, and K_2O, each at rate of 7.2 kg/ha on 28 May. Broccoli seedlings (*Brassica oleracea* L. var. *italica* L. cv. Excelsior) (Northwest Transplants, Molalla, OR) at a four-leaf stage were spaced 30.5 cm apart within rows on a 61 cm center in three-row beds. Beds were spaced 71 cm apart. Weeds were controlled during the season by machine cultivating once and by hand weeding once. Plants were irrigated 1-2 times each week by overhead sprinklers until heads were fully formed.

To determine the amount of broccoli biomass incorporated into the soil, the fresh weight of broccoli heads, stems, and leaves from each plot was determined for four randomly selected 1-m^2 areas of each plot. Broccoli samples for biomass determination were returned to the plots and all plants were flailed down and plowed into the soil to a depth of 15 cm on 22 July. An average of 2.65 kg

broccoli/m^2 was incorporated into each green manured plot. The field was irrigated for several hours to bring the soil moisture up to near field capacity. Trenches about 15 cm deep were dug around plots to be solarized. Half of the broccoli-amended plots and half of the fallow plots were covered with 0.6 mil transparent polyethylene film (PolySource, San Diego, CA) on 23 July.

Soil temperatures. Soil temperatures were monitored for 2 mo using a CR21X data micrologger (Campbell Scientific, Inc., Logan, UT). Probes were placed 10 and 20 cm deep in each of one solarized and one nonsolarized plot without regard for the green manure treatment.

Soil populations. Soil samples were collected from each plot in 1998 at the time of plot establishment, 19 days after soil incorporation of broccoli, 2 mo after the plastic tarps were applied, and in 1999 at planting. At each sampling date, 6-15 soil cores per plot were collected with a soil probe (1.75 cm in diameter x 15 cm long) from nontarped and tarped plots, respectively, using an "N"-shaped sampling pattern. Holes made by the soil sampler in the plastic tarps were patched to prevent heat loss. Soil cores from each plot were bulked and mixed thoroughly by hand to form one sample per plot. Samples were air dried at room temperature for 2-3 wk. Dried samples were ground using a mortar and pestle. Using an Anderson air sampler, aliquots of soil (0.17 g) were deposited onto each of 15 petri plates containing Sorensen's NP-10 medium (Sorensen et al., 1991). After incubation at room temperature in the dark for 3 wk, the adhering soil was rinsed off the agar

surface under running tap water. Number of colony forming units of *V. dahliae* per gram of soil (CFU/g) was determined with the aid of a dissecting microscope.

Susceptible crop bioassay. In mid-April of 1999, plots were fertilized with N, P_2O_5, K_2O, Mg, Zn, and Mn at rates of 56, 56,17, 2, 2, and 2 kg/ha, respectively, and disked. The following day, royal purple smokebush liners (*Cotinus coggygria* L. cv. 'Royal Purple') (0.5 m tall; 10/plot) and amur maple rooted cuttings (*Acer ginnala* L.) (3/16" diameter, 1 m tall, 25/plot) were transplanted by hand with 25 cm between plants within a row. In randomized groups of five smokebush or six or seven amur maple, two rows spaced 1.22 m apart were planted near the center of each plot. Plots were sprinkler irrigated 1-2 times each week and cultivated periodically throughout the summer to control weeds.

Disease severity. Colonization of vascular tissue by *V. dahliae* was confirmed by collecting symptomatic stems of smokebush and maple from several plots on 10 August 1999. Leaves were removed and stems were surface sterilized in 10% bleach for 1 min and rinsed in distilled water. Stems were cut into thin sections to expose vascular tissue and placed on Sorensen's NP-10 medium (Sorensen et al., 1991). Plates were incubated at room temperature in the dark for 1 wk. Stem sections were then examined with the aid of a dissecting microscope for *V. dahliae* colonies.

On 3 September and 8 October, all plants in each plot were assessed for disease severity on a scale of 0-100%, where 0%= symptomless plant and 100%= total foliar senescence and/or defoliation.

Data analyses. Numerical counts of *V. dahliae* soil populations at each sampling date were transformed [ln (x+1)] prior to analysis. Proportional assessments of disease severity in smokebush and amur maple were arcsine-square-root transformed. Population data and disease severity data were subjected to analysis of variance using a general linear model procedure (proc GLM of SAS version 6.12, SAS Inst., Cary, NC), to evaluate the significance of the broccoli green manure and soil solarization main effects and of their interaction. Treatment means were separated by Fisher's protected least significant differences (LSD) analysis. Significant differences were accepted at $P \leq 0.05$.

RESULTS

Soil temperatures. The solarization treatment raised soil temperatures to greater than 45°C at a depth of 10 cm on several days (Table 3.1) compared to the temperatures in the nonsolarized plots, which did not exceed 38°C.

Table 3.1. Mean and maximum soil temperatures and cumulative hours during which soil temperatures exceeded 35, 40, and 45°C in solarized and nonsolarized field plots at 10 and 20 cm depths from 23 July through 22 September 1998.[a]

Depth	Treatment	Mean	Max	>35°C	>40°C	>45°C
10	Solarized	30.1	48.9	435	214	37
20	Solarized	29.1	38.5	117	0	0
10	Nonsolarized	23.3	38.0	21	0	0
20	Nonsolarized	23.5	32.8	0	0	0

[a] Cumulative hours of tarping = 1632.

Soil populations. Initial soil populations of *V. dahliae* before treatment (sampling date 1) were similar among all plots (Table 3.2). Three weeks after the incorporation of the broccoli green manure (sampling date 2), measurable soil populations averaged 50% less (P=0.0031) in broccoli-amended compared to fallow plots (Table 3.2; Table 3.3). At 2 mo after incorporation of the broccoli green manure, which coincided with the end of the soil solarization treatment (sampling date 3), soil populations were still 40% lower in broccoli-amended compared to non-amended plots; there was, however, no additional effect of soil solarization on pathogen populations in the soil (Table 3.4). Seven months after the completion of the treatments (sampling date 4), soil populations of *V. dahliae* did not differ significantly among the four treatments. (Table 3.2). Across sampling date, the mean number of colony forming units per gram detected across all four treatments was 61% higher in the soil samples taken immediately following the end of the soil solarization treatment (sampling date 3) compared to samples taken at the beginning of the soil solarization treatment (sampling date 2) and 49% higher compared to soil samples taken 7 mo after treatment (sampling date 4).

Table 3.2. Effect of a broccoli green manure and soil solarization on soil populations (CFU/g) of *Verticillium dahliae* in field plots located in a commercial nursery on the northern edge of the Williamette Valley of Oregon.

| Treatment | Sampling date | | | |
| | 1 | 2 | 3 | 4 |
	Pre-broccoli crop	Post-broccoli; pre-solarization	Post-broccoli +solarization	7 mo post-treatments
Control	16.31A[c]	10.75A	18.98A	7.67A
Broccoli[a]	24.16A	6.20AB	8.16B	8.22A
Solarization[b]	15.14A	9.88A	12.31AB	8.45A
Broccoli+Solarization[ab]	22.51A	4.16B	10.51B	9.33A

[a] Broccoli was planted on 28 May 98 and incorporated as a green manure on 22 July 98.

[b] Soil solarization tarps were in place from 23 July through 22 Sept 98.

[c] Means within a column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis. Analysis of variance was based on ln CFU/g. Means shown represent nontransformed data.

Table 3.3. General linear model summary of ln CFU/g soil of *Verticillium dahliae* three weeks after incorporation of a broccoli green manure and before soil solarization.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Broccoli	1	3.501419	3.501419	13.60	0.0031
Solarization	1	0.579318	0.579318	2.25	0.1594
Broccoli* Solar	1	0.223327	0.223327	0.87	0.3700
Rep	4	0.845119	0.211280	0.82	0.5363

Table 3.4. General linear model summary of ln CFU/g soil of *Verticillium dahliae* two months after incorporation of a broccoli green manure and the start of soil solarization.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Broccoli	1	1.841264	1.841263	5.45	0.0377
Solarization	1	0.270728	0.270128	0.80	0.3889
Broccoli* Solar	1	0.265207	0.265207	0.79	0.3930
Rep	4	3.071695	0.767924	2.27	0.1216

Disease severity. On 3 September, disease severity in smokebush was 35% less (P=0.0264) in plots which had been solarized compared to nonsolarized plots, but was not affected by the broccoli green manure treatment (Table 3.5). Interactions between broccoli green manure and soil solarization treatments were not significant (Table 3.5; Table 3.6). No treatment differences, however, were observed in smokebush on 8 October or in amur maple on either disease assessment date (Table 3.5).

Table 3.5. Effect of a broccoli green manure and soil solarization on Verticillium wilt (% senescence) of smokebush and amur maple in *Verticillium dahliae*-infested soil on two disease assessment dates.

Species	Treatment	% Senescence	
		3-Sept	8-Oct
Smokebush	Control	27.4A[a]	39.0A
	Broccoli	24.8AB	34.7A
	Solarization	16.4B	27.4A
	Broccoli+Solarization	17.7AB	31.6A
Amur maple	Control	6.3A	30.0A
	Broccoli	6.7A	29.0A
	Solarization	7.1A	26.5A
	Broccoli+Solarization	7.2A	27.3A

[a] Means within a column within each species followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis. Analysis of variance was based on arcsine % foliar senescence. Means shown represent nontransformed data.

Table 3.6. General linear model summary of arcsine percent foliar senescence of smokebush caused by *Verticillium dahliae* on 3 September 1999.

Source	DF	Type I SS	Mean Square	F Value	Pr>F
Broccoli	1	0.000210	0.000210	0.03	0.8625
Solarization	1	0.042931	0.042931	6.40	0.0264
Broccoli* Solar	1	0.002308	0.002308	0.34	0.5683
Rep	4	0.092750	0.023187	3.46	0.0423

DISCUSSION

The number of microsclerotia of *V. dahliae* in the soil was reduced

significantly by the broccoli green manure treatment, regardless of treatment with

soil solarization. Disease severities in smokebush and amur maple, however, were

not affected by the broccoli green manure treatment. In contrast, whereas the soil

solarization treatment did not significantly impact soil populations of *V. dahliae*,

severity of Verticillium wilt in smokebush in September 1999 was significantly less

in plots treated with soil solarization compared to non-solarized plots, regardless of

broccoli green manure treatment. One month later, however, the effect of the soil

solarization on disease suppression was not apparent, and no treatment differences

were observed. Although no significant effects of either the broccoli green manure

or the soil solarization treatments were observed in amur maple, disease severity in

October 1999 was 9% lower in the plots that had received the soil solarization

treatment, regardless of broccoli green manure treatment.

The onset of Verticillium wilt symptoms in smokebush was one month later

in solarized compared to nonsolarized plots, despite the lack of a significant decline

in the number of *V. dahliae* microsclerotia. Elevated temperatures can damage,

rather than kill outright, portions of soil pathogen populations such as

microsclerotia of *V. dahliae* (Katan, 1980). These weakened microsclerotia may be

detectable on culture medium, but lose the ability to infect a plant root. While the

absolute number of microsclerotia measured in our study was unaffected by soil

solarization, we did not test whether the potential of the microsclerotia to cause

root infection and, therefore, disease, was affected. In contrast to our observations, Ashworth and Gaona (1982) reported that soil populations of *V. dahliae* were reduced from 4.6 CFU/g soil to undetectable levels following soil solarization. Similarly, Davis and Sorensen (1986) reported soil pathogen population reductions of 97% from 9.7 to 0.3 CFU/g soil in solarized compared to nonsolarized soil.

Soil solarization tarps in our study were left in place for 2 mo and temperatures at depths of 10 and 20 cm averaged 30.1 and 29.1°C, respectively. In contrast, soil temperatures averaged 37.4°C in a previous study by Katan et al. (1976). Higher temperatures are more effective at suppressing soil pathogens (Katan, 1980). A longer period of soil solarization and higher soil temperatures may have resulted in a significant treatment difference in soil populations in our study. Nelson and Wilhelm (1958) showed that all microsclerotia of *V. dahliae* were killed following 6 mo of incubation at 43-49°C and following 8 mo at 40°C in the laboratory, compared to a shorter time and lower temperatures in our study.

Regardless of soil treatment, soil populations of *V. dahliae* fluctuated across the four sampling dates. Each sample, however, was collected at a different time of the year. Joaquim et al. (1988) also reported seasonal changes in *V. dahliae* soil populations and suggested that comparisons of *V. dahliae* soil populations across years be made using only samples collected at the same time of each year. Soil populations across treatments in our study were lowest in samples collected in April 1999 and higher in samples collected in August or September 1998. Similarly, Joaquim et al. (1988) found that soil populations were low in early spring and peaked in mid to late summer.

Possible explanations why disease severity was less in solarized compared with nonsolarized plots despite the lack of a significant reduction in soil populations of *V. dahliae* are that the soil solarization treatment stimulated populations of microorganisms antagonistic to *V. dahliae* (Katan, 1980) or weakened the microsclerotia directly, resulting in a delayed time of infection and symptom onset. Davis and Sorensen (1986) reported that while soil populations of *V. dahliae* were reduced at a depth of 0-15 cm following solarization, populations were unaffected at a depth of 15-30 cm. Although the roots of the potatoes subsequently planted grew throughout both depth classes and likely came in contact with many microsclerotia at a depth of 15-30 cm, disease severity decreased and yield increased, possibly implicating factors other than the direct suppression of soil pathogen populations in the mechanism of soil solarization.

Possible reasons why the broccoli green manure treatment did not reduce disease severity or enhance the effectiveness of soil solarization in this study are several. The initial soil inoculum density was high (Papavizas and Lewis, 1971) and the amount of broccoli incorporated into the soil was low (Subbarao and Hubbard, 1996), relative to the conditions in previous studies. Papavizas and Lewis (1971) reported that the effectiveness of cruciferous green manures at suppressing disease decreased in soils infested with high pathogen populations. This may indicate that green manures eliminates only a portion of the soil pathogen population and therefore, at high inoculum densities, may not eliminate enough of the fungal microsclerotia to significantly reduce disease severity.

The initial *V. dahliae* inoculum density, which averaged 19.5 CFU/g soil, was high compared to a study by Crowe et al. (2000) who reported significant symptoms of Verticillium wilt in peppermint at a soil inoculum density as low as 0.1 CFU/g. Prior to becoming a commercial nursery, the field in this experiment had been used for commercial potato production for several years. Potatoes are susceptible to Verticillium wilt and growing them several times in the same field can result in increasing disease severity over time (Powelson and Rowe, 1993). Soil populations of *V. dahliae* microsclerotia increase each year if infected plant tissue is allowed to decompose in the soil, thus releasing the microsclerotia to the soil environment. If soil populations build up over the years without using any pathogen suppression strategies such as soil fumigation, crop rotation, or green manures, populations may reach levels at which non-chemical strategies are not effective at reducing soil populations to levels low enough to impact disease severity. While this may mean that treatments such as broccoli green manures and soil solarization are not viable disease management strategies in fields already infested with high soil populations, these treatments may in fact be quite effective at slowing the rate of *V. dahliae* population increase in the soil if used prior to the development of a Verticillium wilt problem, when only low levels of the pathogen are present. Consequently, while alternative disease management strategies may not dramatically reduce soil pathogen populations, further investigation of their value as components of integrated management programs is warranted.

The amount of broccoli green manure biomass incorporated into the soil was 1.2% (weight of broccoli: total weight of broccoli plus soil) or 26.3 metric

tonnes/ha, up to 85% less than the amounts incorporated into other studies (Subbarao, 1996). The broccoli plants in our study appeared to be water-stressed and therefore did not develop as much biomass as expected. While the initial soil inoculum density was high (40-70 CFU/g soil) in a study by Davis et al. (1996), the amounts of fresh green manure biomass were also high (approximately 60-100 metric tonnes/ha) and severity of Verticillium wilt in potato was significantly decreased. In order to more accurately represent broccoli production in the vegetable industry, our study should be repeated with the amount of broccoli biomass increased.

The results of our study indicate that the combination of a green manure and soil solarization is not more effective than a single treatment; however, at larger amounts of green manure biomass, there may be treatment interactions. Coelho et al. (1999) also reported that amendment with a green manure did not enhance the effect of soil solarization at suppressing populations of *Phytophthora* spp., despite amounts of cabbage green manure biomass which were 1.5-2.4 times greater than the amounts incorporated in our study. Various pathogens may be more resistant to the effect of a green manure and therefore require higher amounts of biomass in order to suppress them. In contrast to the findings of our study, Keinath (1996) reported a greater decrease in gummy stem blight of watermelon by soil solarization following a cabbage green manure than without a green manure; however, the amounts of green manure biomass incorporated were not given.

In conclusion, results of this study suggest that both a broccoli green manure and soil solarization show potential for management of Verticillium wilt.

The effectiveness of one treatment, however, was not enhanced by a second treatment. Results of this study should be considered preliminary. This experiment must be conducted again to determine whether these results are repeatable. We hypothesize that the direct effect of a broccoli green manure would be enhanced if larger amounts of broccoli biomass were incorporated into the soil than were used in our study. Larger amounts of green manure biomass might be obtained by importing the broccoli leaf and stem residues produced by nearby vegetable processing plants in addition to growing a crop of broccoli in the field to be treated. Larger amounts of broccoli biomass might also result in a higher likelihood of treatment interactions with soil solarization. Green manures and soil solarization as part of an integrated approach to disease management may help to reduce the amounts of chemical soil fumigants used to suppress Verticillium wilt.

CHAPTER 4

**Aggressiveness of *Verticillium dahliae* Isolates from Potato, Mint, and Maple
on Potato, Eggplant, and Peppermint**

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ABSTRACT

Isolates of *Verticillium dahliae*, the causal agent of Verticillium wilt, are classified into vegetative compatibility groups (VCG) based on isolate aggressiveness on specific hosts. Isolates of *Verticillium dahliae* recovered from symptomatic potato and maple were evaluated in a field study for ability to cause disease in potato. Disease severity was 26% higher (P=0.0329) in soil infested with a potato isolate compared to a maple isolate, however, tuber yield was not affected by isolate (P=0.2232).

An isolate of *V. dahliae* from mint, an isolate from potato, and an isolate from maple were evaluated for two years in a field study for ability to cause disease in eggplant. While root colonization by *V. dahliae* did not differ across isolates, disease severity was highest and fruit yield was lowest in plots infested with the potato isolate, followed by the maple isolate and then the mint isolate.

An isolate of *V. dahliae* from mint and an isolate from potato were evaluated at six inoculum densities (0, 1, 2, 4, 8, or 16 CFU/g of soil) for ability to cause Verticillium wilt of peppermint and potato. Disease severity of peppermint was higher in field plots infested with the mint isolate than with the potato isolate. Disease severity of potato was higher in plots infested with the potato isolate than the mint isolate. In peppermint, inoculum density of the mint isolate (R^2 = 0.9678), but not the potato isolate (R^2 = 0.0291), was a significant predictor of disease severity. Conversely, in potato, inoculum density of the potato isolate (R^2 = 0.8236), but not the mint isolate (R^2 = 0.0302), was a significant predictor of

disease severity. Significant symptoms of disease in both peppermint and potato

were caused by an inoculum density of 2 CFU/g soil of their respective isolate.

INTRODUCTION

Verticillium wilt of peppermint (*Mentha piperita* L.) (Crowe et al., 2000), potato (*Solanum tuberosum* L.) (Cappaert et al., 1992), red maple (*Acer rubrum* L.) (Caroselli, 1957; Harris, 1998), and eggplant (*Solanum melongena* L.) (Elmer and Ferrandino, 1994; Gent et al., 1995), is caused by the soilborne fungus, *Verticillium dahliae* and leads to significant reductions in both yield and quality. Within the dying tissues of infected plants, *V. dahliae* produces microsclerotia, which are long-term survival structures. These microsclerotia infest the soil as the plant tissue decomposes and some may persist in the soil for up to a decade even in the absence of a susceptible host (Schnathorst, 1981).

Current control strategies rely primarily on chemical soil fumigants such as methyl bromide, chloropicrin, and metam sodium to destroy the microsclerotia quickly. These fumigants are effective at killing microsclerotia, but many are expensive and highly toxic (Gamliel et al., 1997; Harris, 1990). The production of methyl bromide, a volatile soil fumigant which contributes to ozone depletion, will be halted in developed countries in the year 2010 (Anonymous, 1995), and the use of other soil fumigants also may be curtailed (Powelson and Rowe, 1993). The development of non-chemical alternative disease management strategies, including viable rotations of crop species not susceptible to *V. dahliae*, is vital to meeting the agricultural and horticultural needs of the future.

Within the species, *V. dahliae*, isolates with varying morphology and aggressiveness have been identified. Isolates have been classified into four or five

vegetative compatibility groups (VCG), some of which may further be divided into two subgroups (Bhat and Subbarao, 2000; Joaquim and Rowe, 1991). Isolates within each VCG are generally most aggressive within a related group of plant hosts and may not be as virulent to plant species outside this group (Bhat and Subbarao, 2000; Subbarao et al., 1995). For example, mint isolates are most aggressive on peppermint, but are generally less so on other species (Green, 1951).

Knowledge of the isolates of *V. dahliae* that make up the fungal population infesting a particular field is valuable information for growers. For example, disease severity of a crop most susceptible to VCG 1 grown in soil infested only with VCG 1 may be high, however, disease severity of a crop less susceptible or resistant to VCG 1 grown in the same soil may potentially be significantly less. Some plant species, however, may be susceptible to isolates from more than one VCG (Bhat and Subbarao, 2000; Subbarao et al., 1995).

Thus, the first objective of our work was to compare the aggressiveness of an isolate of *V. dahliae* obtained from potato to an isolate obtained from maple on a common host, potato. We hypothesize that the isolate from potato is more aggressive on potato than is an isolate from maple, a host which is not related to potato. The second objective was to compare the aggressiveness of an isolate obtained from potato to isolates from mint or maple on eggplant. Both eggplant and potato are classified in the family Solanaceae, while peppermint and maple are classified in the families Lamiaceae and Aceraceae, respectively. We hypothesize that the isolate from potato is more aggressive on eggplant than are isolates from maple or mint.

Preliminary results of another field experiment (Chapter 2) demonstrated that although equal amounts of inoculum of each corresponding isolate of *V. dahliae* were incorporated into the soil, disease severity in peppermint was much higher than disease severity in potato or red maple, leading us to hypothesize that isolates of *V. dahliae* obtained from mint may be more aggressive than isolates from potato or maple. Low numbers of microsclerotia of a highly aggressive isolate of *V. dahliae* may cause as much disease as high numbers of less aggressive isolates and may affect hosts other than the species from which it was obtained. Thus, the third objective was to clarify whether the mint isolate of *V. dahliae* was more aggressive than the potato isolate and to determine the dose-response relationships between inoculum density and disease severity, aerial biomass, and potato tuber yield.

Planting Verticillium wilt-susceptible species year after year leads to a buildup of microsclerotial inoculum in the soil and higher disease severity in subsequent susceptible crops (Powelson and Rowe, 1993). In the absence of susceptible host tissue, *V. dahliae* microsclerotia die naturally over time, often without reproducing (Menzies and Griebel, 1967; Mol et al., 1996). In a field infested with a particular isolate of *V. dahliae*, numerous years of cropping to species not susceptible to that isolate may provide a window of time for a natural reduction in soil pathogen populations. Disease severity in subsequent plantings of species susceptible to that isolate may be lower.

MATERIALS AND METHODS

Field study 1. The study was conducted in 1999 in plots infested in 1997 with potato and maple isolates (putatively VCG 4A and VCG 1, respectively) of *V. dahliae* (see Chapter 2). Treatments were arranged in a randomized complete block design and replicated five times. Fertilizer (N, P_2O_5, K_2O, and boron at 112, 140, 120, and 11 kg/ha, respectively) was broadcast by hand in each plot and rototilled into the soil on 10 May. Potatoes (*Solanum tuberosum* L. cv. Russet Norkotah) (eight single-drop tubers/plot) were planted on 26 May into each plot. Weeds were controlled using hand tools. Plots were irrigated by overhead sprinklers 2-3 times a week as necessary.

Disease severity was assessed weekly from mid-July until late August on a scale of 0 to 100%, where 0%= no symptoms and 100%=total foliar senescence. Tubers in each plot were harvested by hand and weighed on 31 August.

Area under the senescence progress curve (AUSPC) was computed for proportional assessments of symptom severity (Shaner and Finney, 1977). Disease severity and maple height measurements were subjected to analysis of variance using a general linear model procedure (proc GLM, SAS version 6.12, SAS Inst., Cary, NC) to evaluate the significance of the isolate main effect. Treatment means were separated by Fisher's protected least significant difference (LSD) test. Significant differences were accepted at $P \leq 0.05$.

Field study 2. The study was conducted in 1998 and 1999 in plots infested in 1997

and 1998, respectively, with a mint, potato, or maple isolate (putatively VCG 2,

VCG 4, and VCG 1, respectively) of *V. dahliae* (see Chapter 2). In 1999, a non-

infested control was added to the isolate treatment. Treatments were arranged in a

randomized complete block design and replicated five times. Fertilizer (N, P_2O_5,

K_2O, and boron at 112, 140, 120, and 11 kg/ha, respectively) was broadcast by

hand in each plot and rototilled in mid-May of each year. Eggplant (*Solanum*

melongena L. cv. Black Beauty) (6/plot) was planted in early June into plots that

were either infested with *V. dahliae* or fallowed during the previous year. Weeds

were controlled with hand tools. Plots were irrigated by overhead sprinklers 2-3

times a week as necessary.

Root colonization by *V. dahliae* was measured by taking a soil core from

directly beneath four plants in each plot at symptom onset using a metal bulb

planter (10 cm long, 2.5 cm diameter). The four root-containing soil cores were

bulked to form one sample per plot. Samples were kept at 5°C overnight, and then

spread out on plastic trays. Roots were removed from the soil by hand and washed

thoroughly in running distilled water and rinsed with a 1% Tergitol solution to

remove adhering soil particles. A total of 100 cm of root length per sample was

distributed among four petri plates containing Sorensen's NP-10 medium (Sorensen

et al., 1991), in a modification of the technique described by Evans et al. (1974).

Plates were incubated at room temperature in the dark for 2 wk, and colonies of *V.*

dahliae were counted with the aid of a dissecting microscope. Number of colony

forming units of *V. dahliae* per length of root was expressed as CFU/cm.

Severity of Verticillium wilt in eggplant was assessed weekly on a scale of 0 to 100%, where 0%= no symptoms and 100%=total foliar senescence from mid-July until early September. Eggplant fruit yield was determined by harvesting and weighing all fruit in each plot in mid-September.

Counts of *V. dahliae* colonies on roots and yield data were transformed [ln (x+1)] prior to analysis. Area under the senescence progress curve (AUSPC) was computed for proportional assessments of Verticillium wilt symptom severity in each plot (Shaner and Finney, 1977). Root colonization, disease severity, and yield data were subjected to analysis of variance using a general linear model procedure (proc GLM of SAS) to evaluate the significance of the isolate main effect. Treatment means were separated by Fisher's protected least significant difference (LSD) test. Significant differences were accepted at $P \leq 0.05$.

Field study 3. Fungal inoculum of the mint and potato isolates of *V. dahliae* was produced during the winter of 1998-99 as described in Chapter 2. Putative vegetative compatibility groups are VCG 1 and VCG 4 for the mint and potato isolates, respectively. Plots were established in May 1999 at the Botany and Plant Pathology Research Laboratory in Corvallis, OR in a sandy loam soil. The soil did not have a measurable population of *V. dahliae*. Soil samples were collected with a hand trowel in April. Soil nutrients (phosphorous, potassium, calcium, magnesium, manganese, sodium, zinc, percent organic matter, total Kjeldahl nitrogen, pH, and soluble salts) were determined by the Central Analytical Laboratory, Department of Crop and Soil Science, Oregon State University.

Two *V. dahliae* isolates were combined factorially with six inoculum densities for a total of 12 treatments. Forty eight plots were established, each 1.84 m x 1.84 m and separated by a 2.76 m wide border of grass (60% Chewings fine fescue; 40% Nui perennial ryegrass). Each plot was infested with an isolate of *V. dahliae* obtained from either mint or potato at a rate of 0, 1, 2, 4, 8, or 16 CFU/g of soil. Treatments were arranged in a randomized complete block design and replicated four times.

The fertilizers N, P_2O_5, K_2O, and boron were broadcast by hand at rates of 112, 140, 120, and 11 kg/ha, respectively, according to the OSU Extension Service recommendations. On 19 May, the *V. dahliae* inoculum was applied by hand to the surface of each plot, and the fertilizer and inoculum were immediately incorporated to a depth of 15 cm with a rototiller. On 20 May, one half of each plot was planted to peppermint (*Mentha piperita* L. cv. Black Mitcham) (24 rooted cuttings per plot) and the other half to potato (*Solanum tuberosum* L. cv. Russet Norkotah) (eight 50-100 g single eye seed pieces/plot) on 26 May.

Potatoes were sprayed three times during the season with chlorothalonil (Bravo® 720, ISK Biosciences Corporation, Mentor, OH) for control of late blight at a rate of 1 L/ha using a backpack sprayer. Plots were hand weeded throughout the summer.

Soil populations of *V. dahliae* were assayed immediately following infestation. Six soil cores (1.75 cm in diameter x 15 cm long) were collected with a soil probe from each plot in an "N"-shaped sampling pattern. Soil cores from each plot were bulked to form one sample. Samples were kept overnight at 5°C. Each

soil sample was mixed thoroughly by hand and dried at room temperature for 3 wk.

Dried soil samples were ground with a mortar and pestle. Using an Anderson air

sampler (Butterfield and DeVay, 1977), soil aliquots of 0.17 g were plated onto

each of 10 petri plates that contained Sorensen's NP-10 medium (Sorensen et al.,

1991). After incubation at room temperature in the dark for 3 wk, adhering soil

particles were washed off the agar surface under running tap water. Plates were

examined with the aid of a dissecting microscope. Number of colony forming units

of *V. dahliae* per gram of dry soil was expressed as CFU/g.

Disease severity assessments were made at 9 day intervals from the time of

initial symptom observation until early-September in peppermint and at 6 day

intervals from the time of initial symptom observation until mid-August in potato.

In peppermint, symptomatic stems (strikes) in each plot were counted at each

disease assessment date. In potato, three disease assessments were made in each

plot using a scale of 0-100%, where 0% = no symptoms and 100% = total foliar

senescence.

All above-ground potato plant material was harvested on 19 August, placed

in burlap sacks, dried for 48 hr at 100°C, and weighed. Potato tubers were

harvested by hand and weighed on 31 August.

Values for aerial biomass and tuber yield were transformed (ln x) prior to

analysis. Area under the senescence progress curve (AUSPC) was computed for

proportional assessments of Verticillium wilt symptom severity for peppermint and

potato (Shaner and Finney, 1977). Data were subjected to analysis of variance

using a general linear model procedure (proc GLM of SAS) to evaluate the

significance of the isolate and inoculum density main effects and of their

interaction. Treatment means were separated by Fisher's protected least significant

difference (LSD) test. Significant differences were accepted at a P ≤ 0.05. Disease

severity data for peppermint and disease severity, aerial biomass, and tuber yield

data for potato were also regressed by isolate on the transformed inoculum density

of *V. dahliae* [ln (x+1)] using a least squares regression procedure (proc REG of

SAS) to test for linear trends.

RESULTS

Field study 1. Disease severity in potato was 26% higher (P=0.0329) when grown

in soil infested with a potato isolate of *V. dahliae* compared to a maple isolate

(Table 4.1). Tuber yield was 26% higher when potatoes were grown in plots

infested with a potato isolate of *V. dahliae* compared to a maple isolate, however,

this difference was not significant (P=0.2232) (Table 4.1).

Table 4.1. Effect of *Verticillium dahliae* isolate on area under the senescence progress curve (AUSPC) and tuber yield (kg) of potato grown in soil infested with isolates from maple or potato.

Isolate	AUSPC[a]	Yield[a]
Maple	863.5A	4.01A[b]
Potato	1173.0B	5.04A

[a] Means in the same column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis.
[b] Analysis of variance was based on ln kg. Means shown represent nontransformed data.

Field study 2. Colonization of eggplant roots by *V. dahliae* was not detected in 1998. In 1999, root colonization was not significantly different among the mint, potato, and maple isolate treatments (Table 4.2). Colonization was, however, 100% less (P=0.0001) in plots not infested with any isolate of *V. dahliae* compared to all infested plots.

In 1998, disease severity of eggplant grown in soil infested with the potato isolate of *V. dahliae* was 185 and 176% higher (P=0.0001) than with the mint or maple isolates, respectively (Table 4.2). In 1999, eggplant grown in soil infested with the potato isolate again had the highest disease severity, but not significantly more than eggplant grown in soil infested with the maple isolate. Disease severity was 454 and 232% higher (P=0.0001) in eggplant grown in soil infested with the potato or maple isolates than with the mint isolate in 1999.

In 1998, fruit yield of eggplant grown in soil infested with the potato isolate was 60 and 67% less (P=0.0021) than eggplant grown in soil infested with the mint

or maple isolates, respectively (Table 4.2). In 1999, fruit yield was again lowest in

the potato isolate-infested plots, and was significantly less in the potato or maple

isolate-infested plots compared to the mint isolate-infested or noninfested plots

(P=0.0015).

Table 4.2. Effect of *Verticillium dahliae* isolate on root colonization (CFU/cm),
area under the senescence progress curve (AUSPC), and fruit yield (kg) of eggplant
grown in soil infested with either a mint, potato, or maple isolate of *V. dahliae*.

Isolate	Root colonization	AUSPC		Yield	
	1999	1998	1999	1998	1999
Mint	0.0294A[a]	503.0A	164.85A	3.31A	2.80A
Potato	0.0368A	1434.2B	912.74B	1.33B	0.59B
Maple	0.0405A	519.6A	546.99B	3.99A	1.11B
Noninfested	0.0000B	-[b]	138.72A	-[b]	2.40A

[a] Means in the same column followed by the same letter are not significantly
different ($P \leq 0.05$) according to Fisher's protected least significant differences
(LSD) analysis. Analysis of variance was based on ln CFU/cm, AUSPC, and ln kg.
Means shown represent nontransformed data.
[b] No noninfested control plots in 1998.

Field study 3. Measurable soil populations of *V. dahliae* 1 mo after infestation

were proportional to expected population counts based on treated soil volume

calculations made prior to infestation (Table 4.3).

Table 4.3. Soil populations (CFU/g soil) of *Verticillium dahliae* in field plots one month after infestation with either a mint or a potato isolate at an expected inoculum density of either 0, 1, 2, 4, 8 or 16 CFU/g of soil.

V. dahliae isolate	Expected inoculum density	CFU/g
Mint	0	0.00
	1	0.28
	2	1.53
	4	1.39
	8	5.97
	16	6.67
Potato	0	0.00
	1	0.83
	2	2.36
	4	5.56
	8	12.64
	16	14.72

Severity of Verticillium wilt symptoms in peppermint was higher in plots infested with a mint isolate of *V. dahliae* than with a potato isolate; severity of Verticillium wilt symptoms in potato was higher in plots infested with a potato isolate than with a mint isolate (Table 4.4). Both the isolate and the inoculum density treatments were significant main effects, and their interaction was significant for both peppermint (Table 4.5) and potato (Table 4.6). The significant interaction was a result of inoculum density only showing a significant effect on the host from which an isolate was obtained. Thus, for peppermint, inoculum density of the mint isolate (P=0.0004), but not of the potato isolate (P=0.7467), was a significant predictor of symptom severity ($R^2_{mint\ isolate}$ = 0.9678; $R^2_{potato\ isolate}$ = 0.0291) (Figure 4.1). Conversely, in potato, inoculum density of the potato isolate

(P=0.0124), but not of the mint isolate (P=0.7421), was a significant predictor of symptom severity ($R^2_{potato\ isolate}$ = 0.8236; $R^2_{mint\ isolate}$ = 0.0302).

In peppermint grown in soil infested with the mint isolate, disease severity increased as inoculum density increased from 0 to 1 CFU/g (P=0.0588). In potatoes grown in soil infested with the potato isolate, disease severity increased by 120% as inoculum density increased from 0 to 1 CFU/g (P=0.0678). These differences in AUSPC values, however, were not significant. As inoculum density increased from 0 to 16 CFU/g, disease severity of peppermint grown in soil infested with the mint isolate was first significantly different from the noninfested control treatment at an inoculum density of 2 CFU/g, but disease severity of peppermint grown in soil infested with the potato isolate did not increase significantly as the inoculum density. Similarly, as inoculum density increased from 0 to 16 CFU/g, the disease severity of potatoes grown in soil infested with the potato isolate was first significantly different from the noninfested control treatment at an inoculum density of 2 CFU/g, but disease severity of potatoes grown in soil infested with the mint isolate did not increase significantly as the inoculum density increased.

In potato, inoculum density of the potato isolate of *V. dahliae* (*P*=0.0384), but not of the mint isolate (*P*=0.5401), was a significant predictor of aerial biomass ($R^2_{potato\ isolate}$ = 0.6978; $R^2_{mint\ isolate}$ = 0.1006) (Figure 4.2). Inoculum density of the potato isolate of *V. dahliae* (*P*=0.0403), but not of the mint isolate (*P*=0.7113), was a significant predictor of tuber yield ($R^2_{potato\ isolate}$ = 0.6912; $R^2_{mint\ isolate}$ = 0.0380) (Figure 4.2).

Table 4.4. Effect of *Verticillium dahliae* isolate on area under the senescence progress curve (AUSPC) of peppermint and potato grown in soil infested with either a mint or a potato isolate of *V. dahliae*.

Plant species	*V. dahliae* isolate	AUSPC
Peppermint	Mint	183.1A[a]
	Potato	0.1B
Potato	Mint	780.6A
	Potato	1064.2B

[a] Means within each species followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant differences (LSD) analysis.

Table 4.5. General linear model summary for AUSPC of peppermint in field soil infested with either a mint or a potato isolate of *Verticillium dahliae* at an inoculum density of either 0, 1, 2, 4, 8, or 16 CFU/g soil.

Source	DF	Type III SS	Mean square	F value	Pr>F
Isolate	1	20009855.7	20009855.7	85.14	0.0001
Inoculum density	5	7010050.9	1402010.2	5.97	0.0004
Isolate*Inoc. density	5	7026362.3	1405272.5	5.98	0.0004

Table 4.6. General linear model summary for AUSPC of potato in field soil infested with either a mint or a potato isolate of *Verticillium dahliae* at densities of 0, 1, 2, 4, 8, or 16 CFU/g soil.

Source	DF	Type III SS	Mean square	F value	Pr>F
Isolate	1	1290843.6	1290843.6	8.26	0.0068
Inoculum density	5	2040031.9	408006.4	2.61	0.0410
Isolate*Inoc. density	5	2196451.9	439290.4	2.8	0.0304
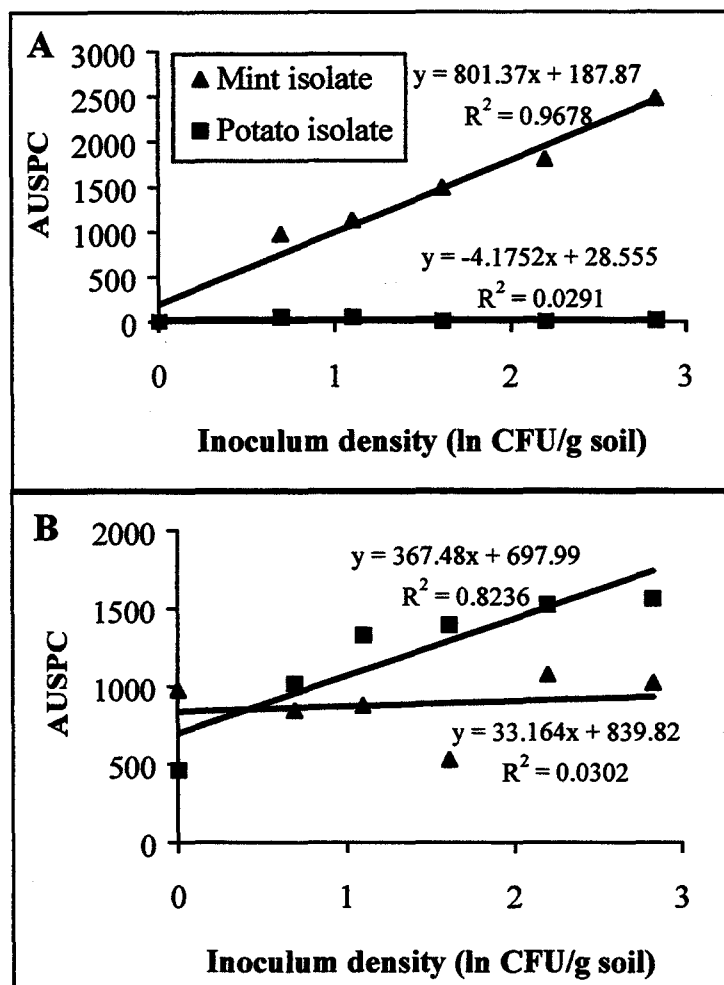
Figure 4.1. Effect of isolate and inoculum density of *Verticillium dahliae* on area under the senescence progress curve (AUSPC) of **A**, peppermint, and **B**, potato, grown in soil infested with either a mint or a potato isolate of *V. dahliae* at an inoculum density of either 0, 1, 2, 4, 8, or 16 CFU/g[a] soil.

[a] Inoculum densities were transformed [ln (x+1)] prior to regression analysis.

Figure 4.2. Effect of isolate and inoculum density of *Verticillium dahliae* on **A**, aerial biomass (ln g) and **B**, tuber yield (ln kg) of potato grown in soil infested with either a mint or a potato isolate of *V. dahliae* at an inoculum density of either 0, 1, 2, 4, 8, or 16 CFU/g[a] soil.

[a] Inoculum densities were transformed [ln (x+1)] prior to regression analysis.
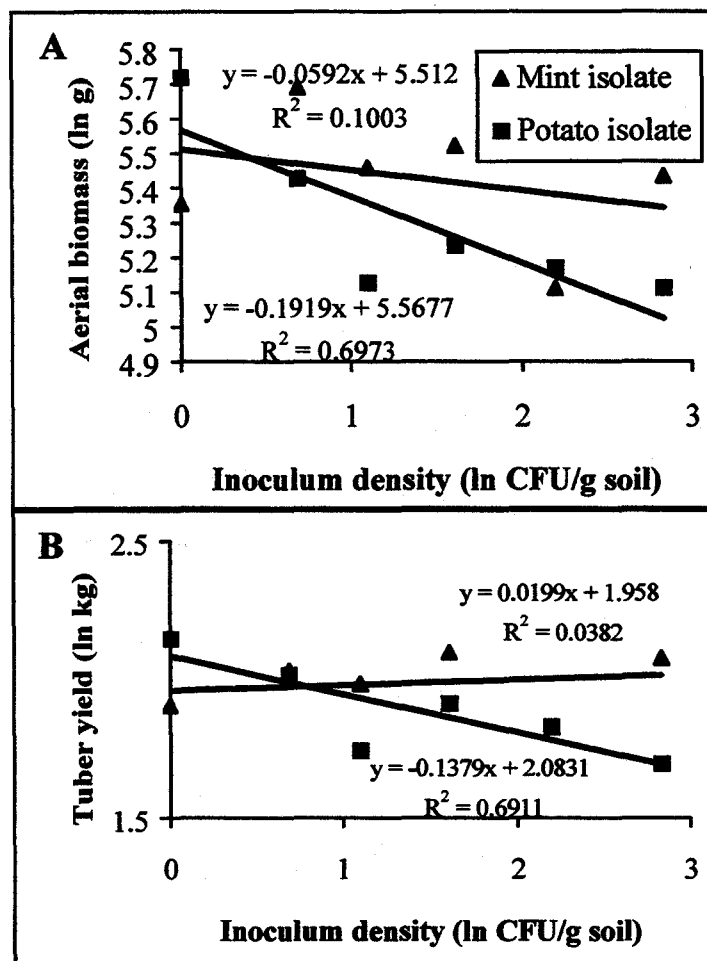
DISCUSSION

Severity of Verticillium wilt symptoms was greater in potatoes grown in soil infested with an isolate of *V. dahliae* from potatoes than with an isolate from maple, although resulting tuber yields were not significantly different among isolates. Potatoes and maples are classified into different taxonomic families, supporting the hypothesis that Verticillium wilt severity in one host is greater in soil infested with an isolate of *V. dahliae* obtained from that host than with an isolate from an unrelated host. Therefore, potatoes planted into soil infested with a potato isolate are at risk for high losses to Verticillium wilt. In soils infested with an isolate from maple, disease severity in potato may be less. This experiment should be repeated to confirm these results. Bhat and Subbarao (2000) also reported that Verticillium wilt was more severe when hosts were inoculated with an isolate from that host than with isolates from unrelated hosts.

On eggplant, the isolate of *V. dahliae* from potato was most aggressive, followed by the maple isolate and the mint isolate. This ranking of host response to isolate was the same, whether results were based on disease severity or fruit yield data. Similarly, Gent et al. (1995) reported that yield of eggplant was correlated with leaf area. Leaf area decreases, due to leaf necrosis and defoliation, as Verticillium wilt severity increases. There were no differences, however, in colonization of eggplant roots among isolates. Previous studies also have reported that the susceptibility of a species to *V. dahliae* is not correlated with the number of colonies per length of root, and that not all root infections lead to colonization of

the vascular system or disease development (Evans and Gleeson, 1973; Huisman, 1988). This may explain why even though root colonization was similar across isolates in our study, disease severity and fruit yield varied.

Since eggplant and potato are both members of the Solanaceae family and maple and peppermint belong to the families Aceraceae and Lamiaceae, respectively, these observations support the hypothesis that plant species are more susceptible to isolates of *V. dahliae* from related hosts than from unrelated hosts. Bhat and Subbarao (2000) reported that *V. dahliae* isolates obtained from eggplant, potato, and tomato (Solanaceous species) were more pathogenic to eggplant than was an isolate from mint. Furthermore, this study reported that isolates from cabbage, cotton, and other unrelated species did not cause disease in eggplant, while isolates from eggplant caused significant disease on eggplant and potato. Bhat and Subbarao (2000) also reported that disease severities in cabbage and cauliflower, both *Brassica oleracea*, were highest when inoculated with either of those isolates compared to inoculation with isolates from non-Cruciferous species. In fields infested with a single isolate of *V. dahliae*, a long rotation with crops not susceptible to that isolate may be an effective disease management strategy when used in combination with other strategies to suppress soil pathogen populations, since the number of *V. dahliae* microsclerotia in the soil declines naturally over time (Mol et al., 1996). A thorough characterization of the aggressiveness of the isolate must first be made.

Inoculum density as a predictor of disease severity of peppermint and potato and aerial biomass and tuber yield of potato varied with the isolate of *V. dahliae*.

Disease severity of peppermint grown in plots infested with a mint isolate increased

with inoculum density, whereas disease severity of peppermint grown in plots

infested with a potato isolate did not. Conversely, disease severity of potatoes

grown in plots infested with a mint isolate did not increase with inoculum density,

whereas disease severity of potatoes grown in plots infested with a potato isolate

did. Although no microsclerotia of *V. dahliae* were detected in the noninfested

control plots, either before or after artificial infestation, the high AUSPC for

potatoes grown in these plots indicates that there may in fact have been a

background level of *V. dahliae*, specifically an isolate which is particularly

aggressive on potato, since peppermint grown in noninfested plots showed no or

very low levels of disease.

Disease severity was significantly higher at an inoculum density of 2 CFU/g

soil than in noninfested control plots for both the mint and potato isolates on their

corresponding hosts. Crowe et al. (2000) found that low inoculum densities (0.1

CFU/g) caused significant disease in peppermint. In contrast, Nnudo and Harrison

(1979), found that the minimum inoculum density of *V. albo-atrum* required to

cause significant tuber yield reductions in potato was 17.5-23 CFU/g soil and that

as air temperature decreased, higher inoculum densities were required to cause

significant disease expression. The low minimum inoculum density (2 CFU/g)

required to cause significant disease and tuber loss in our study may be due to

differences in environment (e.g. air temperature) in Oregon during 1998-99

compared to Colorado during 1975-76; however the air temperature data for these

years in Colorado were not reported.

Bhat and Subbarao (2000) observed that an isolate from mint was pathogenic on mint, but not on potato, and that an isolate from potato was pathogenic on both mint and potato. While these findings suggest that isolates from mint show host specificity and is less aggressive on other hosts and that potato isolate is more aggressive because it attacks multiple hosts, the results from our study suggest that both the mint and potato isolates show both host specificity and differential aggressiveness on peppermint and potato. Neither isolate appeared to be more aggressive than the other.

The results from this field study do not support the hypothesis that isolates obtained from various hosts exhibit differential aggressiveness on their corresponding hosts. The conclusions, however, may have been different if a wider range of hosts and fungal isolates had been used (Bhat and Subbarao, 2000). Therefore, knowledge of previous susceptible crops and/or the VCGs of the *V. dahliae* isolates that make up the fungal soil population in a field may be as or more important than knowledge of pre-plant numbers of *V. dahliae* microsclerotia. Further characterization of isolate aggressiveness and host susceptibility relationships are necessary. A practical method for rapid classification of isolate type also needs to be developed. This kind of information would be of particular importance to the nursery industry, for which land could be used to produce a relatively wide range of crops.

SUMMARY

Broccoli green manure, soil solarization, and long rotations with crops not susceptible to Verticillium wilt are potential alternatives to chemical soil fumigation and may help reduce the usage of toxic chemicals in crop production. Following the incorporation of a broccoli green manure, soil populations of *Verticillium dahliae* generally declined. The severity of Verticillium wilt in potato also was reduced following a broccoli green manure; however, disease severities in peppermint, purple smokebush, and amur maple were not affected. One possible explanation for the inconsistent effectiveness of the broccoli green manure is the low amount of broccoli biomass used in our studies, compared to previous studies on green manures. Despite some inconclusive results, the use of a broccoli green manure to suppress Verticillium wilt retains potential to become an effective disease management strategy. Furthermore, while *V. dahliae* soil populations were not significantly affected by soil solarization, the onset of Verticillium wilt of smokebush was delayed by one month in plots which were solarized, indicating that further investigation into the use of soil solarization as an alternative to chemical soil fumigation is warranted.

When the relative aggressiveness of three isolates of *V. dahliae* on various hosts was examined, isolates were generally more aggressive to the host from which they were obtained or to a related host than were isolates obtained from other, unrelated hosts. Knowledge of the aggressiveness of the *V. dahliae* isolates

in the soil on various crops could help growers decide which crops to plant and which to avoid.

The fungal inoculum densities used to infest the soils in these experiments were higher than generally occur in naturally infested soils, which may have been an important factor contributing to the inconsistent effectiveness of the broccoli green manure and soil solarization treatments. In this regard, green manures, soil solarization, and long crop rotations may be most effective when used to slow the accumulation of microsclerotia of *V. dahliae* in the soil rather than to sanitize a heavily infested field. Additionally, the effect of a broccoli green manure on suppression of *V. dahliae* was shown to be a function of biomass and thus, the effectiveness of the green manure for suppression of disease may potentially be increased by incorporating larger amounts of biomass or by incorporating the green manure during multiple consecutive seasons. Soil solarization may require hotter climates or longer periods of treatment than evaluated in this study in order to significantly affect *V. dahliae* soil populations and Verticillium wilt. Anecdotal evidence from growers suggests that adding broccoli green manure biomass to a field, in a rotation of broccoli with potato, increases potato health and tuber yield, correlating with some of the results of the studies contained in this thesis. Further study is needed before recommendations can be made to growers to replace chemical soil fumigation with broccoli green manuring and soil solarization. However, if green manure biomass and solarization tarps are readily and cheaply available, I suggest that adding these treatments to current disease management

programs will be beneficial to the growth of a crop or, in the worst-case scenario,

have no impact on Verticillium wilt suppression.

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APPENDIX

Table A1. Nutrient analysis of field soil in the spring of each year at the Botany and Plant Pathology Research Farm, Corvallis, OR performed by the Central Analytical Laboratory, Department of Crop and Soil Science, Oregon State University.

Nutrient	1997	1998		1999	
	Fallow[a]	Fallow[a]	Broccoli[b]	Fallow[a]	Broccoli[b]
P (ppm)	19	34.5	29.5	20	33
K (ppm)	187	177.5	162	144	203
Ca (M_{eq}/100g)	6.9	7.1	6.5	6.8	6.1
Mg (M_{eq}/100g)	2.8	3.15	2.9	2.9	2.6
Mn (ppm)	3.2	3.55	3.45	-[c]	-[c]
Na (M_{eq}/100g)	0.08	0.09	0.09	0.13	0.11
Zn (ppm)	0.78	0.96	0.84	-[c]	-[c]
% organic matter	2.33	2.76	2.61	2.91	2.84
total Kjedahl N (%)	0.03	0.04	0.04	0.04	0.04
pH	6.4	6.2	6.2	6.5	5.8
soluble salts(mm_{hos}/cm)	<0.2	0.2	<0.15	0.1	0.1

[a] Soil had been fallowed for several years.

[b] Soil had been amended with a broccoli green manure during the previous year.

[c] No analysis was performed for this nutrient.

Table A2. Mean maximum and minimum daily soil temperatures (°C) in field plots (Botany and Plant Pathology Research Farm, Corvallis, OR) at a depth of 15 cm for the 17 days following broccoli biomass incorporation in 1997, 1998, and 1999.

Year	Minimum	Maximum
1997	18.36	28.68
1998	19.68	28.91
1999	17.49	27.47

Table A3. Mean daily soil temperatures (°C) in broccoli green manure-amended and fallowed field plots (Botany and Plant Pathology Research Farm, Corvallis, OR) at a depth of 15 cm for the 17 days following broccoli biomass incorporation in 1997, 1998, and 1999.

Year	Broccoli	Fallow
1997	22.66	23.88
1998	25.74	24.92
1999	24.78	24.65