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Increasing regulation and costs of soil fumigation to manage diseases such as Verticillium wilt caused by soil-borne pathogens have prompted a search for management alternatives. Single-year microplot experiments were conducted in 2002 and 2003 in central Oregon to evaluate the effects of Austrian winter pea, broccoli, and Sudan grass green manures applied at three rates (6, 12, or 24 Mg ha^{-1}) on soil and potato root populations of *Verticillium dahliae*, severity of Verticillium wilt of potato, and tuber yield. Amendment rate had no effect on inoculum density (ID) or root infection (RI), but affected relative area under the senescence progress curve (RAUSPC). Green manures of broccoli applied at all three rates, Sudan grass applied at 6 or 12 Mg ha^{-1}, and Austrian winter pea applied at 24 Mg ha^{-1} reduced soil populations of *V. dahliae* compared to the unamended control. For green manures of broccoli or Sudan grass, reduction in ID could partially account for reduction of RAUSPC ($r = 0.44$ and 0.46, respectively). Green manures of Austrian winter pea applied at 12 or 24 Mg ha^{-1} and broccoli or Sudan grass applied at 24 Mg ha^{-1} reduced RAUSPC by 74, 70, 70, and 52%, respectively, compared to the unamended control. None of the green manure treatments in either experiment was able to significantly increase yield relative to the unamended control. However, in 2002, Sudan grass applied at 12 and 24 Mg ha^{-1}, and broccoli applied at 24 Mg ha^{-1} resulted in a mean

different from that of the uninfested plots and yield was negatively correlated with disease severity ($r = -0.61$, $P \leq 0.0001$). In 2003, due to an earlier harvest, there were no differences in yield between amended and unamdended plots and no relationship between yield and disease severity.

To explore possible mechanisms of suppression, soil chemical and microbial properties related to severity of Verticillium wilt were identified using regression analyses. We then assessed how these soil properties were affected by the green manure treatments. Soil population of *V. dahliae* predicted 48 and 52% of the variability in disease severity in 2002 and 2003, respectively. In 2002, after accounting for different levels of ID, inclusion of a term for pH improved goodness-of-fit of the regression model to 60%. Severity of Verticillium wilt was overwhelmingly influenced by a wide soil pH gradient (pH 5.2 to 7.5) across the 2002 field. Soil pH was positively related to disease severity and was not affected by green manure treatments. In 2003, after accounting for different levels of ID, inclusion of a term for NO_3-N, microbial biomass (MBC), or microbial respiration (MR), improved fit of the model to 64, 66 or 62%, respectively. All soil properties identified in 2003 were negatively related to disease severity and were positively associated with green manure amendment rate. Finally, stepwise regression using data pooled from both studies resulted in a model which included terms for ID, soil pH, and FDA and accounted for 63% of the variability in RAUSPC.

Effect of Green Manures on Verticillium Wilt of Potatoes and on Soil Properties Related to Disease Suppression.

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CONTRIBUTION OF AUTHORS

Dr. Mary L. Powelson was involved in development of the green manure kind and rate study and was involved in the measurement and interpretation of pathogen and disease variables. Dr. Fred Crowe was involved in research design and implementation. Dr. Richard P. Dick was involved in the analysis and interpretation of soil chemical and biological factors. Naoyuki Ochiai was involved in data collection, laboratory and statistical analyses, and primary development of each manuscript. All authors contributed to the writing of each manuscript.

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INTRODUCTION

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major disease of many cultivated plants, including potato, throughout the temperate regions of the world (102). In North America, yield reduction can range from 10 to as high as 50% (84). Crop rotation is considered to be an impractical management strategy due to the long-term persistence of the pathogen in soil (121) and its wide host range, including non-susceptible bridging hosts (38). One management tactic is soil fumigation to reduce soil populations of *V. dahliae* (85). In North America, metam sodium is used widely to manage this disease (89). In recent years, however, greater regulation of agrochemicals and increasing costs of application have prompted the search for management alternatives.

Green manures and other organic matter (OM) amendments have been used to improve soil tilth and fertility since early times (68). In the last century, organic soil amendments have been shown to suppress some diseases caused by soil-borne pathogens (70,90). Organic matter-mediated disease suppression is thought to result from physiochemical changes in the soil (68) and/or increase in the activity of the general microbial community or of specific members of the microbial community (22,120). More recently, research has focused on the possibility that the OM-derived compounds may have direct deleterious effects on pathogens (17,20).

Little is known about what soil properties are relevant to suppression of Verticillium wilt or how these properties are affected by different soil amendments. Some green manures, particularly those in the brassica family, are thought to act as biofumigants and suppress disease by reducing soil populations of the pathogen (10,107,123). However, suppression of Verticillium wilt by green manures of Sudan grass, Austrian winter peas (28), sweet corn or barley (26) is not always related to changes in inoculum density. Although numerous organic soil amendments have been evaluated for their potential to suppress Verticillium wilt (10,20,28,36,64,80,88), not

all studies concurrently investigated the effects of amendments on soil populations of *V. dahliae* and few have investigated the relation between soil properties and disease severity. Disease suppression by organic soil amendments is often a function of the amount of OM amended (23,47,106) and therefore likely related to the degree of change in soil properties brought about by the amendment. In a greenhouse study, Davis *et al.* (27) reported a negative relationship between the amount of Sudan grass green manure amended and the severity Verticillium wilt of potatoes. To our knowledge, no studies have investigated the effect of amendment rate of green manures on Verticillium wilt at the field scale.

Therefore, two single-year microplot field studies were conducted to compare the effect three green manures on soil populations of *V. dahliae*, severity of Vertillium wilt of potato, and tuber yield. Furthermore, soil properties related to suppression of Verticillium wilt were identified and the effect of green manure treatments on those soil properties were assessed.

CHAPTER 1

REVIEW OF LITERATURE ON VERTICILLIUM WILT OF POTATOES AND THE USE OF GREEN MANURES TO SUPPRESS SOIL-BORNE PLANT PATHOGENS.

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VERTICILLIUM WILT OF POTATO

Etiology. Verticillium wilt is a disease affecting over 200 dicotyledonous plant species, including a number of agronomically important crops, primarily in temperate regions of the world (81). Two species of the taxon *Verticillium* are recognized as the primary causal agents of Verticillium wilt of potato: *Verticillium dahliae* Kleb and *V. albo-atrum* Reinke and Berthold. In the eastern and northern production areas of the United States and Canada, both species are involved in the disease; whereas in the north-central states and the Pacific Northwest, *V. dahliae* predominates (84).

In the absence of host plants, *V. dahliae* forms nutrient-independent microsclerotia (MS), which have been reported to remain viable for up to 13 years (121). While conidia and mycelia also can be found in soil and plant residue, these are relatively short-lived (81); MS are predominant in the soil and are considered to be the primary infective unit. Germination of MS is triggered by exudates from roots of both susceptible and non-susceptible plants (94). Infection occurs when hyphae of germinating microsclerotia penetrate the roots near the root cap or region of elongation and colonize the cortex of the plant (38); the hyphae breach the endodermis and enter the xylem of a susceptible plant (43). Once inside the xylem vessel, the pathogen produces conidia throughout the life of the plant, which are carried by water through the vascular system (83). When the population of the pathogen in the xylem is large enough, the flow of water through the xylem is obstructed, resulting in wilt symptoms and eventually death of the host. Early symptoms of the disease are unilateral chlorosis, wilt, or necrosis of the plant's lower leaves. These symptoms progress upward in the foliage as the disease progresses. Dissection of the lower stem tissue reveals vascular discoloration indicating presence of the pathogen. When symptoms occur early in the growing season, tuber yield and quality are reduced. Upon death of the host tissue, microsclerotia are formed within decomposing tissue and are returned to the soil in plant residue (72). Microsclerotia are released from the tissue to the soil within two years following incorporation of the crop residue.

Verticillium wilt is considered to be a monocyclic disease (94). The relation between inoculum density and disease severity measures such as root colonization, wilt incidence, wilt severity, and stem infection observed in studies are generally consistent with a monocyclic disease (74,76,87,122). For the most part, disease severity is proportional to inoculum density up to a saturation inoculum density level, above which there is no further increase in disease severity; the specific relationship between disease severity and inoculum density lower than the saturation inoculum density, as well as the saturation density, is dependent on pathogen strain, host plant, and a variety of environmental factors (81).

Abiotic factors affecting Verticillium wilt. Environmental factors such as temperature (21,84,113), water potential (42), and soil type (41), are known to have an influence on *V. dahliae* and the severity of Verticillium wilt but will not be emphasized in this review. This review focuses on soil chemical factors that influence the severity of Verticillium wilt and yield.

Verticillium wilt is generally more severe in neutral to alkaline soils (pH 6 to 9) and generally less severe in acidic soils (49,81). Jones and Woltz (57) demonstrated that raising soil pH from 6.0 to 7.5 by liming more than doubled mid-season wilt incidence of tomatoes. Wilhelm (119) and Orellana et al. (77) demonstrated that liming very acidic soils resulted in increased disease incidence and that acidifying soils decreased wilt severity. In some cases, soil pH did not appear to have any effect on disease severity (21,25). In the Davis et al. (25) study, however, the soil pH ranged from 6.0 to 8.2 and did not encompass acidic soils. Although the positive association of soil pH and disease severity is recognized as a general trend (81), cases of Verticillium wilt in acid or neutral soils is not unknown (119). More recently, Brinkerhoff (16) reported Verticillium wilt of cotton in acid clay soils in the Mississippi Delta.

Assuming, however, that there is a fairly consistent relationship between soil pH and severity of Verticillium wilt, it is still difficult to conclude that the concentration of H^+ ions *per se* is responsible for differences in disease severity

(49,82). Soil pH is a "master variable" that controls the availability and form of numerous minerals (104) and microbial community structure (14). The concentration of Ca^{2+} is often strongly correlated with soil pH. It is speculated that high Ca-uptake by plants, which is encouraged by high pH (above 7.8) (73), may play a role in controlling diseases by reinforcing root cell wall structure and inhibiting the polygalacturonase activity of some pathogenic fungi (49,82). High concentrations of soluble aluminum (Al^{3+}) which can occur at low pH (below 5.5) may be toxic to *Verticillium* spp. (49,77). Higher concentrations of ammonia (NH_4) can also be encouraged by low pH reduced ammonia volatilization and slower rates of microbial nitrification (Brady and Weil, 1999); high levels of ammonium have consistently been associated with suppression of Verticillium wilt (1,33,50,81,82,120). Soil pH can also have an influence on the microbial community structure. Fungi, which are often associated with suppression of pathogens, tend to dominate in acid soils, while bacteria dominate in neutral to alkaline soils (14).

Evidence for the direct role of H^+ ions in suppression of *V. dahliae* was presented by Abdel-Razek Osman et al. (1). In an *in vitro* study, they reported optimal germination of *V. dahliae* microsclerotia at pH 7.5, with decreasing germination at pH 6.5, 5.0 and 3.5; abnormal germination and malformed germ tubes were observed at pH 3.5. Optimal conidia production occurred at pH 6.4 and pH 7.5, with decreasing production at pH 5.0 and 3.5. Their findings are consistent with an inference drawn by Pegg and Brady (81) in a review of Verticillium literature that microsclerotial production and survival are completely inhibited at pH 5.5 and below.

The effects of macronutrients on Verticillium wilt can differ significantly by host (82). Deficiency of soil potassium was observed to increase incidence of Verticillium wilt of pistachio trees (5) and cotton (44), but delayed the onset of wilt of alfalfa (53). High levels of nitrogen were associated with greater Verticillium wilt of alfalfa (54), eggplant (101), or hop (59), but reduced disease of potatoes (24,51).

The effect of form of nitrogen fertilizer (NH_4^+ or NO_3^-) has been the subject of many studies. The majority of both laboratory and field research reports that

fertilization with NH_4-N results in inhibition of the pathogen or a reduction of disease severity (1,33,50,81,82,105,120). High NO_3-N is commonly associated with increased wilt severity in potatoes (82), this is not always the case (25,30).

Relatively less is known about the role of micronutrients on Verticillium wilt. According to Davis et al. (28), considerable work by Russian scientists has demonstrated a relationship between Mn, Zn and suppression of Verticillium wilt. Bell (8) also identified Mn, and Zn, as well as Cu and Co as being associated with reduction in wilt. Jones and Woltz (57) concluded that Fe, Mg, and Zn had no effect on Verticillium wilt of tomato.

Modeling factors related to severity of Verticillium wilt. The development of disease models can enhance our understanding of disease etiology and factors influencing disease as well as provide tools to assess disease risk or simulate outcomes of management practices. Models provide an opportunity to synthesize understanding of the biology of disease with empirical data. While there are extensive experimental data for and theoretical interpretations of the dynamics of diseases caused by air-borne pathogens, less is known about diseases caused by soil-borne pathogens (71).

In the case of *V. dahliae*, little theoretical framework exists beyond the classification of the disease as being monocyclic; thus, most attempts to model Verticillium wilt or yield loss due to Verticillium wilt are based solely on preplant inoculum density of *V. dahliae* (112,122) or a combination of *V. dahliae* and other pathogens such as *Pratelynchus penetrans* (40,117). In some studies, the relationship between inoculum density and disease severity was found to be consistent with that predicted by theory, with the specific relationship being described as linear (60,74), negative exponential (60,122), quadratic (60,87), or linear logit (76). These curves have the general characteristics of encompassing a range of inoculum density over which there is a positive relationship with disease severity, and a value of inoculum density above which there is little or no additional increase in disease severity.

In other studies, however, no relationship between inoculum density and severity of Verticillium wilt could be established (6,24,32,60). For the most part, the

studies in which relationships between inoculum density and disease severity were observed involved smaller experimental units such as pots in greenhouses or microplots, while the studies in which relationships were not observed involved field-scale studies or surveys of different fields. Khan et al. (60) reported that although they observed significant correlation between inoculum density and severity of root discoloration of horseradish in greenhouse and microplots, no such correlation was observed in a survey of commercial production fields. The lack of relationship between inoculum density and disease severity at larger scales is likely the result of different environmental factors acting on the pathogen and/or host plant between germination of microsclerotia and appearance of disease symptoms (81,111).

Few studies have attempted to validate models developed on a given data set using data from other studies or to evaluate whether the relationship between inoculum density and disease severity observed in one study is also seen in other studies. Termorshuizen and Rouse (112) found that although their model showed relatively good fit to the data on which it was calibrated, it did not perform well using data from literature. They concluded that the model needed to be calibrated on a larger data set and should include more parameters such as those describing fungal dynamics. Their conclusions, as well as the apparent lack of relationship between inoculum density and disease severity in some studies, strongly suggest that models based only on inoculum density are inadequate to describe disease dynamics under different environments.

Although the effects of a number of environmental factors such as temperature, soil type, and soil pH, and soil chemistry as well as different management practices have been studied individually (81), few attempts have been made to analyze the effects of multiple factors simultaneously. Davis et al. (25) made a commendable effort to identify key soil factors related to disease severity in a survey of 100 commercial potato fields in Southeastern Idaho using multiple regression. They identified root colonization, soil sodium content, and soil organic matter as the most significant predictors of yield loss associated with Verticillium wilt accounting for 49,

53, and 62% of the variability for each of three years of the study. In addition, they reported that factors associated with soil integrity factors (organic matter, organic nitrogen, and increased nutrient availability) were related to suppression of wilt severity and higher yield, whereas factors related to loss of soil integrity (sodium and reduced nutrient availability) were related to increased wilt and reduced yield.

It is clear that the disease dynamics of *V. dahliae*, as well as other soil-borne plant pathogens, depend not only on the pathogen and host, but are influenced significantly by environmental factors. In order to develop a disease model which is robust to different environmental conditions, it is necessary to identify which factors are important and how these factors influence the progress of disease. Understanding these factors will enable development of more reliable disease risk assessment and simulation tools.

Disease significance and management. Verticillium wilt of potatoes is reported to cause reductions in over-all yield of 10 to 15% in moderately infested fields and 30 to 50% in severely infested fields (84). In addition, Verticillium wilt can reduce tuber quality by decreasing average tuber size or causing discoloration of the tuber vascular tissue (89).

From a theoretical standpoint, because Verticillium wilt is a monocyclic disease, the reduction of inoculum should have the greatest effect on reducing the rate of disease development (115). Accordingly, the two most effective methods of managing Verticillium wilt, soil solarization and fumigation, involve drastically reducing soil populations of *V. dahliae*.

From a practical standpoint, the most effective control for large-scale potato production is achieved by soil fumigation. A limited number of broad-spectrum chemical agents are being used currently; these include methyl bromide, metham sodium, chloropicrin, and telone. Currently about 75% of the potato fields in the Pacific Northwest are fumigated (84), with metham sodium being the fumigant of choice (89). A number of factors are motivating the potato industry to develop alternative management strategies: (i) the most effective fumigant, methyl bromide, is

being phased out per provisions of the Kyoto Protocol; because of the limited availability and restriction of its use, costs of using methyl bromide are prohibitively high; (ii) there are growing public concerns about the negative environmental impacts and danger to workers using agrochemicals; (iii) in some areas, metham sodium is becoming less and less effective due to enhanced biodegradation (89).

Crop rotation can be effective as a preventative disease management option, but not a curative one (Berlanger and Powelson, 2000). The wide host range, including non-susceptible bridging hosts (89), make selection of appropriate rotation crops difficult; furthermore, the persistence of MS may require a rotation cycle of 4 to 7 years which may be impractical for many growers (89). Where solarization is possible, it is an effective method of control (10); however, its use is limited by costs and availability of biodegradable plastics. In the past, the potato processing industry has resisted changes in cultivars, citing large capital investment required for switching to new products. In 2000, 75% of the potato acreage in North America was planted to 7 cultivars, 6 of which are moderately or highly susceptible to Verticillium wilt (89). However, new technologies for achieving genetic resistance may allow for insertion of resistance factors into existing varieties, retaining the desirable cultivar characteristics (89).

The remainder of this review focuses on one alternative approach to management of Verticillium wilt: the use of green manures and other organic soil amendments to create soil conditions which are suppressive to soil-borne pathogens. The possibility that these amendments might induce abiotic or general microbial suppression is of particular interest in that it represents a departure from the traditional management objectives of reducing inoculum density.

ORGANIC AMENDMENTS TO SUPPRESS DISEASES CAUSED BY SOIL-BORNE PATHOGENS

Disease-suppressive soils. "Suppressive soils" are defined as soils in which a pathogen either "does not establish; establishes but fails to produce disease; or establishes and causes disease at first, but then disease severity diminishes with continued growing of the same crop" (7). The second kind of suppressive soil, in which pathogen activity is reduced, is typified by soils suppressive to Fusarium-wilts, caused by *Fusarium oxysporum* (22). The third kind of suppression, which develops after continuous croppings of a host plant, is typified by the decline of take-all of wheat, caused by *Gaeumannomyces graminis* (4). The type of the suppressiveness depends on the particular pathogen and environment.

The nature of suppressiveness is particularly difficult to discern due to the complexity of the interactions between biological and abiotic factors in the soil environment. However, in many cases, the involvement of the soil microbiota has been implicitly demonstrated by loss of suppressiveness upon sterilization of the soil and recovery of suppressiveness upon inoculation of sterilized soil with the original microbiota. While a number of mechanisms have been posited for biological suppression – including nutrient competition, signal competition, infection site competition, antibiosis – the nature of suppression seems to depend largely on the pathogen in question, and in some cases more than one mechanism may be at work. Furthermore, it is thought that the above-mentioned mechanisms can result from either activity of the microbial community as a whole or from activity of specific microorganisms. The terms "general" and "specific" suppression are commonly used to indicate this distinction.

Besides biological factors, abiotic or soil physico-chemical factors such as temperature, soil texture, soil structure, soil pH, fertility status, and water availability can affect the pathogen's ability to establish and cause disease. For many of these factors, there is a range in which the pathogen is able to function and optima where the pathogen thrives. These factors can likewise influence both activity and structure of

the soil microbial community. Thus suppression may result from conditions that are unfavorable to the pathogen, and/or are favorable to competitors or antagonists. Furthermore, abiotic factors can affect plant physiology, which can in turn affect the rhizosphere environment and biology.

In recent years, the potential of manipulating the soil microbial community to develop suppressive soils has received much attention, leading to two lines of research: using organic matter amendments to increase activity of the microbial community and developing biocontrol agents that target specific pathogens.

Organic amendments to create suppressive soils. Organic soil amendments, including green manures, have been used to improve soil tilth and fertility since ancient times (68). Organic amendments to soil can improve soil aggregation, resulting in reduced bulk density and better water retention and infiltration characteristics. Additionally, organic amendments improve soil fertility by recycling or adding nutrients necessary for healthy plant growth. Researchers in the early 20th century recognized that some soil amendments could control some diseases caused by soil-borne pathogens (70,90). This potential, however, was overlooked in favor of using synthetic inorganic fertilizers and fungicides which were introduced in the middle of the century (47). Organic soil amendments have received renewed interest from researchers in the past few decades as a possible alternative to chemical management of plant diseases.

Organic soil amendments are thought to suppress diseases caused by soil-borne pathogens in one of two ways: (i) microbial suppression, in which pathogen populations are reduced or inhibited by the activity of the indigenous microbial community which is stimulated by the addition of organic matter; or (ii) biofumigation, in which pathogen populations are reduced by the release of toxic compounds from the decomposing organic matter.

Microbial suppression. The microbial nature of suppression associated with organic soil amendments was recognized by early researchers (19,26,46,58,70,90,114) and could be proven relatively easily by loss of suppression upon sterilization of soils.

Cook and Baker (22) suggested that the suppression of pathogens could result from either increased activity of the general microbial community or of specific organisms, and introduced the terms "general" and "specific" suppression to distinguish the two cases. It is likely that even in the case of "general suppression," only specific organisms or groups of organisms are responsible for suppression of the pathogen (48); thus the categories proposed by Cook and Baker (22) can be thought of as conceptual frameworks for understanding the phenomenon rather than as two distinct mechanisms of suppression. The categories serve as the theoretical bases for the two approaches to "biological" control of plant pathogens currently being pursued: (i) "biocontrol" involves the isolation and use of specific organisms that are antagonistic towards the target pathogen; (ii) the other approach is to manipulate soil or growth media to create conditions that are suppressive to the pathogen, with little regard to the specific organisms involved in suppression. While a substantial amount of literature exists regarding "biocontrol" of soil-borne pathogens, the emphasis in this review will be on the latter approach.

Numerous organic matter amendments, ranging from industrial waste products to green manures, have been evaluated for their potential to induce general suppression of soil-borne pathogens. Consistent suppression of a limited number of pathogens (*Pythium* spp. and *Phytophthora* spp.) has been achieved using composted material in container systems (47). Research using container systems over the course of a few decades has led to the following characterization of this suppression: (i) organic amendment quality, particularly content of labile matter, rather than type or source of amendment determines degree of suppression; (ii) suppression is induced immediately after the amendment of organic matter, but can be lost after a fairly short period of time; and (iii) suppression is related to elevated microbial activity, which is induced and sustained by the presence of labile organic matter (47,106).

In field soils, however, the ability of organic soil amendments to suppress diseases caused by soil-borne pathogens has been highly variable (66), and in some cases has resulted in increased disease (67). As is observed in the container systems,

suppression is related directly to the quality of organic amendment; in addition, the degree of suppression may be modified by environmental/abiotic factors (2). Thus the variability in effectiveness of organic soil amendments observed in field studies is likely due to both variability in the quality of organic amendments within and between studies and environmental conditions under which these trials were conducted. Our understanding of which aspects of organic matter quality, soil biology, and the environment conditions are important is still limited. Understanding how these factors influence the effectiveness of organic soil amendments will allow us to improve the degree and consistency of suppression.

Biofumigation. In addition to inducing microbial suppression of pathogens, a number of green manures are thought to act as biofumigants, releasing toxic compounds during decomposition. The majority of biofumigation research has been conducted on brassicas (such as broccoli, mustard and rape), for which the biofumigant compounds have been identified; brassicas contain a high level of glucosinolates, which break-down into thiocyanates, isothiocyanates (ITC), and nitriles, which are known to have broad range biocidal activity against bacteria (31) and fungi (92). *In vitro* studies have shown ITCs from brassica species to be toxic to a number of soil-borne pathogens, including *Colletotrichum coccodes* (45), *Gaeumannomyces graminis* (3,92), *Phoma exigua* (108), *Phytophthora* spp. (34,45), *Pythium* spp. (96), *Rhizoctonia solani* (45,108), and *V. dahliae* (45). Research has been conducted on profiling the glucosinolate content of various brasssicas (61) and the method of incorporation to affect the most efficient delivery of ITCs to the soil.

Sudan grass has been cited as another potential biofumigant plant species (89). Sudan grass leaf tissues contain high levels of a cyanoglucoside (dhurrin), which breaks down into p-hydroxybenzaldehyde and hydrogen cyanide (HCN) during decomposition. Amendment of soil with Sudan grass residue has been demonstrated to suppress infection of lettuce by the nematode *Meloidogyne hapla* (118); in this study, the release of CN^- from Sudgan grass was identified as the primary factor involved in suppression of *M. hapla*. In a subsequent study, Widmer and Abawi (118)

demonstrated a similar suppression of *M. hapla* by other cyanogenic plants, white clover and flax. While no studies have been conducted to assess the potential of dhurrin breakdown products to suppress soil-borne fungal or bacterial pathogens, production of HCN by fluorescent *Pseudomonas* spp. has been implicated in the suppression of a number of soil-borne pathogens (65,69,78,79).

While "biofumigation" currently refers to the use of brassicas to control soil-borne pathogens, other crops are known to have allelopathic properties; it is expected that some of these crops may also contain compounds that are toxic to the microbiota.

Suppression of Verticillium wilt using organic soil amendments. A variety of organic amendments have been studied as possible inducers of general suppression of *V. dahliae* with varying success. Wilhelm (120) studied the effect of cottonseed meal, barley straw, blood meal, fish meal, conifer wood sawdust, and cow manure, in a variety of combinations with inorganic amendments on Verticillium wilt of tomato. He noted that the degree of control of the disease was related to the amount of nitrogen provided by the amendment, with the only exception being barley straw, which controlled wilt incidence but did not have high nitrogen content. Based on work by previous researchers (19,46,58,70,91,114), Wilhelm speculated, without providing supporting evidence, that the suppression of Verticillium wilt was not due to nitrogen but rather a reduction in inoculum density related to increased microbial activity.

Davis et al. (28,29) assessed the effectiveness of Sudan grass (*Sorghum vulgare* var. *sudanense* 'Monarch'), Austrian winter pea (*Pisum sativum*), two cultivars of rape (*Brassica napus* var. *napus* 'Dwarf Essex' and 'Bridger', rye (*Secale cereale*), oat (*Avena sativa* 'Monida'), and corn (*Zea mays* 'Jubilee') in suppressing Verticillium wilt of potatoes (cv. Russet Burbank). Greatest reductions of Verticillium wilt was achieved with Sudan grass and corn green manure treatments and lesser control with rape, Austrian winter pea, oat or rye. Davis and colleagues concluded that disease reduction was associated with increased microbial activity and changes in

the microbial community structure, reduced root colonization, but not necessarily reduced inoculum density.

Parks (80) evaluated the effect of green manures of Sudan grass on Verticillium wilt of potato in two soils which were imported to the Columbia basin from Washington and Idaho. Parks reported reduction of soil and root population of *V. dahliae* in one type of soil after 2 consecutive years of green manure, but also that she did not observe any differences in stem colonization or disease severity between soil types. Parks speculated that the inability of Sudan grass green manure to suppress Verticillium wilt in the Columbia Basin was due to the warm climate which was favorable to disease development.

La Mondia et al. (64) evaluated the effectiveness of spent mushroom compost or straw mulch on control of Verticillium wilt of potato. They reported decreased wilt severity (AUSPC) and increased marketable tuber weight attributable to the compost amendment but not the straw mulch. Because changes in the inoculum density over the course of the growing season were not assessed, it is not possible to speculate on whether the alleviation of disease symptoms was due to changes in the microbial community or by some other factor.

Rotenberg and Cooperband (88) reported a doubling of Verticillium wilt severity in plots amended with high rate of composted paper mill residue compared to the nonamended soil.

Conn and Lazarovitz (20) studied the effects of chicken, swine, and cattle manure on Verticillium wilt of potato. Inoculum density and disease severity was reduced to near zero by both chicken and swine manure. They speculated that reduction in inoculum density may have resulted from either ammonia toxicity or colonization of *V. dahliae* MS by other fungi stimulated by the amendments. In a subsequent study, Tenuta et al. (110) reported that volatile fatty acids (VFA) released from liquid swine manure were responsible for reduction in inoculum density in acidic soils.

The biofumigation potential of broccoli residue to control Verticillium wilt of cauliflower has been evaluated in a number of studies (63,100,107,123). In these studies, broccoli residue resulted in reduction of inoculum density and wilt severity similar to that achieved by fumigation. While the disease-suppressive effects of brassicas are commonly attributed to the release of toxic isothiocyanates, thiocyanates, nitriles, epithionitriles, and oxazolidine-2-thiones during decompositon (100), this issue remains controversial. Harding (45) demonstrated that extracts from Indian mustard (*Brassica juncea*) and rape (*B. napus*) were able inhibit mycelial growth of *V. dahliae in vitro*. On the other hand, Shetty et al. (100), citing an unpublished *in vitro* study, reported that *V. dahliae* mycelial growth could not be inhibited by broccoli tissue extract and speculated that the high lignin content of broccoli residue may stimulate biological production of lignases that are also capable of cometabolizing the melanin which constitutes the protective structure of microsclerotia.

OBJECTIVES OF THIS RESEARCH

Although previous studies have demonstrated that green manures can suppress Verticillium wilt and alleviate yield losses in infested fields, the results have not been consistent. A better understanding of the impact of green manures on the pathogen and the soil microbial community, as well as a clarification of the mechanisms involved in suppression of the disease, is necessary before green manures can be implemented as a management strategy in commercial potato production. The first objective of this study (Chapter 2) was to determine whether the kind of green manure and/or the amendment rate were significant factors determining disease severity and yield reduction. Three kinds of GM – Austrian winter pea (*Pisum sativum* L. 'Melrose'), broccoli (*Brassica oleracea* L. var botrytis L. 'Excelsior'), and Sudan grass (*Sorghum vulgare* Pers. Var *sudanense* (Piper) Hitche. 'Monarch') – were added at three rates – 6, 12 and 24 Mg ha^{-1} dryweight – to microplots infested with *V. dahliae*. Inoculum density, root colonization, and microbial activity were measured

over the course of the experiment to determine if differences in disease severity could be attributed to changes in soil population, rate of infection, or biological activity.

Numerous environmental factors, both biological and abiotic, can influence the relationship between inoculum density and severity of Verticillium wilt (81). Individual factors have been identified in previous research, but few studies have investigated multiple factors simultaneously (25). The second objective of the study (Chapter 3) was to identify biotic and abiotic soil factors that were predictive of disease severity using multiple regression analyses. A descriptive model was developed including important abiotic and biotic soil factors.

Supplemental experiments dealing with spring-incorporated green manures and soil pH, related to the objectives discussed in chapters 2 and 3, respectively are summarized in Appendices B and C.

CHAPTER 2

EFFECT OF GREEN MANURE KIND AND AMENDMENT RATE ON SEVERITY OF VERTICILLIUM WILT AND YIELD OF RUSSET BURBANK POTATO

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ABSTRACT

Increasing costs as well as environmental concerns over use of fumigants to manage diseases caused by soil-borne pathogens such as *Verticillium dahliae* have prompted a search for ecologically-based means of control. The objective of this study was to determine the effect of type and amendment rate of green manure on soil inoculum density (ID) and infection of potato roots (RI) by *V. dahliae*, severity of Verticillium wilt, and yield of Russet Burbank potatoes. Green manures Austrian winter pea, broccoli, or Sudan grass green manures applied at 6, 12, or 24 Mg ha^{-1} were compared in two single-year field studies. Green manures of broccoli applied at all three rates, Sudan grass applied at 6 or 12 Mg ha^{-1}, and Austrian winter pea applied at 24 Mg ha^{-1} reduced *V. dahliae* ID compared to the unamended control. Amendment rate had no effect on ID or RI by *V. dahliae* but affected relative area under the senescence progress curve (RAUSPC). Green manures of Austrian winter pea applied at 12 Mg ha^{-1} and Austrian winter pea, broccoli, and Sudan grass applied at 24 Mg ha^{-1} reduced RAUSPC by 74, 70, 70, and 52%, respectively, compared to the unamended control. For both experiments, mean total tuber yield was lower in unamended control plots than in plots which were not infested with *V. dahliae*. None of the green manure treatments in either year significantly increased yield relative to the unamended control. In 2002, however, Austrian winter pea applied at all rates, Sudan grass applied at 12 and 24 Mg ha^{-1}, and broccoli applied at 24 Mg ha^{-1} resulted in mean yields which were not significantly different from those of the uninfested plots. In 2002, yield was negatively correlated with disease severity ($r = -0.61$, $P \leq 0.0001$). Overall yield was lower in 2003 than 2002 due to an earlier harvest, and fewer differences were observed between green manure treatments and the unamended control.

List of abbreviations used: ATW = average tuber weight

AUSPC = area under the senescence progress curve

CFU = colony forming unit

ID = inoculum density

OM = organic matter

RAUSPC = relative area under the senescence progress curve

RI = root infection

INTRODUCTION

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major disease of many cultivated plants, including potato, throughout the temperate regions of the world (102). In North America, yield reduction can range from 10 to as high as 50% (84). Crop rotation is considered to be an impractical management strategy due to the long-term persistence of the pathogen in soil (121) and its wide host range, including non-susceptible bridging hosts (38). One management tactic is soil fumigation to reduce soil populations of *V. dahliae* (85). In North America, metam sodium is used widely to manage this disease (89). In recent years, however, greater regulation of agrochemicals and increasing costs of application are motivating the potato industry to seek alternative management strategies.

Green manures and other organic matter (OM) amendments have been used to improve soil tilth and fertility since early times (68). In the early 20th century, it was recognized that some soil amendments could also promote plant health by suppressing some diseases caused by soil-borne pathogens (70,90). Organic matter mediated disease suppression is thought to result from physiochemical changes in the soil (68) and/or increase in the activity of the general microbial community or of specific members of the microbial community (22,120). More recently, research has focused on the possibility that the OM itself or OM-derived compounds may have direct deleterious effects on pathogens. (17,20).

Numerous soil amendments have been evaluated for their potential to suppress Verticillium wilt, including animal manures (20), composted materials (64), industrial byproducts (88), and green manures (10,28,36,80). Different amendments have resulted in a range of responses from nearly complete suppression of disease (20) to no effect on disease (Parks, 1998) or increased disease severity (88), with the majority resulting in partial reduction of disease (10,20,28,36).

It is uncertain what characteristics of soil amendments determine the efficacy in suppressing Verticillium wilt. Among green manures, it is apparent that at least two modes of suppression exist. Broccoli residue, for example, has been shown to

consistently reduce soil populations of *V. dahliae* (10,123), and thereby reduce severity of the disease (63,107,123). However, the suppression of Verticillium wilt by green manures of Sudan grass, Austrian winter peas (28), sweet corn or barley (26) is not always related to changes in ID.

Extensive research on the suppression of *Pythium* spp. using composts in container-grown systems indicates that the availability of readily decomposable organic matter, regardless of source, is a significant determining factor of suppressiveness (12,47). Studies have demonstrated that the suppression of disease (23) or degree of suppression (27) may be a function of the amount of OM amended. In a greenhouse study, Davis *et al.* (27) reported a negative relationship between the amount of Sudan grass green manure amended and the severity Verticillium wilt of potatoes. To our knowledge, however, no studies have investigated the effect of amendment rate of green manures on Verticillium wilt at the field scale.

Therefore, we conducted two single-year field trials to compare the mode of suppression resulting from different green manures and to determine the effect of amendment rate on soil populations of *V. dahliae* and Verticillium wilt of Russet Burbank potatoes.

MATERIALS AND METHODS

Experimental design. The field experiments were conducted in 2002 and 2003 at the Central Oregon Agricultural Research Center in Madras, OR. The soil is classified as a Madras series sandy loam (superactive, mesic Aridic Argixeroll). The experimental design was a randomized complete block (RCB) with four replicates. The treatments comprised a 3 x 3 factorial with three kinds of green manure incorporated at three rates into soil infested with *V. dahliae*, an unamended infested control and four non-infested controls. The three kinds of green manure were Austrian winter pea (*Pisum sativum* L. 'Melrose'), broccoli (*Brassica oleracea* L. var *botrytis* L. 'Excelsior'), and Sudan grass (*Sorghum vulgare* Pers. var *sudanense* (piper) Hitche 'Monarch'); the three amendment rates were 6, 12, and 24 Mg ha^{-1}, dry

biomass; three of the non-infested controls received 12 Mg ha^{-1} of one each of the green manures and the fourth received no green manure (unamended).

The experimental fields had not previously been cropped to potatoes or other hosts of *V. dahliae*, and background levels of *V. dahliae* were low. Microsclerotia of VCG4 (potato strain) of *V. dahliae* were cultivated and harvested in the manner described by Gaudreault *et al.* (42). In August of the year preceding potato cultivation, and immediately prior to the incorporation of the green manures, microsclerotia mixed with fine sand was spread by hand on the experimental plots to achieve a density of approximately 25 to 30 CFU g^{-1} soil and immediately disc-incorporated to a depth of approximately 25 cm.

Austrian winter pea, broccoli, and Sudan grass, representing the three plant families *Leguminosae*, *Brassicaceae*, and *Graminacae*, respectively, were grown in large blocks outside of the experimental sites in the summer of the year preceding potato cultivation. In August, immediately following the infestation of soil with *V. dahliae*, the aerial biomass of the three green manure crops was chopped in the field with a flail, collected, spread within 2 h on plots (3 m x 3.7 m) at one of three rates (6, 12, or 24 Mg ha^{-1} dry matter) and disc-incorporated to a depth of 25 to 30 cm.

In the spring prior to planting, each plot received a basal application of 203 kg ha^{-1} each of N, P_2O_5, and K_2O, and 89 kg ha^{-1} of S in 2002, and 174 kg ha^{-1} each of N, P_2O_5, and K_2O, and 189 kg ha^{-1} of in 2003. Generation III seed potatoes (*Solanum tuberosom* cv. Russet Burbank) were hand-cut into seed pieces weighing 43 to 71 g and were planted on 8 May 2002 and 7 May 2003. Seed pieces were planted in plots consisting of four 3 m long rows. Row width was 93 cm and seed pieces were spaced 23 cm apart within the row. Plots were sprinkler irrigated.

Soil sampling and analysis. Ten soil cores were taken with a 2.5 cm diameter soil probe at a depth of 0 to 15 cm, approximately 1 mo after incorporation of the green manures (October, 2001 or September, 2002), prior to planting potatoes (April, 2002 or May, 2003), and 3 wk after the emergence of potatoes (June, 2002 and 2003).

Soil cores from each plot were bulked and transported in a cooler to Oregon State University where they were air-dried for 2 wk at room temperature.

Pathogen, disease, and yield assessment. Inoculum density (ID) was assessed by plating air-dried soil onto NP10 medium (103) using an Andersen Air sampler (18). Each sample consisted of five plates with approximately 0.36 g of soil per plate. Plates were incubated for a minimum of 10 days in the dark at room temperature and rinsed with running tap water to remove soil particles from the agar surface. The number of *V. dahliae* colonies on each plate was counted using a stereomicroscope and expressed as colony forming units per gram of soil (CFU g^{-1} soil).

Two plants were dug from the middle two rows of each plot 3 wk after emergence and the root ball removed. The root balls were transported in a cooler to Oregon State University. The root balls were rinsed with distilled water containing a small amount of tergitol to remove soil particles and the fine roots were collected on a #10 sieve. Infection of potato feeder roots by *V. dahliae* (RI) was assessed by plating 1 cm long feeder root sections onto NP10 medium (103). The plates were incubated in the dark at room temperature for at least 10 days. The number of root infections was counted using a stereomicroscope and expressed as colony forming units per meter root (CFU m^{-1} root).

Beginning with the appearance of disease symptoms (foliar chlorosis or necrosis), wilt severity for each plot was assessed on a scale of 0 to 100%, where 0% = no symptoms and 100% = all foliage senescent or wilted. Disease readings were taken weekly for 5 wk.

At harvest, the middle two rows of each plot was machine excavated. Tubers from the middle 2.1 m (2002) or 2.4 m (2003) of each row were hand-harvested, counted, weighed and average tuber weight calculated.

Statistical analyses. Area under the senescence progress curve (AUSPC) was calculated based on the weekly disease reading (99) and divided by the number of growing degree-days (base = 10°C, max = 30°C) over the senescence period to

generate a relative area under the senescence progress curve (RAUSPC). Inoculum density averaged across all three sampling dates was used for statistical analysis. Inoculum density and RI were square-root transformed and RAUSPC was log-transformed after addition of 1 (*ln*[x+1]) to approximate normal distribution for these variables. Unless otherwise indicated, all statistical analyses were conducted on transformed data, but raw data are presented in tables and text.

Because the two experiments were conducted in two different fields in different years, each experiment was designated as a single environment ('experiment'). Inoculum density, RI, and RAUSPC were near zero in the non-infested plots and obviously lower than in the infested plots; therefore, analysis of variance of these three response variables were conducted using only data from plots that were artificially infested with *V. dahliae*. Mixed-model ANOVA (PROC GLM; SAS release 8.02) was conducted including main effect terms for experiment, block (nested in experiment), green manure kind, amendment rate, and terms for all interaction of experiment, kind, and rate. For ANOVA the unamended infested control was included as a fourth amendment rate (0 Mg ha^{-1}). Because there were no significant experiment x treatment interactions for ID, RI, or RAUSPC, data from the 2002 and 2003 experiments were pooled for comparison of means. After determining that there were no significant differences in ID, RI, or RAUSPC among unamended and amended non-infested controls, data from all non-infested controls were combined to generate single mean ID, RI, and RAUSPC for the non-infested controls. Three sets of means separation were conducted using Fisher's least squared difference (LSD, $P \leq 0.05$) in PROC GLM. The three sets included the combined uninfested control and (i) all infested treatments (MEANS statement in PROC GLM), (ii) means for green manure kind, averaged across all amendment rates (LSMEANS statement in PROC GLM), or (iii) means for amendment rate, averaged across all green manure kinds (LSMEANS statement in PROC GLM). A correlation matrix was generated for ID, RI, and RAUSPC for each green manure type (PROC CORR).

Mixed-model ANOVA (PROC GLM) was conduced for yield and tuber weight using data from infested and non-infested plots; models included main effect terms for experiment, block (nested in experiment), infestation, green manure kind, amendment rate, and all interaction terms. Because of significant differences in tuber yield between experiments and because of a significant experiment x infestation interaction for average tuber weight, a separate analysis was conducted for each experiment. After determining that there were no significant differences in tuber yield or tuber weight among unamended and amended non-infested controls, data from all non-infested controls were combined to generate single mean tuber yield and tuber weights for the non-infested controls. As in the case of ID, RI, and RAUSPC, three sets of means separations were conducted for the combined non-infested control and (i) infested treatments (MEANS), (ii) means for green manure kind, averaged across all amendment rates (LSMEANS), or (iii) means for amendment rates, averaged across all green manure kinds (LSMEAN). A correlation matrix was generated for RAUSPC, yield, and average tuber size for each experiment (PROC CORR). All statistical analyses were conducted using SAS (release 8.02; SAS Institute, Cary, NC).

RESULTS

Inoculum density, root infection, and disease severity. Inoculum density did not differ significantly by experiment and was significantly affected by green manure kind and amendment rate (Table 2.1). Broccoli and Sudan grass green manures reduced mean ID by 9.3 and 7.7 CFU g^{-1} soil, compared to the unamended control; broccoli green manures reduced mean ID by 3.9 CFU g^{-1} soil relative to the Austrian winter pea green manures (Table 2.2). The 6, 12, and 24 Mg ha^{-1} amendment rates reduced mean ID by 6.4, 6.8, and 6.1 CFU g^{-1} soil relative to the unamended control; there were no differences in mean ID among the three rates. Austrian winter pea green manure applied at 24 Mg ha^{-1} reduced ID by 7.2 CFU g^{-1} soil relative to the unamended control. Broccoli green manures applied at 6, 12, or 24 Mg ha^{-1} at reduced ID by 7.2, 11.2, and 9.2 CFU g^{-1} soil, respectively, compared to the unamended

control. Sudan grass green manures applied at 6 and 12 Mg ha^{-1} reduced ID by 6.6 and 11.1 CFU g^{-1} soil.

Table 2.1 Analysis of variance of ID and RI by *V. dahliae* and severity of Verticillium wilt of potato by experiment, green manure kind (Austrian winter pea, broccoli, or Sudan grass) and amendment rate (0, 6, 12, or 24 Mg ha^{-1}). Pooled data from 2002 and 2003.

Source of variation	d.f.	Inoculum Density†	Root infection	Disease severity
Experiment	1	NS	**	*
Block in experiment	6	NS	NS	NS
Treatment				
Green manure kind	2	*	*	NS
Amendment rate	3	***	NS	**
Kind x rate	4	NS	NS	NS
Experiment by treatment				
Experiment x kind	2	NS	NS	NS
Experiment x rate	3	NS	NS	NS
Experiment x kind x rate	4	NS	NS	NS
Pooled error	54			

† Average inoculum density across all sampling dates.

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 2.2 Effect of green manure treatments on ID of *Verticillium dahliae*. Pooled data from 2002 and 2003.

	Green manure amendment rate				Mean for
Green manure kind	Control	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	kind
Non-infested†	1.5 e,o,z	---	---	---	---
Unamended/infested	20.9 a,m,w	---	---	---	---
Austrian Winter Pea	---	16.3 ab	16.5 ab	13.7 bcd	15.5 wx
Broccoli	---	13.5 bcd	9.7 d	11.7 bcd	11.6 y
Sudan Grass	---	14.3 bcd	9.8 cd	15.3 abc	13.1 xy
Mean for rate	---	14.7 n	12.0 n	13.6 N	---

† Mean of unamended and amended non-infested controls.

‡ Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of controls and all green manure treatments (a,b,c,d,e), amendment rate means (m,n,o), and green manure kind means (w,x,y,z).

Root infection differed significantly by experiment and was affected by green manure kind but not by amendment rate (Table 2.3). There was no significant interaction between green manure kind and experiment, indicating that the effect of

green manure kind was consistent between experiments. On average, mean RI was 3.8 CFU m^{-1} root lower in 2003 than in 2002. None of the green manures resulted in a lower mean RI compared to the unamended control; mean RI for the broccoli green manure was 3.5 CFU m^{-1} root lower than for the Austrian winter pea green manure. The Austrian winter pea green manure applied at 24 Mg ha^{-1} resulted in the highest mean ID, which was significantly higher than the Broccoli green manure applied at either 6 or 24 Mg ha^{-1} and the Sudan grass green manure applied at 24 Mg ha^{-1}.

Table 2.3 Effect of green manure treatments on RI by *Verticillium dahliae*. Pooled data from 2002 and 2003.

	Green manure amendment rate				Mean for
Green manure kind	Control	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	kind
Non-infested[†]	0.1 c,n,y	---	---	---	---
Unamended/infested	9.3 ab,m,wx	---	---	---	---
Austrian Winter Pea	---	8.7 ab	8.0 ab	12.4 a	9.7 w
Broccoli	---	7.1 b	6.6 ab	4.9 b	6.2 x
Sudan Grass	---	8.2 ab	8.5 ab	5.1 b	7.3 wx
Mean for rate	---	8.0 m	7.7 m	7.4 m	---

[†] Mean of unamended and amended non-infested controls.

[‡] Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of controls and all green manure treatments (a,b,c), amendment rate means (m,n), and green manure kind means (w,x,y).

Disease severity differed significantly by experiment and was affected by amendment rate but not by green manure kind (Table 2.1). The experiment x amendment rate interaction was not significant, indicating that the effect of rate was consistent between experiments. On average, median RAUSPC was greater in 2003 than 2002 by 2.2%. Austrian winter pea, broccoli, and Sudan grass green manures reduced RAUSPC by 59, 46, and 45%, relative to the unamended infested control; mean RAUSPC was not different among the three kinds of green manure (Table 2.4). The 12 and 24 Mg ha^{-1} rates reduced RAUSPC by 54 and 63% relative to the unamended infested control, and by 31 and 45% compared to the 6 Mg ha^{-1} rate. The Austrian winter pea green manure applied at 12 Mg ha^{-1} and all green manure kinds applied at 24 Mg ha^{-1} reduced disease severity relative to the unamended control.

Table 2.4 Effect of green manure treatments on severity of Verticillium wilt of potatoes. Pooled data from 2002 and 2003.

	Green manure amendment rate				Mean for
Green manure kind	Control	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	kind
Non-infested[†]	1.4 f,o,y	---	---	---	---
Unamended/infested	21.0 a,m,w	---	---	---	---
Austrian Winter Pea	---	13.8 abcd	5.5 e	6.4 bcde	8.6 x
Broccoli	---	14.3 abc	13.7 abcde	6.3 cde	11.4 x
Sudan Grass	---	14.1 ab	10.1 abcde	10.4 de	11.5 x
Mean for rate	---	14.1 m	9.7 n	7.7 n	---

[†] Mean of unamended and amended non-infested controls.

[‡] Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of controls and all green manure treatments (a,b,c,d,e,f), amendment rate means (m,n,o), and green manure kind means (w,x,y).

Correlation of ID, RI, and RAUSPC. Among the plots that received Austrian winter pea green manure, there were no significant correlations between ID, RI, and RAUSPC (Table 2.5). Among the plots that received broccoli or Sudan grass green manures, ID was weakly positively correlated with root infection and disease severity. Among the plots amended with Sudan grass green manure, root infection was weakly positively correlated with disease severity.

Table 2.5 Simple correlation coefficients (r) for ID or RI by *Verticillium dahliae* and severity of Verticillium wilt of potatoes for three types of green manures. Pooled data from 2002 and 2003.

	Root infection		Disease severity	
Austrian winter pea				
Inoculum density	0.11	NS	0.18	NS
Root infection			0.08	NS
Broccoli				
Inoculum density	0.42	**	0.44	**
Root infection			0.16	NS
Sudan grass				
Inoculum density	0.34	*	0.46	***
Root infection			0.34	*

*, **, ***, and **** indicated significance at $P \leq 0.10$, 0.05, 0.01, and 0.001, respectively.

Yield and average tuber weight. Total tuber yield was significantly lower in 2003 than in 2002 (Tables 2.6 and 2.7). For both the 2002 and 2003 experiments, no difference in mean tuber yield was observed among the unamended and amended non-infested controls and so a single mean for the combined non-infested controls was calculated. For both the 2002 and 2003 experiments, mean tuber yield for the unamended infested controls was estimated to be 20% lower than for the non-infested controls. For both the 2002 and 2003 experiments, neither green manure kind nor amendment rate resulted in a significantly greater tuber yield compared to the unamended infested control. For the 2002 experiment, Austrian winter pea green manure applied at all rates, Sudan grass green manure applied at 12 Mg ha^{-1}, and all green manures applied at 24 Mg ha^{-1} resulted in mean tuber yield that was not significantly different from that of the combined non-infested controls (Table 2.7); for the 2003 experiment, broccoli green manure applied at 6 Mg ha^{-1} and Sudan grass green manure applied at 24 Mg ha^{-1} resulted in mean tuber yield that was not significantly different from that of the combined non-infested controls (Table 2.7).

Table 2.6 Analysis of variance of total tuber yield and average tuber weight of potatoes by experiment, infestation with *Verticillium dahliae*, green manure kind (Austrian winter pea, broccoli, or Sudan grass) and amendment rate (0, 6, 12, or 24 Mg ha^{-1}).

Source of variation	DF	Total tuber yield (Mg ha^{-1})	Average tuber weight (kg $tuber^{-1}$)
Experiment	1	***	***
Block in experiment	6	NS	NS
Treatment			
Infestation	1	***	***
Green manure kind	2	NS	NS
Amendment rate	3	NS	NS
Infestation x kind	1	NS	NS
Kind x rate	4	NS	NS
Experiment by treatment			
Experiment x infestation	1	NS	**
Experiment x kind	2	NS	NS
Experiment x rate	3	NS	NS
Expmt x infestation x kind	5	NS	NS
Experiment x kind x rate	4	NS	NS
Pooled Error	78		

, * and indicate significance at $P \leq 0.01$ and ≤ 0.001, respectively.

Table 2.7 Effect of green manure treatments on potato tuber yield.

Year	Green manure	Control	Green manure amendment rate			Mean for kind
			6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	
2002	Non-infested†	175 ab,m,w	---	---	---	---
	Unamended/infested	140 c,n,x	---	---	---	---
	Austrian Winter Pea	---	150 bc	154 bc	152 bc	152 x
	Broccoli	---	139 c	147 c	151 bc	146 x
	Sudan Grass	---	131 c	152 bc	152 bc	145 x
	Mean for rate	---	140 n	151 n	151 n	---
2003	Non-infested†	152 ab,m,w	---	---	---	---
	Unamended/infested	122 c,n,x	---	---	---	---
	Austrian Winter Pea	---	120 c	111 c	118 c	116 x
	Broccoli	---	134 bc	118 c	127 c	126 x
	Sudan Grass	---	118 c	128 c	136 bc	127 x
	Mean for rate	---	124 n	119 n	127 n	---

† Mean of unamended and amended non-infested controls.

‡ Within each year, means followed by the same letter are not significantly different at $P \leq 0.05$. Means between years were not compared. Different groups of letters are used for comparison of controls and all green manure treatments (a,b,c), amendment rate means (m,n), and green manure kind means (w,x).

Average tuber weight was significantly lower in 2003 than in 2002 (Tables 2.6 and 2.8). For both the 2002 and 2003 experiments, no difference in average tuber weight was observed among the unamended and amended non-infested controls, so a single mean for all non-infested controls was calculated. For the 2002 experiment, average tuber weight for the unamended infested controls was estimated to be 22% lower than for the non-infested controls (Table 2.8); for the 2003 experiment, there was no difference in average tuber weight between the non-infested and infested controls (Table 2.8). For both the 2002 and 2003 experiments, neither green manure kind nor amendment rate resulted in a significantly greater average tuber weight compared to the unamended infested control. For the 2002 experiment, Austrian winter pea green manure applied at 12 Mg ha^{-1} and Sudan grass green manure applied at 24 Mg ha^{-1} resulted in average tuber weights which were not different from that of the combined non-infested controls (Table 2.8); for the 2003 experiment, Austrian

winter pea green manure applied at 6 Mg ha^{-1} resulted in an average tuber weight which was significantly lower than those of both the non-infested and infested controls (Table 2.8).

Table 2.8 Effect of green manure treatments on average potato tuber weight.

Year	Green manure	Control	Green manure amendment rate			Mean for kind	
			6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}		
			2002 experiment				
2002	Non-infested†	0.72 ab,m,w	---	---	---	---	
	Unamended/infested	0.56 c,n,x	---	---	---	---	
	Austrian Winter Pea	---	0.58 c	0.64 bc	0.61 c	0.61	x
	Broccoli	---	0.58 c	0.55 c	0.60 c	0.58	x
	Sudan Grass	---	0.55 c	0.60 c	0.64 bc	0.60	x
	Mean for rate	---	0.57 n	0.60 n	0.62 n	---	
2003	Non-infested†	0.41 a,m,w	---	---	---	---	
	Unamended/infested	0.41 a,m,wx	---	---	---	---	
	Austrian Winter Pea	---	0.32 b	0.34 ab	0.35 ab	0.34	x
	Broccoli	---	0.38 ab	0.40 ab	0.39 ab	0.39	wx
	Sudan Grass	---	0.35 ab	0.37 ab	0.37 ab	0.36	wx
	Mean for rate	---	0.35 m	0.37 m	0.37 ab	---	

† Mean of unamended and amended non-infested controls.

‡ Within each year, means followed by the same letter are not significantly different at $P \leq 0.05$. Means between years were not compared. Different groups of letters are used for comparison of controls and all green manure treatments (a,b,c), amendment rate means (m,n), and green manure kind means (w,x).

Correlation of pathogen, yield, and disease variables. For the 2002 experiment, disease severity was moderately and negatively correlated with yield and weakly and negatively correlated with average tuber weight (Table 2.9). In 2003, although disease severity was negatively associated with yield and average tuber weight, the relationship was not significant. For both experiments, total yield was positively correlated with the average tuber weight.

Table 2.9 Simple correlation coefficients (r) for severity of Verticillium wilt of potatoes, total tuber yield, and average tuber weight for 2002 and 2003 experiments.

Year	Variable	Yield	Average tuber weight
2002	Disease severity	-0.61 ****	-0.27 *
	Yield		0.47 ***
2003	Disease severity	-0.23 NS	-0.11 NS
	Yield		0.29 *

*, **, ***, and **** indicated significance at $P \leq 0.10$, 0.05, 0.01, and 0.001, respectively.

DISCUSSION

The objectives of this study were to compare the effects of different green manures and amendment rate on the *V. dahliae* and the severity of Verticillium wilt. The three green manures affected soil populations of *V.dahliae* differently and there was no consistent effect of amendment rate. None of the green manures reduced Verticillium wilt when applied 6 Mg ha^{-1} but all of the green manures reduced disease severity when applied at 24 Mg ha^{-1}. The green manures resulted in different degree and consistency of disease reduction when applied at 12 Mg ha^{-1}.

Green manure of Austrian winter pea resulted in significant reduction of soil populations of *V. dahliae* relative to the unamended control only when applied at the highest rate (24 Mg ha^{-1}). In contrast, green manures of broccoli at all amendment rates and Sudan grass at the two lowest amendment rates significantly reduced soil populations of *V. dahliae*. Furthermore, although severity of Verticillium wilt within plots receiving green manure of either broccoli or Sudan grass was found to be moderately correlated with ID, no such correlation was observed in Austrian winter pea plots (Table 2.5). Thus, in the case of broccoli or Sudan grass green manures, it seems likely that suppression of disease severity was, in part, due to a reduction in soil population of *V. dahliae*.

Amending soil with broccoli residue has consistently been shown to reduce soil populations of *V. dahliae* and severity of Verticillium wilt of cauliflower (100,107,123) and potato (10). Suppression of Verticillium wilt by broccoli and other

brassica green manures is widely believed to result from the reduction of soil population of *V. dahliae* caused by the release of toxic compounds upon breakdown of glucosinolates (92). Although we observed some correlation between severity of Verticillium wilt and ID, the majority of variability in wilt severity resulting from broccoli green manure was not related to ID. For example, although the 12 Mg ha^{-1} amendment resulted in the highest and most consistent reduction ID, reduction of disease severity was intermediate and highly variable. The greatest reduction of disease severity was at the 24 Mg ha^{-1} rate, although reduction in ID was not significantly different from amendment at 12 Mg ha^{-1}. Based on these discrepancies, we believe that the suppression of Verticillium wilt by broccoli green manure is the result not only of reduction in ID, but also other factors related to the amount of green manure amended.

The effect of Sudan grass on soil populations of *V. dahliae* and suppression of Verticillium wilt is not well understood. Davis, *et al.* (28) reported reduction of *V. dahliae* ID following three consecutive years of a Sudan grass cover crop and green manure. However, in the same study and in a subsequent study, Davis *et al.* (27) concluded that suppression of Verticillium wilt by Sudan grass green manure was not related to the reduction of *V. dahliae* ID. It has been suggested that Sudan grass can act as a biofumigant through the release of p-hydroxybenzaldehyde and hydrogen cyanide (HCN) as a result of the breakdown of dhurrin (118). To our knowledge, no studies have explicitly investigated the potential of dhurrin breakdown products to suppress soil-borne fungal or bacterial pathogens, including *V. dahliae.* This theory rests on the fact that HCN is thought to play a role in the suppression of a number of soil-borne pathogens by fluorescent *Pseudomonas* spp. (65,78,79). Our results suggest that Sudan grass residue may have had some deleterious effect on *V. dahliae* in the soil, although it is not certain that this was the result of biofumigation. However, as in the case of broccoli, severity of Verticillium wilt was only moderately correlated with ID (Table 2.5) and the effects of Sudan grass rates on disease severity did not coincide with effects on ID. Thus, although the suppression of Verticillium wilt by

Sudan grass green manure may have resulted from reduction of ID to some degree, other factors related to the amount of green manure amended also were involved.

Our findings that suppression of Verticillium wilt by Austrian winter pea green manure was not related to reduction in soil population of *V. dahliae,* and that suppression of Verticillium wilt by broccoli or Sudan grass green manures was only partially related to ID, are consistent with the observations of other researchers (26,27,28). Davis *et al.* demonstrated suppression of Verticillium wilt of potato with green manures of Austrian winter pea (28), corn (*Zea mays*), barley (*Hordeum vulgare*) (26) or Sudan grass (27) without concomitant reduction in ID. In the case of broccoli green manures, Shetty *et al.* (100) questioned the assumption that suppression of Verticillium wilt is primarily the result of biofumigation, citing an *in vitro* study in which broccoli tissue extracts were unable to inhibit *V. dahliae* growth and observation of numerous cortical infections and large colonies of *V. dahliae* present in tissue of broccoli grown in infested soil.

Our observation that the degree and consistency of reduction of Verticillium wilt severity increased with higher amendment rates for the three green manures, suggests that suppression was related to the amount of organic matter incorporated into the soil. Organic soil amendments can significantly alter soil physio-chemical (68) and biological properties (97,98) that, in turn, can affect the survival or disease potential of soil-borne pathogens (2,49). Researchers have speculated that organic matter mediated suppression of Verticillium wilt may involve the induction of general suppression (22) by the soil microbial community and/or changes in the nutrient status of the soil (27,29,120). Because the effects of OM amendments on physical, chemical, and biological soil properties are complex and interrelated, it is often difficult to discern which factors are relevant to disease suppression. We further explore the relation between green manures, soil chemical and biological properties, and suppression of Verticillium wilt in the accompanying paper (Chapter 3).

The lack of improvement in yield or tuber size by green manures relative to the unamended control may be explained by the relative late onset of disease and short

growing season in Central Oregon. Following tuber initiation, tubers grow at a fairly constant rate during the tuber bulking (growth stage IV) stage. Approximately 85 to 90 % of tuber weight is accumulated during tuber bulking with the remaining 10 to 15 % being added after the onset of senescence by translocation of carbohydrates from leaf and stem to tubers (62). Final tuber size and therefore yield is primarily a function of the tuber growth rate during tuber bulking and the duration of the linear tuber growth phase; tuber growth rate, in turn, is a function of the photosynthetic activity of the leaf canopy which can be reduced by conditions limiting healthy foliage (35). Verticillium wilt reduces photosynthetic efficiency (13), leaf area, as well as the duration of canopy and can thereby reduce tuber growth rate (56). If disease onset occurs early in the season, there is a longer period over which tuber growth rate is reduced, and consequently a greater difference in final tuber size between affected and non-affected plants. If disease onset, however, occurs later in the season, the difference in tuber size between affected and non-affected plants will be smaller.

In the 2002 experiment, tuber harvest occurred in mid October, approximately 24 wk after planting. The first disease symptoms were observed 15 wk after planting, allowing 9 wks over which differences in tuber growth rate were accumulated. This period may not have been sufficient to result in significant yield differences between non-amended and amended treatments due to varying disease levels. The fact that mean yield and average tuber size for the 12 and 24 Mg ha^{-1} green manure rates were for the most part higher than for the unamended infested control suggests that the green manures applied at the higher rates may have mitigated the reduction of tuber growth rate due to infection by *V. dahliae*. This interpretation is further supported by the strong negative correlation between severity of Verticillium wilt and yield in 2002.

In the 2003 experiment, tuber harvest occurred in late September, 20 wk after planting. The early harvest in 2003 resulted in significantly lower yield and tuber weights than in 2002 and also may explain the lack of difference in average tuber weights between non-infested and infested control. In 2003, disease symptoms were

first observed approximately 14 wks after planting, leaving 6 wks over which difference in tuber growth rate were accumulated.

In summary, severity of Verticillium wilt and yield reduction was mitigated to some degree using green manures. For green manures of broccoli or Sudan grass, reduction in disease severity may in part be attributed to reduction in soil populations of *V. dahliae*. However, much of the reduction in disease severity could not be explained by changes in ID and appeared to be related to the amount of green manure applied. The possibility that suppression of Verticillium wilt involved changes in soil chemical and/or biological properties stimulated by the amendments is further explored in the accompanying paper (Chapter 3). Difference in characteristics such as lignin content or C:N ratio between green manures may explain some of the differences in effect on the disease. Clarification of the relationship between green manure characteristics, their effect on soil properties, and disease suppression will allow for more efficient and consistent management of Verticillium wilt. Although we were able to achieve extremely high rates of amendment in this experiment, such rates are not commercially practical. Thus, the potential for developing suppressive soils over several years of lower rates of amendment should be investigated. Furthermore, it is known that naturally produced MS differ in their pigmentation (32) and viability (9) from artificially produced MS. Thus, it will be necessary to confirm that green manures can also suppress Verticillium wilt in naturally infested soils. Finally, severity of Verticillium wilt is known to be influenced by environmental factors such as soil type and macro-climate (80). Therefore, we recommend that further experiments be conducted in a variety of ecosystems to evaluate the influence of ecosystem on efficacy of suppression of Verticillium wilt by green manures.

CHAPTER 3

SOIL CHEMICAL AND MICROBIAL PROPERTIES RELATED TO THE SUPPRESSION OF VERTICILLIUM WILT OF POTATOES BY GREEN MANURES

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ABSTRACT

The suppression of diseases caused by soil-borne pathogens such as *Verticillium dahliae* by organic amendments is poorly understood. In some cases, organic-matter mediated suppression is the result of reduction in inoculum density (ID), while in other cases it is unrelated to changes in soil populations of the pathogen. Soil amendments can alter soil properties, which in turn may result in soil conditions which are detrimental to the pathogen. The objective of this study was to identify soil factors that were related to suppression of Verticillium wilt of potato following amendment of green manures. Stepwise multiple linear regression (MLR) analysis was used to identify soil chemical and biological properties related to the suppression of Verticillium wilt of potatoes following green manures in two single-year amendment studies. Inoculum density (ID) of *Verticillium dahliae* accounted for 48 and 52% of the variation in relative area under the senescence progress curve (RAUSPC) in 2002 and 2003, respectively. In 2002, in addition to ID, soil pH, Ca, or K contributed to models predicting RAUSPC. Low soil pH, low Ca, and high K were associated with low RAUSPC. These factors were not related to the green manure treatments but rather to a pre-existing soil pH at the 2002 site The best MLR model for 2002 included terms for ID, soil pH, and fluorescein diacetate hydrolysis (FDA) and accounted for 65% of the variation in RAUSPC. In 2003, in addition to ID, NO_3-N, total carbon, FDA, microbial respiration, and microbial biomass C (MBC) were related to RAUSPC. High values of each factor were associated with low RAUSPC and all six of these factors were affected by the green manure treatments. The best MLR model for 2003 included terms for ID, NO_3-N, and MBC and accounted for 66% of the variation in RAUSPC. The best 4-parameter MLR model fit to data pooled from both studies included terms for ID, soil pH, and FDA and accounted for 63% of the variability in RAUSPC.

List of abbreviations used: AS = arylsulfatase activity
AUSPC = area under the senescence progress curve
CFU = colony forming unit
FDA = rate of hydrolysis of fluorescein diacetate
ID = inoculum density
MBC = microbial biomass carbon
MLR = multiple linear regression
MR = microbial respiration
MS = microsclerotia
RAUSPC = relative area under the senescence progress curve
RI = root infection
TC = total carbon

INTRODUCTION

Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae* Kleb., is a major disease of many cultivated plants, including potato, throughout the temperate regions of the world (102). The long-term persistence of the pathogen in the soil and its wide host range, including some non-susceptible bridging hosts, make crop rotation an impractical method of managing this disease (89). Soil fumigation with metham sodium alone or incombination with 1,3-dichloropropene is used widely in the United States to reduce soil populations of *V. dahliae* (89). In recent years, however, greater regulation of agrochemicals and increasing costs of application are motivating the potato industry to seek alternative management stategies.

One potential alternative to fumigation is the plow-down of green manures, or the incorporation of other organic soil amendments, to create soil conditions that are suppressive to the disease. A number of organic soil amendments such as animal manures (20), mushroom compost (64) or green manures of Austrian winter pea, broccoli, Sudan grass, barley, or corn (10,28,chapter 2) have been shown to reduce the severity Verticillium wilt of potatoes. In some cases, the reduction in wilt severity was associated with a lowering of *V. dahliae* inoculum density (10,20); whereas, in other cases, suppression was independent of changes in soil population of the pathogen (26,28,chapter 2).

The incorporation of plant residues or other organic soil amendments can significantly alter the physical and chemical properties of a soil ecosystem (14,68,97). These changes, in turn, could affect the inoculum potential of *V. dahliae* in a number of ways: the resulting soil environment may be (i) less conducive to pathogen germination or growth, (ii) more optimal for the host plant, resulting in healthier plants (68) which are less susceptible to attack by the pathogen, or (iii) more conducive to the growth and activity of soil microbial community members that can inihibit the ability of *V. dahliae* to germinate or infect roots of host plants (22).

In some cases, suppression of Verticillium wilt of potato in the absence of reduction in inoculum density using organic amendments has been attributed to

improved soil fertility (27) or mineral content of plants (37). While it has long been suspected that the suppression of Verticillium wilt by organic soil amendments is microbial in nature (120), only a few studies have investigated changes in soil microbial properties following organic soil amendments and the relation between microbial properties and Verticillium wilt severity (20,28,29,37). These studies reported increased total microbial activity or biomass in amended soils and a negative relationship between total microbial activity or biomass and disease severity. These observations, in addition to examples of general suppression of diseases caused by other soil-borne pathogens using organic amendments (47), support the hypothesis that suppression of Verticillium wilt by green manures also may involve inhibition of *V. dahliae* by the general microbial community.

It is uncertain what properties of the soil environment contribute to suppression of *V. dahliae* and Verticillium wilt, and how these properties are affected by the incorporation of green manures. Therefore the objectives of this study were to identify soil chemical and biological properties related to reduction of disease severity and to investigate if and how these properties were affected by the amendment of green manures.

MATERIALS AND METHODS

Field site and experimental design. The field experiment was conducted in 2002 and 2003 at the Central Oregon Agricultural Research Center in Madras, OR. The soil is classified as a Madras series sandy loam (superactive, mesic Aridic Argixeroll). The experimental design was a randomized complete block (RCB) with four replicates. The treatments comprised a 3 x 3 factorial with three kinds of green manure incorporated at three rates into soil infested with *V. dahliae*, and four non-infested controls. The three kinds of green manure were Austrian winter pea (*Pisum sativum* L. 'Melrose'), broccoli (*Brassica oleracea* L. var *botrytis* L. 'Excelsior'), or Sudan grass (*Sorghum vulgare* Pers. var *sudanense* (piper) Hitche 'Monarch'). The three amendment rates were 6, 12, or 24 Mg ha^{-1}, dry biomass and three of the non-

infested controls received 12 Mg ha^{-1} of one each of the green manures and the fourth received no green manure (unamended).

The experimental fields had not previously been cropped to potatoes or other hosts of *V. dahliae*, and background levels of *V. dahliae* were low. Average and maximum inoculum densities for non-infested plots were 1.2 and 8.3 CFU g^{-1} soil in 2002, and 1.3 and 9.8 CFU g^{-1} soil in 2003. Microsclerotia of VCG4 (potato strain) of *V. dahliae* were cultivated and harvested in the manner described by Gaudreault *et al.* (42). In August of the year preceding potato cultivation, microsclerotia mixed with fine sand was spread by hand on the experimental plots at a rate of approximately 25 to 30 CFU g^{-1} soil and immediately disc-incorporated to a depth of approximately 15 cm.

Austrian winter pea, broccoli, and Sudangrass, representing three families *Leguminosae*, *Brassicae*, and *Graminacae*, respectively, were grown in large blocks outside of the experimental sites in the summer of the year preceding potato cultivation. In August, the aerial biomass of the three green manure crops was chopped in the field with a flail, collected, spread on plots (3 m x 3.7 m) at one of three rates (6, 12, or 24 Mg ha^{-1} dry matter) and disc-incorporated to a depth of 25 to 30 cm.

In the spring prior to planting, each plot received a basal application of 203 kg ha^{-1} each of N, P_2O_5, and K_2O, and 89 kg ha^{-1} of S in 2002, and 174 kg ha^{-1} each of N, P_2O_5, and K_2O, and 189 kg ha^{-1} of in 2003. Generation III seed potatoes (*Solanum tuberosom* cv. Russet Burbank) were hand-cut into seed pieces weighing 28 to 56 g and were planted on 8 May 2002 and 7 May 2003. Rows were spaced 93 cm apart, with 4 rows per plot and within row spacing of the seed pieces was 23 cm.

Soil sampling and analysis. Ten soil cores were taken with a 2.5 cm diameter soil probe at a depth of 0 to 15 cm, approximately 1 mo after incorporation of the green manures (October, 2001 or September, 2002), prior to planting potatoes (April, 2002 or May, 2003), and 3 wk after the emergence of potatoes (June, 2002 and 2003). Soil cores from each plot were bulked and transported in a cooler to Oregon State

University and stored at 5°C. Analyses of microbial properties were conducted on fresh soils within a week of sampling; otherwise a subsample of each sample was air-dried for 2 wk at room temperature before the remaining analyses were conducted.

Microbial properties. Arylsulfatase activity of fresh soils sampled in spring was assessed in duplicate as described by Tabatabai (109). In brief, the substrate *p*-nitrophenyl sulfate solution was incubated with 1 g soil (buffer pH 5.8) for 1 h at 37°C. After filtration, the product *p*-nitrophenol (PNP) was determined by measuring absorbance at 420 nm. Controls were run without the substrate, and the absorbance subtracted from the samples with substrate. Arylsulfatase activity was reported as μg PNP converted g^{-1} soil hr^{-1}.

Fluorescein diacetate hydrolysis (FDA) of fresh soils sampled in summer was assessed colorimetrically using a method modified from Schnürer and Rosswall (95). Each soil sample was analyzed in duplicate. To each sample, sodium phosphate buffer (20 mL) was added to each sample to adjust solution pH to 7.6, fluorescein diacetate stock solution (100 μL of 4.8 mM) was added as a substrate for the enzymes. Samples were incubated at 25°C on a rotary shaker for 2 hr. Hydrolysis was stopped by addition of 20 mL acetone. The solution was centrifuged at 6,000 rpm for 4 min, filtered, and absorbance measured at 490 nm. FDA hydrolysis was reported as the amount of FDA hydrolyzed g^{-1} soil hr^{-1}.

Microbial respiration (MR) and microbial biomass C (MBC) of soils sampled in spring were assessed at the same time using the chloroform-fumigation-incubation method (55). Each soil sample was weighed (10 g) into two scintillation vials, one of which was fumigated for 24 h with ethanol-free chloroform and the other left untreated. Samples were incubated in sealed vials for 10 days at 25°C. The CO_2 evolved was measured with a gas chromatograph. Microbial respiration was reported as the CO_2-C evolved by the non-fumigated sub-sample g^{-1} soil 10 day^{-1}. A k_c of 0.41 (116) was used to calculate MBC without the subtraction of the control and reported as MBC g^{-1} soil.

Soil chemical properties. Soil pH of soils sampled in summer was measured in a 1:2 (w/v) solution of 0.05 M $CaCl_2$ using an Orion pH meter. Exchangeable cations were extracted with KCl and analyzed by inductively coupled plasma spectrometry (ICP-AES) at the Central Analytical Laboratory, Oregon State University, in 2002, and at the W.M. Keck Collaboratory, Oregon State University, in 2003. Ammonium and nitrate were extracted from the soil with 2.0 N KCl and analyzed by steam distillation as described by Bremner (15). Percentage total carbon (TC) was analyzed by dry combustion as described by Nelson and Sommers (74). Exchangeable cations, ammonium, and nitrate were reported as mg kg^{-1} and percent TC was reported as g kg^{-1}soil.

Pathogen and disease severity. Inoculum density (ID) was assessed for fall, spring, and summer soil samples by plating air-dried soil onto NP10 medium (103) using an Andersen Air sampler (18). Each sample consisted of five plates with approximately 0.36 g of soil per plate. Plates were incubated for a minimum of 10 days in the dark at room temperature and rinsed with running tap water to remove soil particles from the agar surface. The number of *V. dahliae* colonies was counted using a stereomicroscope and expressed as colony forming units per gram of soil (CFU g^{-1}soil).

Two plants were dug from the middle two rows of each plot 3 wk after mergence an the root ball removed. The root balls were transported in a cooler to Oregon State University. The root balls were rinsed with distilled water containing a small amount of tergitol to remove soil particles and fine roots were collected on a #10 sieve. Infection of potato feeder roots by *V. dahliae* (RI) was assessed by plating 1 cm feeder root sections onto NP10 medium (103) and incubating and counting *V. dahliae* colonies. Number of root infections was expressed as colony forming units per meter root (CFU m^{-1} root).

Beginning with the appearance of disease symptoms (foliar chlorosis or necrosis), wilt severity for each plot was assessed on a scale of 0 to 100%, where 0%

= no symptoms and 100% = all foliage senescent or wilted. Disease readings were taken weekly for 5 wk.

Statistical analyses. Area under the senescence progress curve (AUSPC) was calculated based on the weekly disease reading (99) and divided by the number of growing degree-days (base = 10°C, max = 30°C; USBR, online) over the senescence period to generate a relative area under the senescence progress curve (RAUSPC). Inoculum density was averaged across the three sampling dates. The following data transformations were performed to give normal distributions for each variable: (i) all soil chemical variables, excluding soil pH and TC, were natural-log- transformed; (ii) ID and RI were square-root transformed; (iii) RAUSPC was log-transformed after addition of 1.

Data from both infested and non-infested plots were included in the analyses, because the background level of *V. dahliae* in the non-infested plots was high enough to cause disease (Figure 3.1). Each experiment was designated as a single environment ('experiment'). Analyses of variance were conducted to investigate the degrees to which variation in soil chemical properties, soil microbial properties, ID, or RI were related to the green manure treatments or experimental environment.

The objective of the first series of statistical analyses was to identify soil factors that made a significant contribution to the model in predicting of RAUSPC in the two experiments. Relative AUSPC was regressed on ID and RI to determine the predictive ability of both *V. dahliae* measures. Correlation matrices of all the variables were generated for individual experiments. Stepwise regression and BIC-based model selection were used to identify the variable(s) from two variable categories (soil chemical properties and soil microbial properties) which were important in the model to predict RAUSPC for individual experiments. The possibility of multicollinearity between variables was assessed by investigating bivariate correlations. Variables exhibiting strong correlation ($r > 0.65$) were mutually excluded from variable pools for stepwise regression analysis.

The objective of the second series of analyses was to develop and evaluate a linear model that was capable of predicting RAUSPC for both experiments. Stepwise regression was used to select a set of variables from among the subset of variables identified in the first series of analyses to be included in a final predictive model. Finally, a table of predicted disease severities was generated based on a matrix of values for the variables selected in the final predictive model. All statistical analyses were conducted using SAS (release 8.02; SAS Institute, Cary, NC).

RESULTS

Inoculum density and root infection. For the 2002 and 2003 experiments, inoculum density of the non-infested control plots was sufficient to cause disease (Fig. 3.1). Both ID and root infection predicted disease severity to a limited degree, although predictive abilities varied by year and sampling date (Table 3.1). In some cases, estimates of ID averaged across sampling dates (fall and spring, spring and summer, or all dates) were better predictors of disease severity than measurements from individual sampling dates. In the 2002 study, all estimates of ID including data from the fall sampling were good predictors of disease severity; and in the 2003 study, summer ID was the strongest predictor. Root infection also predicted disease severity in both years, but was not as good a predictor of disease severity as the best estimate of ID. In models predicting RI, ID from all sampling dates was a significant factor (R^2 = 0.34 to 0.57). For the 2002 and 2003 studies, holding all other variables constant, the estimated effect of increasing ID (averaged across all sampling dates) by 1 CFU g^{-1} soil was a 60 and 57% increase, respectively, in median RAUSPC (Table 3.2).

Soil chemical properties. In the 2002 study, inclusion of a main effect term for Ca, pH, or K significantly increased the power of the model to predict disease severity relative to the reduced model including only a main effect term for ID (Table 3.2). Reduction in disease severity was associated with decreasing Ca or soil pH and increasing K. Soil pH, Ca, and K were strongly intercorrelated ($r > 0.65$; Table 3.3). In the 2003 study, inclusion of a main effect term for NO_3-N or TC significantly

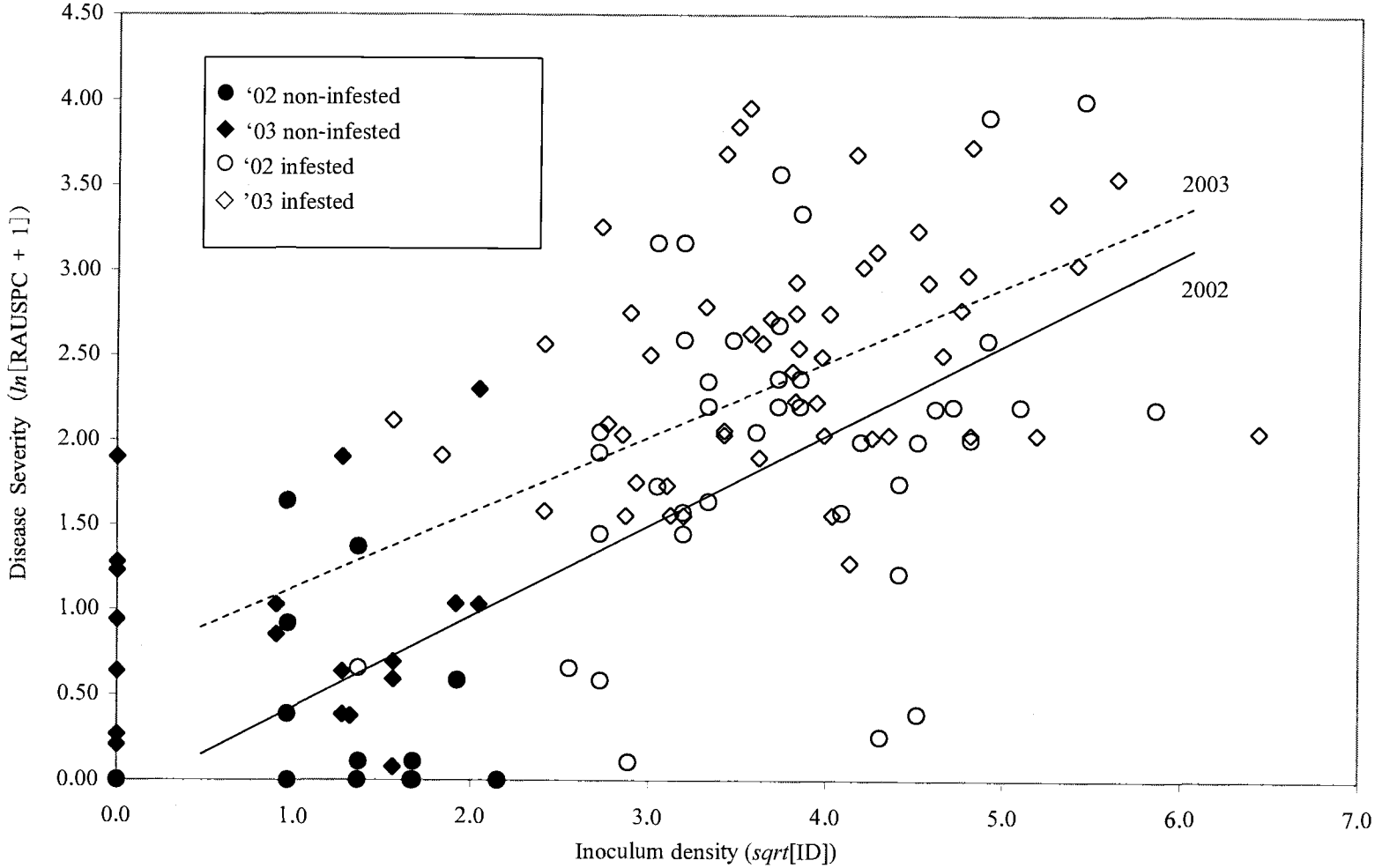

Figure 3.1 Relationship between IDof *Verticillium dahliae* (averaged across all sampling dates) and severity of Verticillium wilt of potato, grouped by experiment and infestation. Lines represent estimated effect of inoculum density on disease severity for 2002 and 2003 experiments.

increased the power of the model to predict disease severity in comparison to the reduced model including model including only a main effect term for ID (Table 3.2). Reduction in disease severity was associated with increasing values of any both properties. TC and NO_3-N were weakly correlated (Table 3.3).

Table 3.1 Goodness-of-fit (R^2) of regressions of root infection by *Verticillium dahliae* or Verticillium wilt of potatoes on inoculum density and infection of potato root by *Verticillium dahliae*.

Pathogen variable	2002 experiment		2003 experiment	
Sampling date	Root infection	Disease severity	Root infection	Disease severity
Inoculum density				
Fall	0.44	0.48	0.38	0.35
Spring	0.37	0.33	0.35	0.31
Summer	0.34	0.19	0.42	0.54
Fall-spring average[†]	0.51	0.48	0.46	0.40
Spring-summer average	0.48	0.34	0.44	0.50
Overall average	0.57	0.48	0.50	0.52
Root infection		0.39		0.41

Soil microbial properties. In the 2002 study, after accounting for the effect of ID in the model, there was suggestive but inconclusive evidence ($P \leq 0.10$) that FDA or MBC had an effect on disease severity (Table 3.2). Models including terms for soil microbial properties resulted in only modest improvement of goodness-of-fit over the reduced model. Assuming that the effects of FDA and MBC were real, reduction of disease severity was associated with increasing rates of FDA hydrolysis and increasing microbial biomass.

In the 2003 study, after accounting for the effect of ID, all three microbial properties were identified as having a significant effect on disease severity (Table 3.2). Inclusion of MR in the model resulted in the greatest improvement of the goodness-of-fit, followed by lesser improvements by inclusion of MBC or FDA. Reduction in disease severity was associated with increasing MBC, MR, or FDA. Microbial respiration, MBC, and FDA were moderately intercorrelated (Table 3.3).

Stepwise model selection for individual studies. For the 2002 study, stepwise regression selected a model for Verticillium wilt severity including terms for ID, soil pH, Mg, and FDA (Table 3.2). Reduction in disease severity was associated with decreasing ID or soil pH and increasing Mg or FDA. For the 2003 study, stepwise regression selected a model that included only terms for ID and MR. Based on the BIC statistic, the best model with both soil chemical and microbial property variables included terms for ID, NO_3-N, and MBC; for this model, reduction in disease severity was associated with decreasing ID and increasing NO_3-N or MBC (Table 3.2).

Effects of green manures on soil properties. The effects of green manure and rate on ID and RI are described in detail in chapter 2 of this thesis. Of the soil chemical properties identified above as being predictive of disease severity, only those identified in the 2003 study (NO_3-N and TC) were affected by the green manure amendments. Nitrate-N was affected both by green manure and rate, but the effect was not the same in the two studies (Table 3.4). In 2002, NO_3-N for all green manure treatments and unamended controls were the same (data not shown). In 2003, the highest levels of NO_3-N were associated with the Austrian winter pea green manure and increased NO_3-N with amendment rate (Table 3.5). Total carbon was also affected by both green manure and rate; the effect of green manure was not consistent between the two studies (Table 3.4). In 2002, highest TC was associated with the Austrian winter pea and Sudan grass green manures (data not shown), whereas in 2003, mean TC for all green manures was similar (Table 3.6). In both studies, increasing levels of TC were associated with increasing rates of green manure. Soil pH, Ca, and K were not affected by the amendment of green manure in either study, but differed significantly between studies.

Table 3.2 Multiple linear regression models predicting severity of Verticillium wilt of potato using inoculum density of *Verticillium dahliae* and soil chemical or soil microbial property variables.

	Multiple regression models						R^2	BIC
				2002 experiment				
Inoculum density	RAUSPC =	– 0.08^{NS}	+ 0.54 (ID) ***				0.48	-17.2
Soil chemical property variables	RAUSPC =	– 10.6 ***	+ 0.53 (ID) ***	+ 1.34 (Ca) ***			0.62	-33.1
	RAUSPC =	– 3.59 ***	+ 0.53 (ID) ***	+ 0.61 (pH) ***			0.60	-29.5
	RAUSPC =	– 9.89 ***	+ 0.52 (ID) ***	– 1.43 (K) ***			0.59	-23.5
Soil microbial property variablss	RAUSPC =	0.67 ^{NS}	+ 0.53 (ID) ***	– 0.008 (MR) $^{0.06}$			0.51	-18.8
	RAUSPC =	3.05 $^{0.07}$	+ 0.55 (ID) ***	– 0.88 (FDA) $^{0.06}$			0.51	-18.7
	RAUSPC =	0.20 ^{NS}	+ 0.54 (ID) ***	– 0.002 (MBC) ^{NS}			0.49	-15.9
All variables[†]	RAUSPC =	0.06 ^{NS}	+ 0.54 (ID) ***	+ 0.66 (pH) ***	– 1.1 (FDA)**		0.65	-34.9
	[‡]RAUSPC =	20.9**	+ 0.56 (ID) ***	+ 0.68 (pH) ***	– 3.2 (Mg)**	– 1.3 (FDA) **	0.69	-40.3
				2003 experiment				
Inoculum density	RAUSPC =	0.72 ***	+ 0.45 (ID) ***				0.52	-46.4
Soil chemical property variables	RAUSPC =	3.01 ***	+ 0.45 (ID) ***	– 0.62 (NO_3-N) ***			0.64	-59.9
	RAUSPC =	3.16 ***	+ 0.49 (ID) ***	– 2.99 (TC) **			0.59	-52.7
Soil microbial property variablss	RAUSPC =	1.91 ***	+ 0.46 (ID) ***	– 0.0044 (MBC) ***			0.66	-62.8
	[‡]RAUSPC =	1.42 ***	+ 0.46 (ID) ***	– 0.0087 (MR) ***			0.62	-56.1
	RAUSPC =	2.85 ***	+ 0.45 (ID) ***	– 0.73 (FDA) ***			0.61	-54.8
All variables	[§]RAUSPC =	2.73 ***	+ 0.46 (ID) ***	– 0.32 (NO_3-N) $^{0.09}$	– 0.003 (MBC) *		0.66	-63.0

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively. *P* values between 0.05 and 0.10 are specified.

[†] Ca and K were excluded from the pool of variables available for inclusion in the model because of strong correlation with soil pH.

[‡] Model selected by stepwise regression with both soil chemical and microbial variables available for inclusion in model.

[¶] Bayesian information criteria statistic. This statistic penalizes goodness-of-fit values for increasing number of parameters in model, allowing for comparison of models with different numbers of parameters (Ramsey and Schafer, 2002).

Table 3.3 Simple correlations coefficients (r) of soil chemical and microbial properties in 2002 and 2003.

	PH	NH_4-N	NO_3-N	N	Ca	Mg	K	Na	TC	FDA	AS	MR
PH												
NH_4-N	-0.43 ***											
NO_3-N	0.04	-0.30 **										
N	-0.18	0.64 ***	0.31 ***									
Ca	0.66 *** (0.90) (***)	-0.58 ***	0.61 ***	0.03								
Mg	0.08	-0.13	-0.13	-0.15	0.06							
K	-0.30 ** (0.69) (***)	0.27 **	-0.10	0.16	-0.31 *** (-0.70) (***)	-0.05						
Na	0.01	-0.35 ***	0.48 ***	0.10	0.66 ***	-0.03	0.04					
TC	-0.18	-0.00	0.23 *	0.08	-0.00	-0.13	0.15	0.01				
FDA	-0.09	-0.33 ***	0.69 ***	0.08	0.52 ***	-0.21 *	-0.09	0.52 ***	0.30 **			
AS	0.48 ***	-0.05	-0.01 **	0.04	0.05	0.00	-0.12	-0.27 **	0.14	-0.11		
MR	-0.26 **	0.12	0.45 ***	0.23 *	-0.02	-0.04	0.20 *	-0.05	0.45 ***	0.39 ***	0.19 *	
MBC	-0.20 *	-0.28 **	-0.21 *	0.06	0.41 ***	-0.02	0.17	-0.63 ***	0.21 *	0.30 **	0.56 ***	0.41 ***

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

( ) r-values and asterices in paraentheses are indicate correlations in 2002 experiment.

Table 3.4 Effects of green manure and amendment rate on soil chemical and microbial properties.

Source of error	d.f.	pH	NH_4	NO_3	N	Ca	K	Mg	Na	TC	Soil microbial properties: AS	FDA	MR	MB
Experiment	1	*	***	***	NS	***	NS	NS	***	NS	***	***	***	***
Block in Experiment	6	NS	NS	***	NS	NS	NS	***	NS	NS	***	***	**	NS
Treatment														
Green manure	2	*	NS	***	NS	NS	NS	NS	NS	*	***	NS	***	*
Amendment rate	3	NS	NS	***	NS	NS	NS	NS	NS	***	***	***	**	***
Green manure x rate	4	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Experiment x Treatment														
Experiment x green manure	2	*	NS	***	NS	NS	NS	NS	NS	*	**	NS	NS	*
Experiment x rate	3	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	***	*
Experiment x green manure x rate	4	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*
Pooled Error	86													

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

All three microbial properties differed between studies and were significantly affected by amendment rate (Tables 3.4, 3.7, 3.8, and 3.9). Total microbial activity, measured by FDA hydrolysis, was not affected by green manure in either study. In both studies, increasing rates of FDA hydrolysis were associated with increasing amendment rates. The highest levels of MR were associated with the Austrian winter pea green manure in 2002 (data not shown), followed by Sudan grass and broccoli (data not shown). In 2003, the effects of the three green manures were similar (Table 3.8). In both studies, increasing levels of MR were associated with increasing levels of green manure amendment. The highest levels of MBC were associated with the Austrian winter pea green manure in 2002 (data not shown), and with the broccoli green manure in 2003. In both studies, increasing levels of MBC was associated with increasing amendment rates.

Table 3.5 Effect of green manure and amendment rate on mid-season soil NO_3-N in 2003.

	Amendment rate								Mean for kind	
Green manure	0 Mg ha^{-1}		6 Mg ha^{-1}		12 Mg ha^{-1}		24 Mg ha^{-1}			
Unamended control	22	e,n,z	---		---		---		---	
Austrian winter pea	---		35	cd	64	b	95	a	65	w
Broccoli	---		28	de	31	de	49	b	35	x
Sudan grass	---		25	de	51	bc	59	b	46	y
Mean for rate	---		29	n	50	m	66	l	---	

† Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of all green manure treatments (a,b,c,d,e), amendment rate (l,m,n), and green manure (w,x,y,z).

Table 3.6 Effect of green manure and amendment rate on TC in 2003.

	Amendment rate				Mean for kind
Green manure	0 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	
Unamended control	0.78 c,n,x	---	---	---	---
Austrian winter pea	---	0.85 abc	0.87 ab	0.94 a	0.88 w
Broccoli	---	0.87 abc	0.85 abc	0.86 abc	0.86 w
Sudan grass	---	0.82 bc	0.83 bc	0.93 a	0.85 w
Mean for rate	---	0.84 nm	0.85 m	0.91 l	---

† Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of all green manure treatments (a,b,c), amendment rate (l,m,n), and green manure (w,x,y).

Table 3.7 Effect of green manure and amendment rate on rate of FDA in 2003.

Green manure	Amendment rate				Mean for kind
	0 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	
Unamended control	13.1 d,n,x	---	---	---	---
Austrian winter pea	---	15.2 cd	18.3 bc	24.5 ab	19.1 w
Broccoli	---	15.9 cd	19.9 bc	22.1 abc	19.5 w
Sudan grass	---	16.4 bcd	21.3 abc	28.8 a	22.0 w
Mean for rate	---	15.9 n	19.8 m	25.1 l	---

† Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of all green manure treatments (a,b,c,d) amendment rate (l,m,n), and green manure (w,x).

Table 3.8 Effect of green manure and amendment rate on MR in 2003.

Green manure	Amendment rate				Mean for kind
	0 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	
Unamended control	44 f,n,y	---	---	---	---
Austrian winter pea	---	60 ef	99 c	148 a	102 w
Broccoli	---	57 ef	74 de	101 c	76 x
Sudan grass	---	60 ef	79 d	125 b	86 wx
Mean for rate	---	59 n	84 m	125 l	---

† Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of all green manure treatments (a,b,c,d,e,f), amendment rate (l,m,n), and green manure (w,x,y).

Table 3.9 Effect of green manure and amendment rate on MBC in 2003.

Green manure	Amendment rate				Mean for kind
	0 Mg ha^{-1}	6 Mg ha^{-1}	12 Mg ha^{-1}	24 Mg ha^{-1}	
Unamended control	162 g,o,x	---	---	---	---
Austrian winter pea	---	230 ef	307 cd	357 b	300 w
Broccoli	---	226 f	283 cd	412 a	302 w
Sudan grass	---	224 f	274 de	329 bc	275 w
Mean for rate	---	227 n	288 m	366 l	---

† Means followed by the same letter are not significantly different at $P \leq 0.05$. Different groups of letters are used for comparison of all green manure treatments (a,b,c,d,e,f), amendment rate (l,m,n,o), and green manure (w,x).

DISCUSSION

Inoculum density and root infection. Theory predicts that the severity of monocyclic diseases such as Verticillium wilt will be related to preplant soil populations of *V. dahliae*. While relationships between ID and severity of Verticillium wilt are apparent in some empirical studies (60,74,76), in others, no such relationship is discernable (6,24,32,60). In our study, simple regression models which included terms for ID were able to predict disease severity to some degree. Models including an estimate of ID averaged over two or three sampling dates tended to result in a better fit to the data than models using ID from one sampling date, suggesting that the amount of soil assayed at each sampling may have been too small to comprise the spatial variability of ID within the plots. However, even the best models including ID could account only for about half of the variability in disease severity in either experiment (48% in 2002 and 54% in 2003).

In some cases, RI was observed to be a better predictor of disease severity than ID (25). In other studies, however, RI was found to be proportional to ID (39,52) or to have similar predictive ability as ID (73). In our study, simple regression models including RI were predictive of disease severity, but to a lesser degree than those including ID.

Soil chemical properties. Different soil chemical properties were identified as being related to disease severity in 2002 and 2003. Soil properties selected in 2003 were significantly affected by green manure amendments, while the factors selected in 2002 were unaffected.

2002 experiment. The difference between years was likely due to a pre-existing gradient in soil properties associated with soil pH in the 2002 field which had an overwhelming effect on severity of Verticillium wilt. In the 2002 field, soil pH ranged widely from 5.2 at one end of the field to 7.5 at the other end. Calcium and K varied with soil pH. Severity of Verticillium wilt also appeared to vary with soil pH, with highest disease severity in plots with soil pH 7.0 and above and mild disease in plots with soil pH 5.5 and below. These observations are consistent with previous

findings that Verticillium wilt is most severe in neutral to alkaline soils (pH 6 to 9) and less severe in acidic soils (49,81). Studies have also demonstrated that raising soil pH by liming results in increased disease severity (57) and acidifying soil results in reduced disease severity (77,119).

Despite the fairly consistent relationship between soil pH and severity of Verticillium wilt, it is possible that H^+ or OH^- ions are not directly involved in suppression or enhancement of the disease (49). Soil pH is a "master variable" that controls the availability and forms of numerous minerals and inorganic species (104), which, in turn, may have a direct effect on the disease. As was observed in the 2002 experiment, concentration of Ca can be strongly correlated with soil pH. High concentrations of Ca, however, are generally associated with reduced disease severity (49,73), contradicting the relationship observed in our study. Concentration of K also can be negatively correlated with soil pH (104) but is believed to affect severity of Verticillium wilt only when it is deficient (86). Thus, it is unlikely that Ca or K was directly related to the gradient in disease severity observed in 2002. Abdel-Razek Osman *et al.* (1) presented evidence of a direct link between H^+ ion concentration and changes in *in vitro* behavior of *V. dahliae*. In a study on growth medium pH, they reported optimal MS germination and conidia production at pH 7.5, with decreasing and abnormal germination and reduced conidia production at pH 5.0 and 3.5. These observations strongly suggest that H^+ or OH^- ion concentration are biologically significant but do not preclude the possibility that other factors which vary with pH but were not measured also affect the pathogen or disease. For example, below pH 5.2, Al and Mn become soluble and may be toxic to certain organisms.

A term for Mg was included in the model selected by stepwise regression (Table 3.2). To our knowledge, there have been no reports of an association between Mg and Verticillium wilt. In fact, Jones and Woltz (57) concluded that Mg did not have an effect on Verticillium wilt of tomato. Magnesium was not correlated with soil pH, Ca, or K in 2002 eliminating collinearity with those factors as a possible explanation. The effect of micronutrients on Verticillium wilt is relatively poorly

understood. To date, only Mn, Zn, Cu, and Co have been identified being associated with Verticillium wilt (8,27,81).

2003 experiment. In contrast to the 2002 study, there was a much narrower range of pH in the field used in 2003. Also in contrast to the 2002 study, the soil chemical properties, NO_3-N and TC selected by modeling disease severity in 2003 were significantly affected by the green manure amendments (Table 3.5). The highest levels of NO_3-N were found in plots which had received a green manure of Austrian winter peas and also plots which had received the highest rate of green manure. Similarly, the highest level of TC were found in plots receiving the Austrian winter pea green manure and those receiving the highest rates of green manure.

There are several plausible explanations for the observed negative relationship between NO_3-N and severity of Verticillium wilt. Higher levels of soil NO_3-N may have directly improved health of the potato plants and therefore increased their resistance to attack by *V. dahliae*. Trials involving the amendment of soils with inorganic NO_3-N fertilizers show the direct effects of the NO_3^- anion on Verticillium wilt. Davis *et al.* (29) demonstrated that amendment of soil with 240 or 300 kg ha^{-1} NO_3-N increased resistance in potatoes relative to those grown in N-deficient soils. This may be an example of improved plant resistance to the disease due to improved plant health. However, an alternative explanation of those results is that the disease symptoms in the nutrient-deficient soil were compounded by those of N-deficiency and fertilization where N simply alleviated the N-deficiency symptoms. Thus, it remains uncertain whether increasing levels of NO_3-N actually confers increased resistance to Verticillium wilt relative to adequate levels of NO_3-N.

A second explanation for the negative relationship of NO_3-N with disease severity is that NO_3-N, in this case, may be an indicator of a cluster of changes in soil fertility due to the addition of green manures. Decomposition of plant residue releases macronutrients such as N, P, K, and S, micronutrients and other organic compounds to the soil environment (25). Decomposition of green manures released more NO_3-N than control soils, which may have resulted in increased vigor of the potato plants and

therefore increased resistance to Verticillium wilt. Davis *et al.* (27) concluded that reduced severity of Verticillium wilt and increased tuber yield following green manures of Sudan grass were due to improved availability of N, Mn, and K to the host plants rather than direct effects of the green manure on *V. dahliae.*

A third explanation is that increase in C and availability of nutrients such as NO_3-N or TC stimulated microbial activity and biomass, which in turn contributed to general suppression of *V. dahliae.*

Soil Microbial Properties. For the 2003 study, inclusion of microbial properties in regression models predicting RAUSPC resulted in significant improvement in goodness-of-fit. Although the effects of the microbial properties were not significant in 2002, we suspected the lack of significance was due to the overwhelming influence of the soil pH gradient in the field used in 2002. Three pieces of evidence suggested that the effects of microbial properties in 2002 on disease severity were real and similar to those in 2003: (i) the estimated effects of FDA and MBC were not significant at $P \leq 0.05$, but were significant at $P \leq 0.10$; (ii) the estimated effects of all three microbial properties in 2002 were similar to those in 2003; and (iii) inclusion FDA in addition to soil pH resulted in significant improvement in the power of the model to predict RAUSPC (Table 3.2).

The concept of 'general suppression' of soil-borne pathogens was proposed by Cook and Baker (22) to contrast with the idea of specific suppression. Specific suppression refers to the inhibition of a pathogen by antagonistic or competitive activity of a specific organism or group of organisms. Several mechanisms of specific suppression have been proposed, including (i) destruction of pathogen propagules; (ii) prevention of propagule germination; (iii) antibiosis; (iv) hyperparasitism; (v) competition for nutrients; or (vi) competition for infection sites (Stone *et al.*, 2004). General suppression refers to the reduction in the ability of a pathogen to infect or cause disease to a host plant resulting from the cumulative activity of the microbial community which cannot be linked to any one type of organism (22).

Many studies have demonstrated the principle of general suppression on other soil-borne pathogens (47). However, relatively few have investigated microbial suppression of Verticillium wilt. Wilhelm (120) recognized the possibility of the general microbial suppression *V. dahliae* induced by organic soil amendments, but did not present any evidence for this. Davis *et al.*, (29) reported a significant negative correlation between colonization of potato stems by *V. dahliae* and FDA hydrolysis. Conn and Lazarovitz (20) reported a coincidence of increased microbial populations of soil and decreased germination of microsclerotia due to animal manures but only speculated briefly on the possibility of suppression. Elmer *et al.* (37) noted reduced severity of Verticillium wilt of potatoes and also higher microbial diversity in potato rhizosphere soil following amendment with mushroom compost. While associations of high microbial activity or biomass with decreased disease severity are consistent with general suppression, general suppression is difficult to prove.

Hoitink and Boehm (47) have demonstrated that general suppression can be induced by many different kinds of organic matter and that the salient quality of the organic matter is having a high content of labile material that can serve as an energy source for soil microorganisms (12). Stone *et al.* (106) characterized organic matter-mediated suppression as follows: (i) many types and sources of organic amendments consistently generate suppression; (ii) suppression is generated immediately after a high rate of organic amendment; and (iii) suppression is positively related to microbial activity. In our study, all microbial properties were strongly affected by amendment rate, with higher activities associated with higher rates of amendment. Microbial properties were also correlated with TC in both years and NO_3-N in 2003 which may represent the nutrient status of the soil. The association of high microbial activity and biomass with low severity of Verticillium wilt provides strong circumstantial, although not conclusive, evidence of the general suppression of Verticillium wilt.

Descriptive/Predictive model for combined 2002 and 2003 experiments. The subset of variables available for stepwise regression for the predictive model was selected as described above and included ID, soil pH, Mg, NO_3-N, TC, FDA, MR and

MBC. Stepwise regression was used to generate the best models with 3, 4, or 5 parameters (Table 3.10). For all models a visual assessment of the relationship between predicted and observed RAUSPC confirmed that the residuals were both normally distributed and constant (data not shown).

Table 3.10 Multiple linear regression models predicting severity of Verticillium wilt of potato using ID of *Verticillium dahliae*, soil chemical, and soil microbial properties. Data from 2002 and 2003 studies pooled.

No. of parameters	Model selected by R^2 maximization				R^2
2	RAUSPC†= 2.37***	+ 0.50 (ID)***			0.45
3	RAUSPC†=–2.82***	+ 0.50 (ID)***	– 0.77 (FDA)***		0.57
4	RAUSPC†=–0.53^{NS}	+ 0.50 (ID)***	+ 0.54 (pH)***	– 0.72 (FDA)***	0.63
5	RAUSPC†=–0.22^{NS} (MBC)*	+ 0.50 (ID)***	+ 0.59 (pH)***	– 0.81 (FDA)*** – 0.02	0.65

† RAUSPC = *ln*(RAUSPC+1)

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table 3.11 gives predicted RAUSPC outcomes for a matrix of ID, pH, and FDA values based on the predictive model (Table 3.10). The table shows maximum relative wilt severity with high ID, high pH, and low FDA. Relative AUSPC decreases with decreasing ID, decreasing pH, and increasing FDA. It is important to keep in mind that because the different levels of the soil properties could not be randomly assigned to the plots, it is not possible to draw causal links between the soil factors and RAUSPC. Furthermore, the model was generated based on data from two experiments that were conducted in the same location, with the same climate and same soil type. Therefore, this model needs to be tested on other soils and in other environments.

Table 3.11 Predicted RAUSPC at given levels of *Verticillium dahliae* inoculum density, soil pH, and FDA hydrolysis.

Inoculum density ($CFUg^{-1}soil$)	Soil pH	Levels of FDA hydrolysis (μg FDA g^{-1} soil hr^{-1})		
		60	35	10
		Predicted RAUSPC[†]		
1	5.2	0	0	2
1	6.5	1	2	5
1	7.5	2	3	10
9	5.2	1	2	2
9	6.5	4	6	6
9	7.5	7	11	28
25	5.2	5	8	22
25	6.5	12	18	45
25	7.5	21	31	77

[†] Linear model on which predictions were based:

$$ln(\text{RAUSPC}+1) = -0.53 + 0.50\ (sqrt\text{ID}) + 0.54\ (\text{pH}) - 0.72\ (ln\text{FDA})$$

In summary, ID significantly contributed to the model predicting RAUSPC and could account for approximately half of the variability in disease severity in both experiments. For the 2002 experiment, soil pH, Ca, or K and Mg and no microbial properties were identified as potential predictors of RAUSPC; for the 2003 experiment, NO3–N or TC and MR, MBC, or FDA hydrolysis were identified. The discrepancy between experiments was due to the overriding influence of a pre-existing pH gradient across the field used in 2002. Analysis of the two experiments together identified ID, soil pH and FDA as the three factors that contributed most to predicting RAUSPC. Decreasing soil pH was associated with decreasing disease severity, confirming observations by other researchers. Increasing FDA hydrolysis was associated with decreasing disease severity, which is consistent with, although not proof, that general suppression contributed to reduction of Verticillium wilt. The best regression model could account for only 63% of the variability in disease severity observed in the two experiments, indicating that other factors not identified in our analyses also influenced disease severity. Further research is necessary to confirm these results in a broad range of soil types and ecosystems and to identify other factors relevant to suppression of Verticillium wilt.

GENERAL CONCLUSIONS

Analyses in Chapter 2 indicated that the three green manures had different effects on soil populations of *Verticillium dahliae*, suggesting that different modes of suppression were involved. For broccoli or Sudan grass green manures, which are suspected of acting as biofumigants, reduction in severity of Verticillium wilt was related, in part, to reduction in inoculum density (ID). Factors other than reduction in ID were responsible for reduction of disease severity by Austrian winter pea green manure. For all three green manures, higher amendment rates resulted in more consistent or greater reduction of Verticillium wilt severity, suggesting that suppression of the disease was related to the degree to which soil properties were altered by the amendments.

In Chapter 3, several soil chemical and biological properties (soil pH, Ca, K, NO_3-N, total carbon (TC), microbial respiration (MR), microbial biomass C (MBC), and FDA hydrolysis) were identified as being related to severity of Verticillium wilt of potato. In 2002, disease severity was overwhelmingly influenced by the wide pH gradient (pH 5.2 to 7.5) across the field. The association of low soil pH with low disease severity observed in this study is consistent with previous research and suggests a mechanism of abiotic suppression involving H^+ ion concentration. The chemical properties identified in 2003 were negatively related to disease severity and positively associated with green manure amendment rate. Differences in NO_3-N or TC may have resulted in different levels of plant vigor and resistance to Verticillium wilt or may simply have indicated different levels of soil fertility, which, in turn, could affect microbial activity and biomass. In 2002, two microbial properties, MR and MBC, were negatively associated with disease severity ($P = 0.06$) and in 2003, all three microbial properties were significantly and negatively related to disease severity. In both years, the estimated effects of the properties on disease severity were of similar magnitude. The consistent relationship between microbial properties and disease severity strongly suggest that general microbial suppression stimulated by

amendment of soils with green manures contributed to suppression of Verticillium wilt.

Soil population of *V. dahliae* and the soil chemical and biological properties that were measured in this study could account for approximately two-thirds of the variability in disease severity. Further investigation of a wider range of soil properties is necessary to develop a more complete understanding abiotic and biological suppression of Verticillium wilt. Understanding soil properties relevant to disease suppression will, in turn, enable selection of organic soil amendments based on their effect on specific soil properties. In addition, priority should be given to investigation of serial amendments, as opposed to single-applications, to develop disease suppressive soils. In this study, consistent suppression of Verticillium wilt required high rates of amendment (24 Mg ha^{-1}) which would not be achievable by a single cropping of green manure. It may be possible, however, to attain the same degree of suppressiveness with lower amendment rates over several seasons. Multi-year studies will also allow investigation of temporal changes in soil properties relevant to disease suppression which could yield information regarding durability and maintenance of suppression. Finally, because Verticillium wilt is influenced by larger-scale environmental factors such as macroclimate, further experiments should be conducted in a variety of ecosystems to evaluate the influence of ecosystem on efficacy of disease suppression by green manures.

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APPENDICES

Appendix A: Raw data for field experiment

Table A.1. Soil chemical properties by treatment for 2002 experiment

Plot	Trtmt	Block	pHfall	pHsp	pHsu	NH_4	NO_3	N	Ca	K	Mg	Na	TOC	POM
									mg kg^{-1}				g g^{-1}	
4	00_00_0	A	.	.	5.5	134	68	202	2084	1120	596	62	0.87	0.55
21	00_00_0	B	.	.	5.6	84	73	157	2385	998	571	71	0.83	0.49
40	00_00_0	C	.	.	6.9	4	109	113	3667	680	608	80	0.69	0.46
50	00_00_0	D	.	.	5.4	25	70	94	2144	1080	596	64	0.92	0.59
5	AP_12_0	A	.	.	5.6	23	48	71	2325	1020	632	64	0.90	0.65
18	AP_12_0	B	.	.	6.4	4	89	93	3226	740	705	64	0.84	0.51
34	AP_12_0	C	.	.	5.4	27	93	120	2144	1180	620	60	1.00	0.72
46	AP_12_0	D	.	.	5.5	92	109	201	2565	1040	596	64	0.86	0.71
8	Br_12_0	A	.	.	5.6	-7	52	45	2485	947	596	69	0.84	0.52
28	Br_12_0	B	.	.	5.4	38	110	148	2224	1110	644	60	0.79	0.52
32	Br_12_0	C	.	.	5.2	44	107	151	1804	1220	608	62	0.91	0.60
49	Br_12_0	D	.	.	5.3	53	86	139	2244	1080	583	64	0.87	0.57
13	SG_12_0	A	.	.	7.3	2	153	155	4770	722	681	103	0.77	0.57
19	SG_12_0	B	.	.	5.9	22	113	135	2986	929	571	74	0.93	0.57
41	SG_12_0	C	.	.	7.4	9	96	105	4609	781	547	76	0.86	0.57
47	SG_12_0	D	.	.	5.8	229	85	313	2425	1500	535	71	0.96	0.60
6	00_00_1	A	.	.	5.8	21	67	88	2325	949	644	67	0.81	0.48
27	00_00_1	B	.	.	5.4	70	78	148	1984	1060	632	57	0.74	0.50
42	00_00_1	C	.	.	7	6	154	159	4349	602	559	90	0.69	0.41
55	00_00_1	D	.	.	5.3	10	42	52	1583	957	620	55	0.69	0.46
12	AP_06_1	A	.	.	6.2	25	107	133	3788	690	766	87	0.70	0.48
25	AP_06_1	B	.	.	5.2	58	94	152	2024	1270	583	57	1.00	0.64
37	AP_06_1	C	.	.	5.5	55	99	154	2445	1090	547	69	0.88	0.68
48	AP_06_1	D	.	.	5.6	33	69	101	2385	991	547	69	0.83	0.59
11	AP_12_1	A	.	.	6.1	1	88	90	3246	721	754	78	0.76	0.56
23	AP_12_1	B	.	.	5.4	132	88	220	2184	1210	632	67	0.93	0.57
33	AP_12_1	C	.	.	5.3	59	108	166	2084	1250	608	145	1.11	0.67
56	AP_12_1	D	.	.	5.3	18	67	84	1683	938	620	48	0.71	0.42
9	AP_24_1	A	.	.	5.5	3	71	74	2625	1030	559	62	0.91	0.65
22	AP_24_1	B	.	.	5.2	107	126	233	2244	1310	656	57	1.14	0.76
38	AP_24_1	C	.	.	5.5	46	133	179	2665	1360	547	67	1.02	0.77
44	AP_24_1	D	.	.	6.6	2	141	143	3908	961	547	76	1.06	0.46
3	Br_06_1	A	.	.	5.7	145	68	213	2044	1300	608	64	0.81	0.53
16	Br_06_1	B	.	.	7.5	1	120	120	5170	603	620	92	0.74	0.48
36	Br_06_1	C	.	.	5.5	28	85	113	2345	1050	596	69	0.92	0.58
53	Br_06_1	D	.	.	5.4	50	45	95	1603	1100	656	53	0.79	0.55
10	Br_12_1	A	.	.	6	33	71	103	3086	875	583	71	0.79	0.52
24	Br_12_1	B	.	.	5.6	184	103	286	2224	1400	656	64	0.95	0.62
30	Br_12_1	C	.	.	5.3	6	98	104	1784	1150	608	55	0.77	0.57
54	Br_12_1	D	.	.	5.3	99	68	166	1543	1200	596	55	0.80	0.44
7	Br_24_1	A	.	.	5.8	56	68	124	2405	1180	608	71	0.91	0.61
26	Br_24_1	B	.	.	5.2	46	103	150	2024	1370	656	57	0.96	0.64
39	Br_24_1	C	.	.	6.1	2	140	142	3066	1050	620	74	0.80	0.48
43	Br_24_1	D	.	.	6.2	40	161	200	3667	1060	583	78	0.76	0.56
2	SG_06_1	A	.	.	5.7	90	82	171	2144	1110	620	16	0.84	0.49
17	SG_06_1	B	.	.	6.4	30	136	166	3868	870	681	80	0.74	0.47
35	SG_06_1	C	.	.	5.4	150	134	283	2184	1240	632	67	0.95	0.58
51	SG_06_1	D	.	.	5.4	83	69	152	2004	1200	596	60	0.89	0.59
14	SG_12_1	A	.	.	7.2	0	166	165	5010	911	656	87	0.85	0.61
20	SG_12_1	B	.	.	5.5	89	106	194	2585	1140	559	80	1.01	0.59
31	SG_12_1	C	.	.	5.3	42	88	130	1583	1310	620	60	0.99	0.63
52	SG_12_1	D	.	.	5.6	41	64	105	1904	1170	644	60	0.96	0.57
1	SG_24_1	A	.	.	6.7	1	33	34	2285	1370	669	53	1.15	0.83
15	SG_24_1	B	.	.	7.3	3	157	160	5170	923	559	80	1.10	0.73
29	SG_24_1	C	.	.	5.4	3	101	104	1844	1370	669	57	1.19	0.73
45	SG_24_1	D	.	.	6	41	89	130	3026	1200	608	64	0.88	0.68

Appendix A: Raw data (Continued)

Table A.2. Soil microbial properties by treatment for 2002 experiment

Plot	Trtmt	Block	FDAfall	FDAsp	FDAsu	ASsp	ASsu	MRsp	MRsu	MBsp†	MBsu†
			---------- FDA g^{-1} soil hr^{-1} ----------			--- PNP g^{-1} soil hr^{-1} ---		----------	$-CO_2$-C g^{-1} soil 10 d^{-1}		----------
4	00_00_0	A	.	.	33	3.4	1.8	68	32	36	84
21	00_00_0	B	.	.	31	2.6	2.0	90	27	40	56
40	00_00_0	C	.	.	30	2.7	2.4	59	22	45	51
50	00_00_0	D	.	.	31	2.4	1.3	80	20	46	56
5	AP_12_0	A	.	.	43	4.3	2.6	102	32	64	72
18	AP_12_0	B	.	.	42	4.7	2.7	72	13	58	68
34	AP_12_0	C	.	.	45	2.9	2.5	100	24	89	100
46	AP_12_0	D	.	.	43	3.4	2.1	101	30	80	58
8	Br_12_0	A	.	.	31	3.8	3.0	56	36	45	85
28	Br_12_0	B	.	.	31	4.1	2.2	120	28	62	53
32	Br_12_0	C	.	.	32	2.8	2.0	86	38	54	92
49	Br_12_0	D	.	.	35	2.7	2.1	90	44	48	52
13	SG_12_0	A	.	.	41	6.9	4.0	82	20	30	58
19	SG_12_0	B	.	.	37	4.8	2.6	77	19	44	79
41	SG_12_0	C	.	.	37	5.8	3.5	86	22	72	68
47	SG_12_0	D	.	.	54	3.4	2.2	95	25	73	70
6	00_00_1	A	.	.	31	2.4	2.0	63	29	31	68
27	00_00_1	B	.	.	30	1.6	1.7	63	19	38	58
42	00_00_1	C	.	.	31	3.2	2.5	47	12	51	42
55	00_00_1	D	.	.	33	1.2	1.3	61	16	47	42
12	AP_06_1	A	.	.	34	4.2	2.8	96	25	66	67
25	AP_06_1	B	.	.	25	3.2	1.9	100	35	63	73
37	AP_06_1	C	.	.	35	4.4	2.5	122	36	72	85
48	AP_06_1	D	.	.	45	2.3	1.5	109	16	56	61
11	AP_12_1	A	.	.	42	5.2	4.0	108	25	55	90
23	AP_12_1	B	.	.	43	3.6	2.2	141	32	66	61
33	AP_12_1	C	.	.	43	3.3	1.5	83	22	70	101
56	AP_12_1	D	.	.	43	1.9	1.6	82	17	80	44
9	AP_24_1	A	.	.	48	4.3	2.9	97	33	67	86
22	AP_24_1	B	.	.	48	3.8	2.6	162	41	120	85
38	AP_24_1	C	.	.	57	4.8	3.3	136	69	78	131
44	AP_24_1	D	.	.	46	5.9	3.1	89	35	110	90
3	Br_06_1	A	.	.	37	4.3	2.7	74	21	46	79
16	Br_06_1	B	.	.	29	5.2	4.0	58	12	47	57
36	Br_06_1	C	.	.	33	1.8	1.6	93	24	58	65
53	Br_06_1	D	.	.	36	1.4	1.0	66	13	56	45
10	Br_12_1	A	.	.	36	6.1	3.9	85	26	49	76
24	Br_12_1	B	.	.	23	6.0	1.8	116	30	56	75
30	Br_12_1	C	.	.	31	2.6	2.2	80	29	70	89
54	Br_12_1	D	.	.	31	1.8	1.8	91	20	55	56
7	Br_24_1	A	.	.	45	3.2	3.1	84	22	44	91
26	Br_24_1	B	.	.	44	0.6	2.8	120	25	67	73
39	Br_24_1	C	.	.	41	5.2	2.8	101	35	72	75
43	Br_24_1	D	.	.	49	5.1	2.4	47	28	59	70
2	SG_06_1	A	.	.	27	4.9	2.6	77	29	52	72
17	SG_06_1	B	.	.	24	5.7	2.3	41	16	68	55
35	SG_06_1	C	.	.	45	2.6	1.3	119	16	84	74
51	SG_06_1	D	.	.	47	2.4	1.4	85	25	10	65
14	SG_12_1	A	.	.	37	5.2	4.0	39	22	49	57
20	SG_12_1	B	.	.	24	4.6	2.1	113	28	88	74
31	SG_12_1	C	.	.	31	2.7	1.4	86	34	64	97
52	SG_12_1	D	.	.	37	2.7	1.5	95	25	95	82
1	SG_24_1	A	.	.	51	7.9	6.1	112	60	98	130
15	SG_24_1	B	.	.	70	11.3	6.2	82	34	93	92
29	SG_24_1	C	.	.	32	3.6	2.7	83	33	56	99
45	SG_24_1	D	.	.	57	4.5	3.2	121	32	93	88

† Amount of CO_2-C generated by samples fumigated with chloroform for 24 h.

Appendix A: Raw data (Continued)

Table A.3. Soil populations and infection of potato roots by *Verticillium dahliae,* severity of Verticillium wilt of potato, total tuber yield, and average tuber weight for 2002 experiment.

Plot	Trtmt	Block	IDfall	IDsp	IDsu	RI	Severity	Yield	ATW
			---------- CFU g^{-1} soil ----------			CFU cm^{-1}	RAUSPC	kg ha^{-1}	kg $tuber^{-1}$
4	00_00_0	A	0.0	5.6	0.0	0.0	0.0	184	0.64
21	00_00_0	B	0.0	0.0	5.6	0.0	0.1	194	0.71
40	00_00_0	C	8.3	2.8	0.0	0.0	0.8	161	0.72
50	00_00_0	D	0.0	0.0	0.0	0.0	0.0	171	0.72
5	AP_12_0	A	0.0	5.6	8.3	0.0	0.0	191	0.68
18	AP_12_0	B	2.8	0.0	5.6	1.0	0.0	179	0.79
34	AP_12_0	C	2.8	0.0	0.0	0.0	0.0	199	0.81
46	AP_12_0	D	2.8	0.0	0.0	0.0	0.0	168	0.64
8	Br_12_0	A	2.8	0.0	5.6	0.0	0.1	170	0.76
28	Br_12_0	B	0.0	0.0	0.0	0.0	0.0	189	0.80
32	Br_12_0	C	0.0	0.0	0.0	0.0	0.0	174	0.71
49	Br_12_0	D	0.0	2.8	0.0	0.0	0.5	123	0.58
13	SG_12_0	A	2.8	0.0	0.0	0.0	1.5	169	0.82
19	SG_12_0	B	0.0	2.8	2.8	0.0	2.9	181	0.74
41	SG_12_0	C	0.0	2.8	0.0	0.0	4.1	164	0.74
47	SG_12_0	D	0.0	0.0	8.3	0.0	0.0	177	0.68
6	00_00_1	A	22.2	13.9	5.6	3.8	13.6	151	0.52
27	00_00_1	B	38.9	16.7	16.7	1.3	12.3	128	0.48
42	00_00_1	C	33.3	25.0	30.6	22.9	53.7	108	0.58
55	00_00_1	D	41.7	22.2	38.9	17.5	7.9	171	0.64
12	AP_06_1	A	44.4	8.3	11.1	5.6	7.9	136	0.58
25	AP_06_1	B	25.0	8.3	5.6	8.8	6.7	133	0.57
37	AP_06_1	C	8.3	19.4	16.7	16.0	9.6	164	0.58
48	AP_06_1	D	27.8	11.1	22.2	11.0	6.3	167	0.59
11	AP_12_1	A	5.6	19.4	19.4	8.0	8.0	118	0.61
23	AP_12_1	B	8.3	16.7	5.6	21.0	3.2	183	0.72
33	AP_12_1	C	19.4	36.1	2.8	3.0	2.4	156	0.53
56	AP_12_1	D	2.8	19.4	33.3	6.0	0.3	157	0.70
9	AP_24_1	A	13.9	5.6	22.2	5.7	9.6	120	0.65
22	AP_24_1	B	11.1	30.6	19.4	17.1	0.5	181	0.47
38	AP_24_1	C	19.4	13.9	19.4	22.5	6.3	161	0.59
44	AP_24_1	D	11.1	8.3	13.9	17.5	8.0	146	0.72
3	Br_06_1	A	16.7	8.3	5.6	4.0	12.3	131	0.53
16	Br_06_1	B	13.9	11.1	16.7	3.0	34.4	105	0.49
36	Br_06_1	C	22.2	11.1	2.8	27.0	12.3	183	0.67
53	Br_06_1	D	16.7	36.1	16.7	14.0	6.4	135	0.63
10	Br_12_1	A	8.3	16.7	2.8	6.0	22.6	140	0.56
24	Br_12_1	B	13.9	5.6	2.8	9.0	3.2	142	0.60
30	Br_12_1	C	0.0	2.8	2.8	4.0	0.9	162	0.53
54	Br_12_1	D	25.0	25.0	8.3	11.0	4.7	145	0.50
7	Br_24_1	A	13.9	8.3	5.6	1.3	4.6	127	0.47
26	Br_24_1	B	16.7	0.0	5.6	6.3	0.8	186	0.71
39	Br_24_1	C	19.4	11.1	2.8	11.3	9.4	175	0.64
43	Br_24_1	D	25.0	8.3	0.0	11.3	4.1	115	0.58
2	SG_06_1	A	44.4	5.6	16.7	3.8	8.0	136	0.56
17	SG_06_1	B	19.4	5.6	5.6	10.0	22.6	115	0.58
35	SG_06_1	C	16.7	5.6	0.0	3.8	6.7	135	0.52
51	SG_06_1	D	38.9	2.8	8.3	14.0	3.8	137	0.54
14	SG_12_1	A	22.2	2.8	16.7	13.0	8.0	139	0.70
20	SG_12_1	B	2.8	2.8	38.9	15.0	27.1	131	0.53
31	SG_12_1	C	8.3	2.8	11.1	3.0	5.8	169	0.55
52	SG_12_1	D	16.7	5.6	8.3	14.0	3.8	171	0.62
1	SG_24_1	A	2.8	0.0	16.7	3.8	0.9	201	0.75
15	SG_24_1	B	33.3	36.1	2.8	6.3	48.6	100	0.49
29	SG_24_1	C	0.0	16.7	8.3	1.3	0.1	150	0.60
45	SG_24_1	D	30.6	11.1	36.1	6.3	8.0	156	0.73

Appendix A: Raw data (Continued)

Table A.4 Soil chemical properties by treatment for 2002 experiment.

Plot	Trtmt	Block	pHfall	pHsp	pHsu	NH_4	NO_3	N	Ca	K	Mg	Na	TOC	POM
									mg kg^{-1}				g g^{-1}	
2	00_00_0	A	6.3	6.0	5.8	154	25	179	1348	1677	525	69	0.84	0.77
35	00_00_0	B	6.3	6.5	6.2	169	15	184	1914	989	678	62	0.81	0.71
59	00_00_0	C	6.3	6.6	6.0	217	19	236	1867	1049	686	49	0.68	0.80
69	00_00_0	D	6.1	6.4	5.8	139	27	166	1491	1199	585	26	0.75	0.68
1	AP_12_0	A	6.2	6.0	5.9	23	67	90	1505	3504	580	35	0.59	0.85
9	AP_12_0	B	6.0	6.3	5.9	133	64	197	1760	1017	608	25	1.02	0.96
54	AP_12_0	C	6.1	6.3	5.7	102	70	173	1861	957	658	29	0.84	0.95
58	AP_12_0	D	6.1	6.4	5.8	148	73	222	1709	1047	642	32	0.89	0.96
6	Br_12_0	A	6.3	6.5	6.1	106	32	138	1788	929	598	25	0.90	0.86
16	Br_12_0	B	6.4	6.7	6.5	15	34	49	1755	869	614	32	0.76	0.83
43	Br_12_0	C	6.3	6.6	6.4	3	27	30	2426	5179	714	82	0.88	0.85
57	Br_12_0	D	6.2	6.6	6.0	169	40	208	1802	1092	641	26	0.91	0.83
4	SG_12_0	A	5.9	6.0	6.0	22	60	82	1480	981	581	30	0.88	0.87
15	SG_12_0	B	6.3	6.5	6.2	95	66	161	1712	1016	624	28	0.86	0.77
48	SG_12_0	C	6.2	6.4	6.0	16	63	79	1528	989	607	31	0.80	0.77
62	SG_12_0	D	6.3	6.6	6.2	18	37	55	1776	960	642	26	0.79	0.78
3	00_00_1	A	6.2	6.1	5.9	45	20	66	1472	1050	589	35	0.78	0.79
10	00_00_1	B	6.3	6.6	6.2	57	19	76	1625	818	569	35	0.83	0.79
46	00_00_1	C	6.3	6.4	5.9	98	25	124	1546	927	627	32	0.78	0.76
65	00_00_1	D	6.1	6.4	5.9	112	22	134	1775	5860	605	89	0.76	0.84
18	AP_06_1	A	6.1	6.3	5.9	243	42	286	1887	1075	631	50	0.83	0.91
27	AP_06_1	B	6.2	6.3	5.8	281	22	303	1475	1068	592	33	0.84	0.86
53	AP_06_1	C	6.1	6.4	6.0	32	36	68	1775	833	650	37	0.83	0.87
66	AP_06_1	D	5.9	6.5	5.8	142	39	181	1792	893	635	28	0.88	0.81
22	AP_12_1	A	6.2	6.0	6.0	21	66	87	1532	945	614	13	1.01	0.87
31	AP_12_1	B	6.2	6.2	6.0	55	54	109	1791	923	641	27	0.91	0.95
41	AP_12_1	C	6.0	6.0	5.7	162	54	216	1676	1442	610	20	0.94	0.94
50	AP_12_1	D	6.1	6.2	5.6	190	65	255	1565	1088	618	34	0.77	0.84
24	AP_24_1	A	6.0	6.1	5.8	88	86	174	1556	1022	584	32	0.86	0.84
33	AP_24_1	B	6.1	6.2	6.0	77	81	158	1702	1191	604	47	0.98	1.07
52	AP_24_1	C	5.8	6.1	5.6	130	108	238	1733	2182	622	35	0.90	1.04
64	AP_24_1	D	6.0	6.2	5.8	43	105	148	1744	1133	619	17	1.00	1.04
7	Br_06_1	A	6.2	6.4	6.0	174	19	193	1817	1120	633	52	0.87	0.82
30	Br_06_1	B	6.4	6.5	6.3	13	28	41	1905	901	667	44	0.97	0.84
63	Br_06_1	C	6.4	6.6	6.2	27	29	56	1650	900	611	21	0.81	0.91
67	Br_06_1	D	6.3	6.5	6.0	80	37	117	1984	1041	682	35	0.83	0.86
5	Br_12_1	A	6.3	6.6	6.2	62	18	80	1787	980	630	26	0.88	0.83
17	Br_12_1	B	6.4	6.6	6.3	130	46	176	1803	939	623	40	0.87	0.87
38	Br_12_1	C	6.5	6.6	6.3	23	18	41	1900	867	683	25	0.79	0.70
51	Br_12_1	D	6.2	6.5	6.0	93	35	127	1614	1018	629	39	0.79	0.83
14	Br_24_1	A	6.7	6.7	6.7	44	51	95	1956	1033	669	35	0.83	0.82
23	Br_24_1	B	6.4	6.7	6.4	77	44	122	1354	990	509	20	0.94	0.90
47	Br_24_1	C	6.5	7.0	6.4	85	56	141	1622	1127	590	32	0.85	0.87
60	Br_24_1	D	6.6	7.1	6.3	506	46	552	2009	1435	648	34	0.82	0.88
21	SG_06_1	A	6.1	6.3	6.0	78	28	106	1390	874	565	22	0.87	0.83
36	SG_06_1	B	6.4	6.6	5.9	81	26	107	1905	834	651	23	0.81	0.74
39	SG_06_1	C	6.3	6.4	6.2	9	23	32	1755	887	635	32	0.81	0.81
44	SG_06_1	D	6.3	6.4	5.8	268	20	288	1751	1011	645	49	0.78	0.80
11	SG_12_1	A	6.3	6.5	6.3	87	44	131	1680	956	576	42	0.82	0.82
28	SG_12_1	B	6.2	6.3	5.8	331	49	379	1653	2705	591	41	0.85	0.90
56	SG_12_1	C	6.1	6.4	6.0	23	41	65	1827	987	636	28	0.91	0.85
71	SG_12_1	D	6.1	6.5	5.9	58	49	107	1563	1030	619	21	0.73	0.89
13	SG_24_1	A	6.4	6.6	6.2	298	60	358	1897	1502	625	31	0.95	0.99
19	SG_24_1	B	6.2	6.4	6.1	54	39	94	1799	1428	631	21	0.98	0.97
42	SG_24_1	C	6.1	6.4	6.0	43	51	94	1771	1206	653	23	0.93	0.98
72	SG_24_1	D	6.1	6.5	5.7	405	86	491	1659	1654	629	39	0.85	0.96

Appendix A: Raw data (Continued)

Table A.5 Soil microbial properties by treatment for 2002 experiment.

Plot	Trtmt	Block	FDAfall	FDAsp	FDAsu	ASsp	ASsu	MRsp	MRsu	MBsp†	MBsu†
			FDA g^{-1} soil hr^{-1}			PNP g^{-1} soil hr^{-1}		$-CO_2$-C g^{-1} soil 10 d^{-1}			
2	00_00_0	A	16.0	18.8	13.4	4.1	.	53	22	65	59
35	00_00_0	B	20.9	14.4	4.8	1.9	.	47	23	72	56
59	00_00_0	C	25.1	14.5	14.9	2.1	.	37	25	74	59
69	00_00_0	D	21.0	19.5	9.4	2.9	.	49	25	69	54
1	AP_12_0	A	26.6	32.5	20.4	10.4	.	117	30	144	94
9	AP_12_0	B	52.1	30.8	23.4	5.9	.	128	50	137	119
54	AP_12_0	C	33.2	24.3	13.6	6.5	.	79	41	108	83
58	AP_12_0	D	32.0	23.4	23.3	3.8	.	98	60	123	107
6	Br_12_0	A	33.2	26.6	30.6	7.8	.	64	41	130	109
16	Br_12_0	B	34.1	24.5	24.9	6.9	.	76	44	118	103
43	Br_12_0	C	26.5	28.3	13.2	17.4	.	78	51	121	95
57	Br_12_0	D	33.4	25.1	16.2	3.5	.	73	35	126	98
4	SG_12_0	A	35.5	25.2	25.1	5.8	.	60	37	90	101
15	SG_12_0	B	11.6	27.1	18.1	6.4	.	102	44	151	112
48	SG_12_0	C	32.6	28.0	15.6	6.3	.	80	59	113	99
62	SG_12_0	D	28.3	20.0	18.9	3.4	.	108	48	106	95
3	00_00_1	A	22.9	19.0	18.5	4.1	.	45	19	55	54
10	00_00_1	B	20.6	19.7	18.3	3.3	.	42	29	78	64
46	00_00_1	C	28.0	19.6	13.5	9.4	.	42	38	68	74
65	00_00_1	D	20.9	15.4	12.2	2.0	.	35	27	49	48
18	AP_06_1	A	30.8	25.3	22.3	4.7	.	82	28	116	68
27	AP_06_1	B	31.9	21.8	12.7	4.7	.	52	27	87	66
53	AP_06_1	C	28.3	22.6	13.4	5.8	.	57	40	95	69
66	AP_06_1	D	22.0	22.4	12.5	2.9	.	50	39	80	65
22	AP_12_1	A	22.9	30.1	15.8	6.9	.	74	56	122	118
31	AP_12_1	B	30.1	24.5	16.9	3.3	.	97	56	125	92
41	AP_12_1	C	35.4	26.7	18.4	6.1	.	100	44	144	93
50	AP_12_1	D	39.2	26.7	14.4	5.5	.	98	47	103	84
24	AP_24_1	A	56.6	36.4	24.4	7.7	.	173	80	144	113
33	AP_24_1	B	49.6	30.2	27.1	5.1	.	136	83	170	127
52	AP_24_1	C	40.8	34.3	23.8	7.1	.	137	111	135	144
64	AP_24_1	D	39.3	34.4	22.5	3.4	.	146	110	136	138
7	Br_06_1	A	30.1	22.4	25.9	4.1	.	46	32	89	77
30	Br_06_1	B	20.5	20.6	9.4	6.4	.	70	38	103	83
63	Br_06_1	C	30.1	22.3	13.3	2.6	.	65	52	103	108
67	Br_06_1	D	25.2	22.0	15.0	4.8	.	46	37	76	75
5	Br_12_1	A	23.0	23.7	24.9	7.6	.	88	40	109	98
17	Br_12_1	B	32.3	25.5	20.2	9.8	.	94	43	133	106
38	Br_12_1	C	28.3	23.0	13.1	5.6	.	64	46	109	85
51	Br_12_1	D	29.8	23.3	16.3	6.2	.	57	36	85	81
14	Br_24_1	A	39.2	26.4	26.2	10.8	.	104	54	179	148
23	Br_24_1	B	40.7	31.4	24.5	10.7	.	89	56	179	147
47	Br_24_1	C	49.9	33.9	17.5	10.4	.	111	67	169	139
60	Br_24_1	D	36.9	25.8	20.2	5.4	.	98	45	149	121
21	SG_06_1	A	30.8	22.8	22.0	5.0	.	57	41	87	81
36	SG_06_1	B	39.0	20.1	13.0	2.1	.	78	29	102	69
39	SG_06_1	C	34.0	22.9	17.4	2.4	.	55	39	90	78
44	SG_06_1	D	25.1	20.1	13.3	4.6	.	50	33	88	81
11	SG_12_1	A	29.2	29.9	32.3	5.2	.	78	74	135	124
28	SG_12_1	B	27.6	24.4	24.1	6.6	.	68	39	100	74
56	SG_12_1	C	38.0	19.7	15.3	3.6	.	69	27	101	92
71	SG_12_1	D	33.4	37.7	21.3	7.1	.	66	44	105	96
13	SG_24_1	A	36.5	36.8	36.5	9.0	.	153	67	161	146
19	SG_24_1	B	33.0	32.7	34.6	10.2	.	112	50	120	156
42	SG_24_1	C	38.7	31.7	21.3	6.0	.	114	56	151	122
72	SG_24_1	D	50.2	28.5	22.7	8.5	.	122	-9	107	107

† Amount of CO_2-C generated by samples fumigated with chloroform for 24 h.

Appendix A: Raw data (Continued)

Table A.6 Soil populations and infection of potato roots by *Verticillium dahliae,* severity of Verticillium wilt of potato, total tuber yield, and average tuber weight for 2003 experiment.

Plot	Trtmt	Block	IDfall	IDsp	IDsu	RC	Severity	Yield	ATW
			---------- CFU g^{-1} soil ----------			CFU cm^{-1}	RAUSPC	kg ha^{-1}	kg $tuber^{-1}$
2	00_00_0	A	0.0	0.0	0.0	0.0	2.6	156	0.41
35	00_00_0	B	2.8	9.8	0.0	2.5	9.0	111	0.33
59	00_00_0	C	0.0	2.4	2.4	0.0	5.7	142	0.30
69	00_00_0	D	0.0	2.4	2.4	0.0	5.7	194	0.54
1	AP_12_0	A	0.0	0.0	2.4	0.0	1.8	131	0.36
9	AP_12_0	B	0.0	0.0	0.0	0.0	2.4	156	0.42
54	AP_12_0	C	2.8	4.9	4.9	0.0	1.8	154	0.35
58	AP_12_0	D	0.0	2.4	2.4	0.0	0.9	168	0.35
6	Br_12_0	A	0.0	4.9	2.4	0.0	0.1	141	0.39
16	Br_12_0	B	0.0	0.0	0.0	0.0	0.2	152	0.45
43	Br_12_0	C	0.0	2.4	0.0	0.0	1.3	152	0.42
57	Br_12_0	D	0.0	0.0	0.0	0.0	1.6	177	0.37
4	SG_12_0	A	0.0	0.0	0.0	0.0	0.9	134	0.51
15	SG_12_0	B	0.0	4.9	0.0	0.0	0.5	155	0.36
48	SG_12_0	C	2.8	2.4	0.0	0.0	0.5	183	0.37
62	SG_12_0	D	0.0	0.0	0.0	0.0	0.3	132	0.55
3	00_00_1	A	8.3	12.2	2.4	2.0	7.1	131	0.43
10	00_00_1	B	13.9	14.6	24.4	15.0	19.5	112	0.39
46	00_00_1	C	5.6	26.8	19.5	11.0	38.9	114	0.38
65	00_00_1	D	16.7	29.3	22.0	1.0	15.0	131	0.42
18	AP_06_1	A	2.8	22.0	14.6	3.0	5.7	133	0.29
27	AP_06_1	B	8.3	4.9	22.0	15.0	38.9	72	0.27
53	AP_06_1	C	0.0	39.0	22.0	7.0	24.4	140	0.30
66	AP_06_1	D	8.3	17.1	22.0	3.0	11.1	134	0.41
22	AP_12_1	A	5.6	31.7	19.5	6.0	6.6	120	0.31
31	AP_12_1	B	16.7	4.9	7.3	5.0	4.7	91	0.38
41	AP_12_1	C	22.2	36.6	22.0	9.0	6.6	124	0.42
50	AP_12_1	D	5.6	19.5	14.6	6.0	12.1	109	0.25
24	AP_24_1	A	2.8	14.6	7.3	4.0	3.7	106	0.25
33	AP_24_1	B	11.1	7.3	7.3	17.0	4.8	122	0.41
52	AP_24_1	C	16.7	17.1	9.8	8.0	10.1	129	0.23
64	AP_24_1	D	2.8	26.8	17.1	7.0	8.3	116	0.50
7	Br_06_1	A	2.8	12.2	2.4	0.0	3.9	148	0.37
30	Br_06_1	B	25.0	29.3	14.6	7.0	18.7	133	0.29
63	Br_06_1	C	2.8	12.2	12.2	2.0	11.2	141	0.45
67	Br_06_1	D	11.1	14.6	7.3	0.0	15.3	116	0.40
5	Br_12_1	A	0.0	0.0	7.3	0.0	7.3	112	0.29
17	Br_12_1	B	8.3	22.0	4.9	14.0	6.8	117	0.40
38	Br_12_1	C	13.9	19.5	4.9	2.0	12.9	130	0.44
51	Br_12_1	D	11.1	9.8	17.1	7.0	51.3	111	0.45
14	Br_24_1	A	8.3	9.8	17.1	4.0	6.6	128	0.34
23	Br_24_1	B	0.0	29.3	19.5	4.0	3.7	126	0.47
47	Br_24_1	C	5.6	34.1	14.6	0.0	6.5	110	0.42
60	Br_24_1	D	5.6	0.0	19.5	1.0	14.6	146	0.33
21	SG_06_1	A	16.7	22.0	9.8	14.0	14.6	127	0.31
36	SG_06_1	B	2.8	9.8	9.8	2.0	24.9	105	0.24
39	SG_06_1	C	13.9	31.7	17.1	14.0	17.8	126	0.43
44	SG_06_1	D	13.9	12.2	14.6	4.0	14.1	115	0.43
11	SG_12_1	A	2.8	2.4	4.9	7.0	5.7	125	0.39
28	SG_12_1	B	2.8	26.8	14.6	1.0	11.7	83	0.25
56	SG_12_1	C	0.0	19.5	4.9	8.0	6.6	147	0.35
71	SG_12_1	D	2.8	2.4	12.2	7.0	12.0	157	0.49
13	SG_24_1	A	33.3	22.0	9.8	10.0	11.2	128	0.31
19	SG_24_1	B	11.1	14.6	22.0	3.0	6.6	156	0.40
42	SG_24_1	C	0.0	12.2	17.1	5.0	3.7	149	0.37
72	SG_24_1	D	11.1	9.8	9.8	5.0	3.7	109	0.40

Appendix B: Supplemental field experiment on the effect of spring green manure on Verticillium wilt of potato

INTRODUCTION

A field study was conducted in 2003 to investigate the effects of different rates of spring manure on Verticillium wilt of potato. The two objectives of the study were to: (i) determine the effects of different rates of spring green manure on soil populations and infection of potato roots by *Verticillium dahliae*, the severity of Verticillium wilt of potato, total tuber yield, and average tuber weight, and (ii) compare the effects of spring green manure with fall green manures at two rates (6 and 12 Mg ha^{-1}). The raw data and preliminary results are presented in this appendix.

MATERIALS AND METHODS

Experimental design. The spring green manure trial was conducted in 2003 at the COARC in conjunction with the 2003 fall green manure experiment (Appendix B). The study location and analytical methods are as described in chapter 2 and 3 of this thesis. A mix of crown vetch (*Coronilla varia* L.), phacelia (*Phacelia juss.*), and mustard (*Brassica rapa* L.) was harvested on 30 April, 2003 in Albany, OR, transported to COARC and incorporated into soil on 1 May at one of three rates (3, 6, or 12 Mg ha^{-1}, dryweight).

Data analyses. Manipulation of the data were performed as described in chapters 2 and 3 of this thesis. For all pathogen, disease, and yield variables, as well as soil properties variables for which amendment rate was found to be significant, means separations was conducted using Fisher's least squared difference (LSD). For all pathogen, disease, yield and soil microbial property variables, means separations were conducted for the 6 and 12 Mg ha^{-1} amendment rates of spring green manure and the three kinds of fall green manures. All analyses were conducted using SAS (release 8.02, Cary, NJ).

Appendix B: Supplemental field experiment (Continued)

Table B.1 Comparison of mean inoculum density and infection of potato roots by *Verticillium dahliae*, severity of Verticillium wilt of potato, total tuber yield, and average tuber weight for spring green manure at three rates.

	Inoculum density		Root	Disease		Average
	Spring	Summer	infection	severity	Yield	tuber weight
	CFU g^{-1} soil		CFU m^{-1} root	RAUSPC	kg ha^{-1}	kg $tuber^{-1}$
Unamended control	21 a	17 a	7.3 a	20 b	122 ab	0.41 a
3 Mg ha^{-1}	30 a	18 a	6.8 a	37 a	100 b	0.37 a
6 Mg ha^{-1}	26 a	13 a	8.5 a	13 b	121 ab	0.35 a
12 Mg ha^{-1}	30 a	16 a	7.8 a	11 b	136 a	0.38 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table B.2 Comparison of mean summer nitrate, spring and summer FDA hydrolysis, microbial respiration, and microbial biomass carbon for spring green manure at three rates.

		FDA hydrolysis		Microbial respiration		Microbial biomass-C	
	NO_3-N	Spring	Summer	Spring	Summer	Spring	Summer
	mg kg^{-1}	μg FDA g^{-1} h^{-1}		mg CO_2-C g^{-1} soil 10 d^{-1}		mg C g^{-1}	
Unamended control	52 a	17.6 b	13.1 a	44 c	26 c	162 b	143 c
3 Mg ha^{-1}	26 b	18.4 b	17.5 a	57 bc	33 bc	191 ab	171 b
6 Mg ha^{-1}	29 ab	19.4 b	16.6 a	72 ab	40 b	206 a	187 b
12 Mg ha^{-1}	43 a	23.4 a	18.3 a	71 b	52 a	196 ab	233 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Appendix B: Supplemental field experiment (Continued)

Table B.3 Comparison of mean inoculum density and infection of potato roots by *Verticillium dahliae*, severity of Verticillium wilt of potato, total tuber yield, and average tuber weight for spring green manure and three kinds of fall green manure applied at 6 Mg ha^{-1}.

	Inoculum density		Root	Disease		Average
	Spring	Summer	infection	severity	Yield	tuber weight
	CFU g^{-1} soil		CFU m^{-1} root	RAUSPC	kg ha^{-1}	kg $tuber^{-1}$
Unamended control	21 a	17 ab	7.3 ab	20 a	122 a	0.41 a
Spring green manure	26 a	13 ab	8.5 a	13 a	122 a	0.35 a
Austrian winter pea	21 a	20 a	7.0 b	20 a	120 a	0.32 a
Broccoli	17 a	9 b	2.3 ab	12 a	135 a	0.38 a
Sudan grass	19 a	12 ab	8.5 ab	18 a	118 a	0.35 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table B.4 Comparison of mean spring arylsulfatase activity, spring and summer FDA hydrolysis, microbial respiration, and microbial biomass carbon for spring green manure and three kinds of fall green manure applied at 6 Mg ha^{-1}.

	Aryl-	FDA hydrolysis		Microbial respiration		Microbial biomass-C	
	sulfatase	Spring	Summer	Spring	Summer	Spring	Summer
	μg PNP g^{-1} h^{-1}	μg FDA g^{-1} h^{-1}		mg CO_2-C g^{-1} soil 10 d^{-1}		mg C g^{-1}	
Unamended control	3.6 ab	17.6 c	13.1 a	44 b	26 b	162 b	143 b
Spring green manure	2.3 b	19.5 b	16.6 a	72 a	40 a	206 a	187 a
Austrian winter pea	4.8 a	23.8 a	13.3 a	60 a	34 ab	230 a	163 ab
Broccoli	4.5 a	21.8 ab	15.8 a	57 ab	40 a	226 a	209 a
Sudan grass	3.5 ab	21.5 ab	16.3 a	60 ab	36 a	224 a	188 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Appendix B: Supplemental field experiment (Continued)

Table B.5 Comparison of mean inoculum density and infection of potato roots by *Verticillium dahliae*, severity of Verticillium wilt of potato, total tuber yield, and average tuber weight for spring green manure and three kinds of fall green manure applied at 12 Mg ha^{-1}.

	Inoculum density					
	Spring	Summer	Root infection	Disease severity	Yield	Average tuber weight
	CFU g^{-1} soil		CFU m^{-1} root	RAUSPC	kg ha^{-1}	kg $tuber^{-1}$
Unamended control	21 a	17 a	7.3 a	20 a	122 a	0.41 a
Spring green manure	30 a	16 a	7.8 a	11 a	136 a	0.38 a
Austrian winter pea	23 a	16 a	6.5 a	8 a	111 a	0.34 a
Broccoli	13 a	9 a	5.8 a	20 a	118 a	0.40 a
Sudan grass	13 a	9 a	5.8 a	9 a	128 a	0.37 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table B.6 Comparison of mean spring arylsulfatase activity, spring and summer FDA hydrolysis, microbial respiration, and microbial biomass carbon for spring green manure and three kinds of fall green manure applied at 12 Mg ha^{-1}.

	Aryl-sulfatase	FDA hydrolysis		Microbial respiration		Microbial biomass-C	
		Spring	Summer	Spring	Summer	Spring	Summer
	μg PNP g^{-1} h^{-1}	μg FDA g^{-1} h^{-1}		mg CO_2-C g^{-1} soil 10 d^{-1}		mg C g^{-1}	
Unamended control	3.6 c	17.6 b	13.1 b	44 c	26 b	162 b	143 b
Spring green manure	4.3 bc	23.3 a	18.3 a	71 b	52 a	196 b	234 a
Austrian winter pea	6.0 ab	27.4 a	18.1 a	99 a	48 a	307 a	241 a
Broccoli	8.3 a	25.0 a	19.9 a	74 b	42 a	284 a	236 a
Sudan grass	5.5 ab	26.5 a	21.3 a	79 b	47 a	275 a	242 a

Within columns, means followed by the same letter are not significantly different at $P \leq 0.05$.

Appendix B: Supplemental field experiment (Continued)

Table B.7 Raw data for spring green manures (soil chemical properties)

Plot	Block	Trtmt	pHsp	pHsu	NH_4	NO_3	N	Ca	K	Mg	Na	TOC	POM
								mg kg^{-1}				g g^{-1}	
2	A	00_00_0	6.0	5.8	154	25	179	1348	1677	525	69	0.84	0.77
35	B	00_00_0	6.5	6.2	169	15	184	1914	989	678	62	0.81	0.71
59	D	00_00_0	6.6	6.0	217	19	236	1867	1049	686	49	0.68	0.80
69	C	00_00_0	6.4	5.8	139	27	166	1491	1199	585	26	0.75	0.68
3	A	00_00_1	6.1	5.9	45	20	66	1472	1050	589	35	0.78	0.79
10	B	00_00_1	6.6	6.2	57	19	76	1625	818	569	35	0.83	0.79
46	C	00_00_1	6.4	5.9	98	25	124	1546	927	627	32	0.78	0.76
65	D	00_00_1	6.4	5.9	112	22	134	1775	5860	605	89	0.76	0.84
8	B	VPM_06_0	6.4	6.0	70	30	100	1690	872	616	25	0.84	0.83
29	A	VPM_06_0	6.3	6.2	33	24	57	1773	924	650	31	0.75	0.82
45	C	VPM_06_0	6.5	6.0	103	26	129	1605	948	611	36	0.77	0.81
55	D	VPM_06_0	6.4	5.9	149	47	196	1804	1118	648	40	0.79	0.86
12	B	VPM_03_1	6.6	6.5	19	25	44	1977	838	607	45	0.76	0.82
25	A	VPM_03_1	6.3	6.2	.	.	.	1537	902	625	27	0.66	0.72
40	D	VPM_03_1	6.3	5.9	104	27	132	1676	867	599	27	0.82	0.84
70	C	VPM_03_1	6.5	5.8	80	26	107	1565	918	627	30	0.75	0.80
20	A	VPM_06_1	6.5	6.4	86	25	111	1738	892	632	29	0.82	0.79
32	B	VPM_06_1	6.4	6.0	81	34	115	1669	1105	620	34	0.77	0.81
37	D	VPM_06_1	6.5	6.1	49	15	65	1913	896	664	26	0.72	0.73
49	C	VPM_06_1	6.5	5.9	149	28	177	1580	899	638	30	0.65	0.74
26	A	VPM_12_1	6.3	6.2	97	40	138	1689	2592	613	76	0.83	0.77
34	B	VPM_12_1	6.6	6.1	151	44	195	1803	1105	640	94	0.76	0.80
61	D	VPM_12_1	6.6	6.1	130	44	174	1966	1123	686	25	0.78	0.81
68	C	VPM_12_1	6.5	6.1	62	45	106	1630	832	604	23	0.82	0.81

Table B.8 Raw data for spring green manures (soil microbial properties)

Plot	Block	Trtmt	FDAsp	FDAsu	ASsp	MRsp	MRsu	MBsp	MBsu
			FDA g^{-1} soil h^{-1}		PNP g^{-1} h^{-1}		CO_2-C g^{-1} soil 10 d^{-1}		
2	A	00_00_0	18.8	13.4	4.1	53	22	65	59
35	B	00_00_0	14.4	4.8	1.9	47	23	72	56
59	D	00_00_0	14.5	14.9	2.1	37	25	74	59
69	C	00_00_0	19.5	9.4	2.9	49	25	69	54
3	A	00_00_1	19.0	18.5	4.1	45	19	55	54
10	B	00_00_1	19.7	18.3	3.3	42	29	78	64
46	C	00_00_1	19.6	13.5	9.4	42	38	68	74
65	D	00_00_1	15.4	12.2	2.0	35	27	49	48
8	B	VPM_06_0	20.8	21.3	3.9	76	51	86	80
29	A	VPM_06_0	18.6	14.3	5.5	55	40	80	69
45	C	VPM_06_0	21.2	16.7	-0.8	64	51	104	95
55	D	VPM_06_0	18.4	17.1	1.9	62	36	94	74
12	B	VPM_03_1	17.0	21.0	5.2	54	37	74	81
25	A	VPM_03_1	14.0	18.8	5.4	51	30	69	55
40	D	VPM_03_1	13.8	17.0	0.9	68	32	85	73
70	C	VPM_03_1	28.7	13.4	5.3	54	32	86	72
20	A	VPM_06_1	22.3	24	-0.4	66	38	59	77
32	B	VPM_06_1	19.4	14	2.3	76	35	96	83
37	D	VPM_06_1	18.4	13	1.8	66	29	83	69
49	C	VPM_06_1	16.7	13	4.2	111	38	74	67
26	A	VPM_12_1	23.3	14	5.0	62	47	68	95
34	B	VPM_12_1	22.3	19	3.0	64	44	64	93
61	D	VPM_12_1	19.1	21	4.1	71	60	87	95
68	C	VPM_12_1	28.7	19	4.6	87	55	103	100

Appendix B: Supplemental field experiment (Continued)

Table B.9 Raw data for spring green manures (soil populations and infection of potato roots by *Verticillium dahliae,* Verticillium wilt of potato, total tuber yield, and average tuber weight.

Plot	Block	Trtmt	IDsp	IDsu	RI	Severity	Yield	ATW
2	A	00_00_0	0.0	0.0	0.0	2.6	156	0.41
35	B	00_00_0	9.8	0.0	2.5	9.0	111	0.33
59	D	00_00_0	2.4	2.4	0.0	5.7	142	0.30
69	C	00_00_0	2.4	2.4	0.0	5.7	194	0.54
3	A	00_00_1	12.2	2.4	2.0	7.1	131	0.43
10	B	00_00_1	14.6	24.4	15.0	19.5	112	0.39
46	C	00_00_1	26.8	19.5	11.0	38.9	114	0.38
65	D	00_00_1	29.3	22.0	1.0	15.0	131	0.42
8	B	VPM_06_0	4.9	0.0	0.0	0.8	138	0.37
29	A	VPM_06_0	7.3	0.0	0.0	1.8	150	0.35
45	C	VPM_06_0	0.0	4.9	0.0	1.0	157	0.37
55	D	VPM_06_0	0.0	0.0	0.0	5.7	160	0.33
12	B	VPM_03_1	24.4	22.0	7.0	40.8	107	0.39
25	A	VPM_03_1	17.1	7.3	6.0	45.8	65	0.27
40	D	VPM_03_1	41.5	22.0	9.0	33.8	104	0.35
70	C	VPM_03_1	36.6	19.5	5.0	28.9	124	0.45
20	A	VPM_06_1	14.6	19.5	15.0	2.6	140	0.32
32	B	VPM_06_1	26.8	9.8	6.0	21.5	131	0.29
37	D	VPM_06_1	22.0	7.3	4.0	8.3	113	0.40
49	C	VPM_06_1	41.5	17.1	9.0	19.9	102	0.39
26	A	VPM_12_1	17.1	12.2	12.2	17.8	90	0.23
34	B	VPM_12_1	19.5	26.8	17.0	6.6	125	0.30
61	D	VPM_12_1	26.8	2.4	0.0	14.6	170	0.51
68	C	VPM_12_1	58.5	24.4	2.0	6.8	160	0.47

Appendix C: Greenhouse experiment on the effect of soil pH and inoculum density on Verticillium wilt of potato

INTRODUCTION

A greenhouse study was conducted to (i) investigate the effects two nitrogen sources, urea and potassium nitrate, and (ii) soil pH on soil populations of *Verticillium dahliae* and severity of Verticillium wilt of potato; (ii) determine the relationship between preplant soil populations of *V. dahliae* and severity Verticillium wilt. A brief description of the study is presented in this appendix, along with preliminary data analysis.

MATERIALS AND METHODS

Research Design. The greenhouse study was conducted at Oregon State University (Corvallis, OR) from June to October, 2003. The experiment was a completely randomized design with 2 replicates. The treatments were arranged as a 10 x 5 factorial, with amendment of urea or lime and rate of *Verticillium dahliae* infestation as the two factors. The soil was obtained from the Central Oregon Agricultural Research Center (COARC) in Madras, Oregon, and is classified as a Madras series sandy loam (Superactive, mesic Aridic Argixeroll). The field from which the soil was obtained was used for the green manure trial in 2002 (Chapter 2) and had been planted to dryland wheat for the previous 20 years. Soil was excavated (0 to 45 cm) from a unamended control plot, with an inoculum density of *V.dahliae* of approximately 25 CFU g^{-1} soil. Soils were transported to Corvallis, OR, passed through a 6.35 mm sieve to remove rocks and plant debris and placed into 15-liter pots with drainage holes that were lined with paper towels.

Approximately 60 days prior to planting, pots received one of the following amendments: no amendment; 2, 14, 25, 212, or 366 kg ha^{-1} of urea; or 11, 24, 570, or 1260 kg ha^{-1} of hydrated lime. Thirty days prior to planting of potatoes, the soil was infested with microsclerotia of *V. dahliae* that was cultured in the manner described in Chapter 2 of this thesis at one of five rates: (i) no additional infestation; (ii) 5 to 10

CFU g^{-1} soil; (iii) 15 to 20 CFU g^{-1} soil; (iv) 25 to 30 CFU g^{-1} soil; (v) 35 to 40 CFU g^{-1} soil.

Just prior to planting, composite soil samples were taken from a depth of 5 to 10 cm from each pot, air dried, and analyzed for preplant inoculum density, soil pH, NH_4-N, and NO_3-N.

Greenhouse, Potatoes, and Verticillium Wilt Severity. The greenhouse was maintained at approximately 21°C with ambient light; day-length ranged from 14 to 11 hours. Thirty days prior to planting of potatoes, a base rate of 300 kg ha^{-1} of K_2SO_4 and 215 kg ha^{-1} of super phosphate was applied to each pot; enough KNO_3 was added so that the total nitrogen added as KNO_3 and/or urea equaled at least 310 kg ha^{-1}. Eyes of first generation Russet Norkota seed were removed from tubers with a sterilized melon ball scooper, allowed to suberize for 24 hours, and planted, three per pot, on August 14th, 2003. During the experiment water was applied until saturation every other day and allowed to drain. After the first appearance of wilt symptoms, the percentage of senescent leaves in each pot was visually assessed weekly for 4 weeks using a scale of 0, 1, 5 10, 25, or 75%.

Data Analyses. Area under the senescence progress curve (AUSPC) was calculated based on the weekly disease reading (99) and divided by the number of growing degree days (base = 10°C, max = 30°C) over the senescent period to generae a relative area under the senescence progress curve (RAUSPC). The following transformations were performed to approximate normal distribution for each variable: (i) square root of preplant inoculum density (ID); (ii) natural log of AUSPC + 1; (iii) natural log of NH_4-N and NO_3-N. The change in ID between infestation and planting was calculated by taking the difference of the infestation rate and measured preplant ID.

Analyses of variance were performed to determine the significance of the main effects of the two factors, urea or lime amendment and infestation rate, on all other variables. The means of the different levels of the two factors were compared using Fisher's least significant difference (LSD) means separation ($P \leq 0.05$). A matrix of

correlations and scatterplots were generated to assess the relationships between soil pH, preplant NH_4-N, NO_3-N, changes in ID between infestation and preplant sampling, preplant ID, and RWS. All statistical analyses were conducted using SAS (release 8.02; SAS Institute, Cary, NC)

Table C.1 effect of urea and lime amendments and infestation rate on siol chemical, soil population of *Verticillium dahliae*, and severity of Verticillium wilt of potato.

Source of error	d.f.	Soil pH	Preplant NH_4-N	Preplant NO_3-N	Chainge in ID	Preplant ID	RAUSPC
Amendment	9	***	***	NS	***	***	**
Infestation rate	4	NS	NS	NS	***	NS	NS
Pooled	36						

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

Table C.2 Comparisons of mean soil chemical properties, soil populations of *V. dahliae*, and severity of Verticilliu wilt, by type and rate of amendment.

Amendment type and rae	Soil pH†	Preaplant NH_4-N†	Preplant NO_3-N†	Change in ID†	Preplant ID†	RAUSPC†
		$\mu g\ g^{-1}$ soil	$\mu g\ g^{-1}$ soil	CFU g^{-1} soil	CFU g^{-1} soil	
Urea 366 kg ha^{-1}	5.2 f	148 a	134 a	-50 d ***	0 c	5 d
Urea 213 kg ha^{-1}	5.3 e	25 b	110 ab	-40 cd ***	2 c	32 cd
Urea 25 kg ha^{-1}	5.7 d	4 c	67 bc	-7 ab	41 ab	39 bc
Urea 14 kg ha^{-1}	5.7 d	5 c	74 abc	11 a	56 a	50 abc
Urea 2 kg ha^{-1}	5.8 d	4 c	55 bc	13 a	58 a	53 abc
No amendment	5.8 d	3 c	67 abc	15 a	61 a	61 ab
Lime 11 kg ha^{-1}	5.9 d	5 c	55 bc	1 ab	45 a	61 ab
Lime 24 kg ha^{-1}	6.1 c	3 c	82 abc	-23 bc *	21 b	67 a
Lime 570 kg ha^{-1}	6.6 b	4 c	55 c	-5 ab	37 ab	57 abc
Lime 1260 kg ha^{-1}	7.3 a	3 c	82 abc	-8 ab	40 ab	61 ab

† Within colums, means follwed by the same letter are not significantly different at $P \leq 0.05$.

*,and *** indicate that the mean change in inID is different from zero at $P \leq 0.05$ or 0.001, respectively.

Table C.3 Simple correlations (r) of soil pH, nitrogen, soil population of V.dahliae, and severity of Verticillium wilt

	Soil pH	NH_4-N	NO_3-N	Change in ID	Preplant ID
Preplant NH_4-N	-0.49				
	***				
Preplant NO_3-N	-0.23	0.34			
		*			
Change in ID	0.20	-0.62	-0.33		
		***	*		
Preplant ID	0.37	-0.81	-0.38	0.86	
	**	***	**	***	
RAUSPC	0.39	-0.55	-0.41	0.27	0.44
	**	***	**		**

*, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001, respectively.

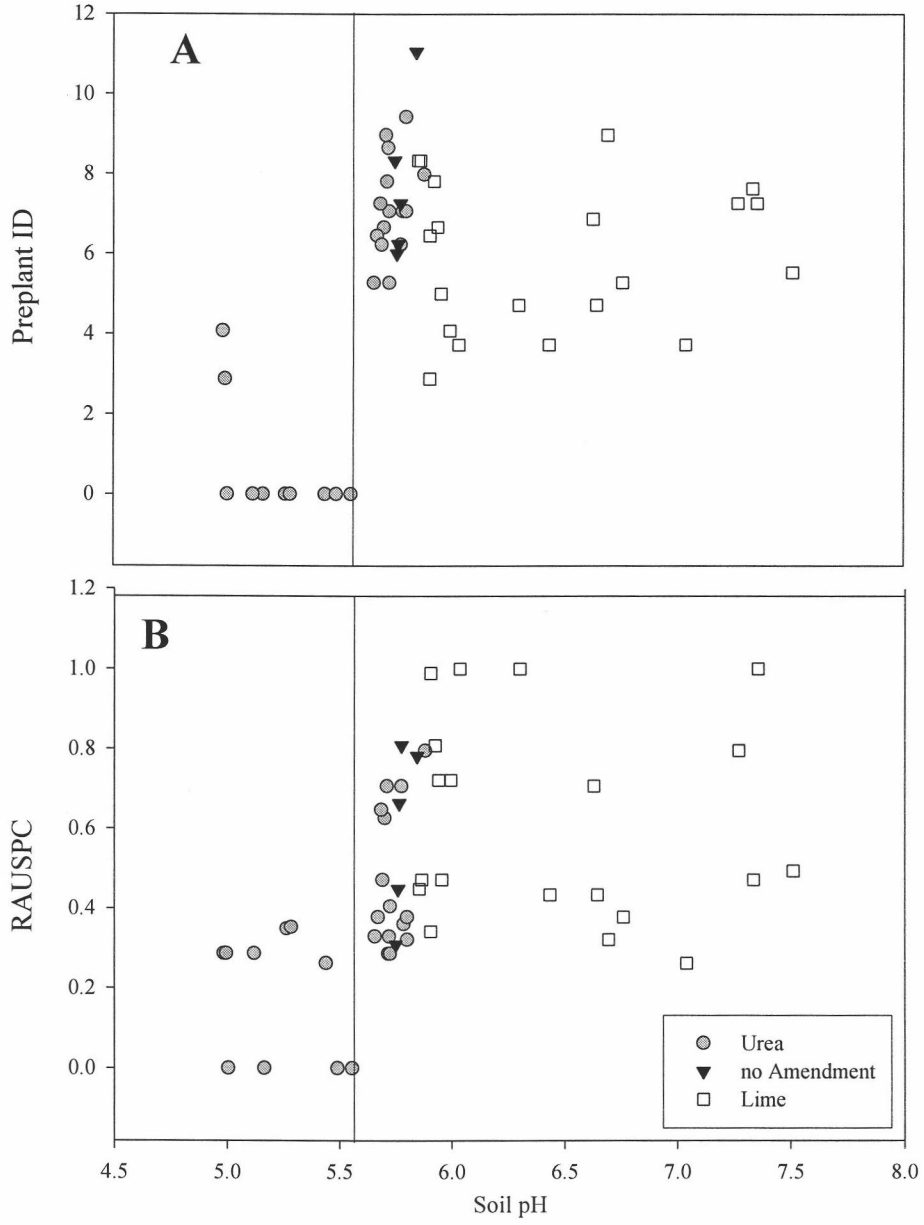

Figure C.1 Scatterplots of soil pH vs. (A) preplant ID and (B) RAUSPC. Data grouped by type of amendment.

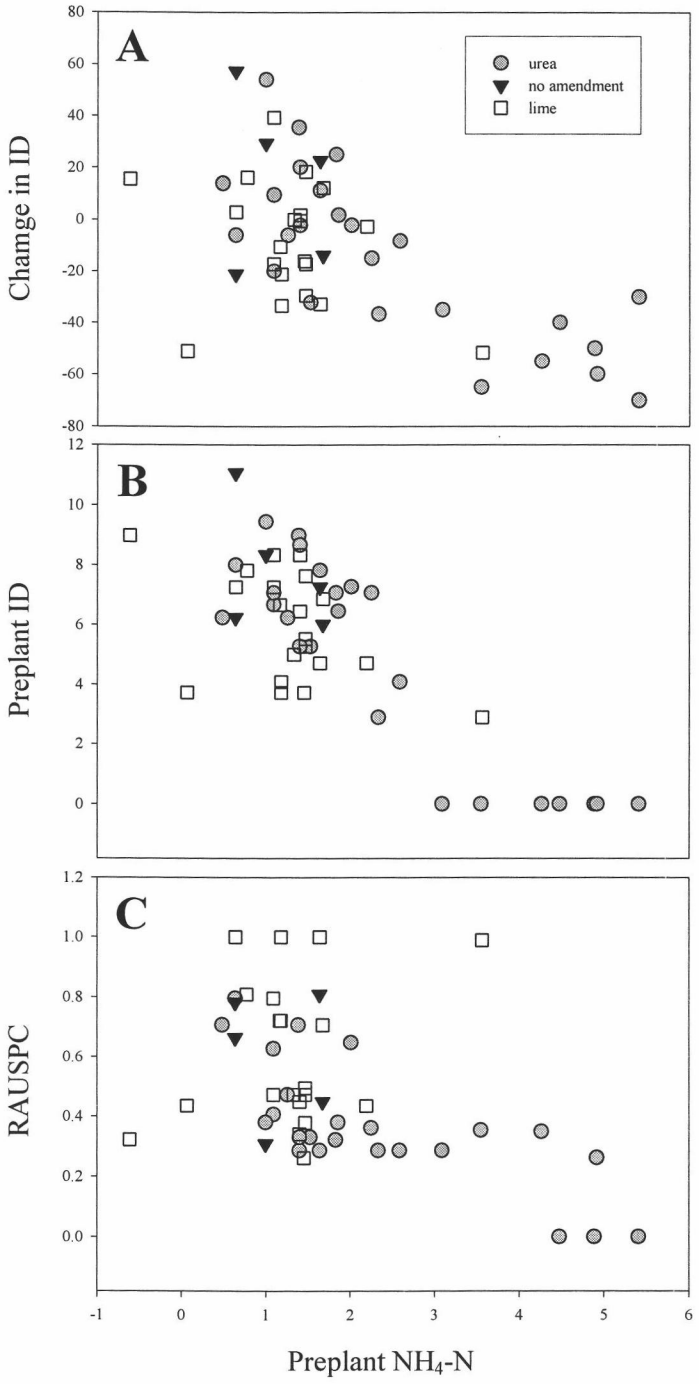

Figure C.2 Scatterplots of preplant NH_4-N vs. (A) change in ID between infestation and planting, (B) preplant ID, and (C) RAUSPC. Data grouped by amendment type

sdfd

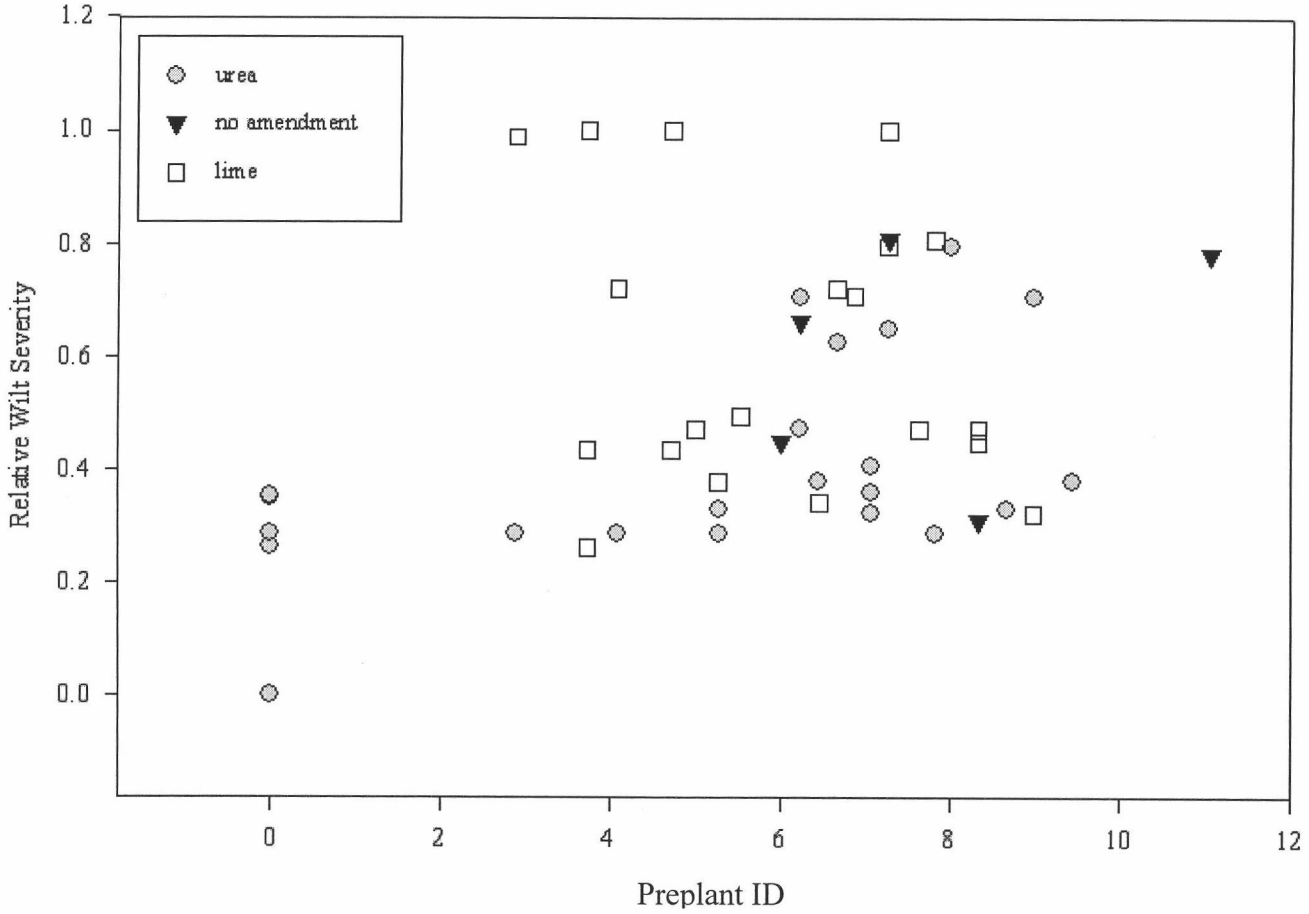

Figure C.3 Scatterplot of prepland soil population of *V.dahliae* vs. Severity of Verticillium wilt. Data grouped by amendment type