Effects of Green Manure Type and Amendment Rate on Verticillium Wilt Severity and Yield of Russet Burbank Potato

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ABSTRACT

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Increasing restriction of agrochemicals is motivating development of ecology-based cropping systems, including green manures, to manage soilborne diseases. Green manures have shown promise in suppressing *Verticillium dahliae*, but information about effect of different green manures and optimal application rates remains limited. Therefore, we conducted two single-year field experiments comparing effects of Austrian winter pea (*Pisum sativum* 'Melrose'), broccoli (*Brassica oleracea* var. *botrytis* 'Excelsior'), and Sudan grass (*Sorghum vulgare* var. *sudanense* 'Monarch'), amended at 6, 12, or 24 Mg ha⁻¹, on soil populations and root infection by *V. dahliae*, wilt severity, and yield of Russet Burbank potato. Inoculum density was reduced relative to the nonamended, infested control by all broccoli treatments, Austrian winter pea applied at 12 and 24 Mg ha⁻¹, and Sudan grass applied at 12 Mg ha⁻¹. Root infection was not reduced by any green manure treatment. Median wilt severity was reduced approximately 70% by all green manures applied at 24 Mg ha⁻¹ and 74% by Austrian winter pea applied at 12 Mg ha⁻¹. Tuber yield was reduced approximately 20% in nonamended, *V. dahliae*-infested controls relative to the noninfested controls. No green manure treatment improved yield relative to the nonamended, infested control.

Additional keywords: cover crop, disease suppression

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major disease of many cultivated plant species, including potato, throughout temperate regions of the world (47). In North America, potato yield reduction due to Verticillium wilt can range from 10 to 50% (37). The long-term persistence of the pathogen in the soil (56) and its wide host range, including some bridging hosts (16), complicate use of crop rotation for managing this disease (39). In North America, periodic reduction of soil populations of *V. dahliae* by soil fumigation is widely practiced as a means of managing this disease (39). In recent years, however, greater regulation of agrochemicals and increasing costs of application are motivating the development of alternative management strategies.

Green manures and other organic matter (OM) amendments have been used to improve soil tilth and fertility since early times (29). Studies conducted in the mid-twentieth century demonstrated the poten-

tial of OM amendments to promote plant health by suppressing diseases caused by soilborne pathogens (20,31,55). Numerous soil amendments have been evaluated for their potential to suppress Verticillium wilt, including animal manures (5), composted materials (27), industrial byproducts (38), and green manures (1,11,15,35). Different amendments have resulted in a range of responses from nearly complete suppression (5) to no effect (35) or increased severity (38), with the majority resulting in partial reduction of disease (1,11,15).

Characteristics of OM that determine their efficacy in suppressing Verticillium wilt and the underlying mechanisms of suppression are not fully understood. From previous research, it appears that at least two modes of suppression exist. Suppression of Verticillium wilt by broccoli residues or animal manures is attributed to reduction of soil populations of *V. dahliae* (5,46). In these cases, it is believed that compounds toxic to *V. dahliae* are released during decomposition of the amendment (28,46,52). For other amendments—such as Sudan grass, Austrian winter pea, sweet corn, or barley residues—there was no association between reduction in inoculum density (ID) and suppression of Verticillium wilt (9,11,12) or the association was not determined (27).

In cases of OM-mediated suppression of other diseases, degree of disease reduction often appears to be related to the amount of material amended (7,8,14,21,33,49). In an in vitro study, Berlinger (1) demonstrated a positive correlation between amendment rate of broccoli residue and reduction of soil populations of *V. dahliae*. In a greenhouse study, Davis et al. (10) observed a negative correlation between the amount of Sudan grass residue amended and severity of Verticillium wilt of potato. The rate effect likely is related to a general suppression, where increased carbon inputs increases microbial activity and soil quality. However, there is limited information about the quantitative relationship between amendment rate and suppression of Verticillium wilt (1,10), particularly at the field scale.

Although the use of green manures to suppress Verticillium wilt shows promise, the phenomenon remains poorly understood and has had inconsistent results. Information is needed on the disease suppressive potential of diverse plant materials that can be grown on site. In addition, although it is suspected that increased amendment rates will result in greater suppression of Verticillium wilt, it is not known to what degree suppression depends on the amount of biomass amended rather than the type of material amended. At the same time, the response of soil properties can provide insights into suppressive mechanisms. Therefore, the objective of this investigation was to evaluate the effect of three green manures amended at three rates on ID, root infection by *V. dahliae*, severity of Verticillium wilt, and tuber yield of Russet Burbank potato.

MATERIALS AND METHODS

Experimental design. Field experiments were conducted in 2002 and 2003 in adjacent fields at the Central Oregon Agricultural Research Center in Madras, OR. The experimental design was a randomized complete block (RCB) with four replications. Treatments comprised a 3-by-3 factorial with three types of green manure amended at three rates into soil infested with *V. dahliae* microsclerotia, a nonamended infested control, and four noninfested controls. The three types of green manure used, representing the three plant families *Leguminosae*, *Brassicaceae*, and

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Graminaceae, 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) Hitchc 'Monarch'), respectively; the three amendment rates were 6, 12, and 24 Mg ha⁻¹ dry biomass; three of the noninfested controls received one each of the green manures at 12 Mg ha⁻¹ and the fourth received no green manure (nonamended). Austrian winter pea is considered a winter cover crop which can improve nitrogen fertility after incorporation. Broccoli is commonly grown as a crop, but the remaining aboveground matter potentially could be plowed down after harvest. Sudan grass is considered a suitable green manure for hot, dry climates, producing a significant amount of biomass. The lowest amendment rate (6 Mg ha⁻¹) used was considered to be a reasonable estimate of dry-matter accumulation for a typical green manure in a single season based on various published reports (17,30).

Infestation and green manure incorporation. The experimental fields, mapped as Madras series sandy loam (superactive, mesic, Aridic Argixeroll), had not been cropped previously to potato or other hosts of *V. dahliae*, and background levels of *V. dahliae*, determined by plating samples of air-dried soil collected from random locations within each field, were low (1.8 and 0.8 CFU g⁻¹ of soil in 2002 and 2003, respectively). Microsclerotia of VCG4 (potato strain) of *V. dahliae* were cultivated on potato dextrose agar and harvested in the manner described by Gaudreault et al. (18). In August of the year preceding potato cultivation, microsclerotia mixed with fine sand were spread by hand on the experimental microplots (3 by 3.7 m) to achieve an approximate density of 25 to 30 CFU g⁻¹ of soil, which is a level expected to cause significant Verticillium wilt severity. Austrian winter pea, broccoli, and Sudan grass were grown for approximately 45 days in large blocks outside of the experimental sites in the summer of the year preceding potato cultivation. In August, at the same time as infestation of soil with *V. dahliae*, the aerial biomass of the three green manures was chopped in the field with a tractor flail, collected, and spread within 2 h on plots at one of three rates (6, 12, or 24 Mg ha⁻¹ dry matter). Both microsclerotia and green manures were immediately disc-incorporated to a depth of 25 to 30 cm.

Cultural practices for potato. In the spring prior to planting, each plot received a base application of N, P₂O₅, and K₂O at 203 kg ha⁻¹ each and S at 89 kg ha⁻¹ in 2002, and N, P₂O₅, and K₂O at 174 kg ha⁻¹ each and S at 189 kg ha⁻¹ in 2003, based on soil tests following amendment. Generation III seed potato (*Solanum tuberosum* 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 throughout the season as necessary.

Soil sampling and analyses. Ten soil cores were taken with a 2.5-cm-diameter soil probe at a depth of 0 to 15 cm approximately 1 month after incorporation of the green manures (27 September 2001 and 25 September 2002), prior to planting potato (23 April 2002 and May 7 2003), and 3 weeks after the emergence of potato plants (25 June 2002 and 16 June 2003). Soil cores from each plot were composited, passed through a 4-mm sieve, and air dried at room temperature or, in the case of microbial analyses, processed within 2 days while the soil was still moist.

Soil pH was measured in a 1:2 (wt/vol) solution of 0.05 M CaCl₂ 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. Total organic carbon (TOC) was analyzed by dry combustion as described by Nelson and Sommers (32).

Fluorescein diacetate (FDA) hydrolysis of soils sampled in summer was assessed colorimetrically using a method modified from Schnürer and Rosswall (42) within 10 h of collection. Microbial respiration (MR) and microbial biomass carbon (MBC) of soils sampled in spring were assessed simultaneously using the chloroform-fumigation-incubation method (23).

Pathogen, disease, and yield assessment. Inoculum density was assessed by plating air-dried soil onto NP10 medium (48) using an Andersen Air sampler (4). 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 CFU per gram of soil.

Two plants were dug from the middle two rows of each plot 3 weeks after emergence and the root ball removed. Root balls were transported in a cooler to Oregon State University and rinsed with distilled water containing a small amount of tergitol to remove soil particles. Fine roots were collected on a number-10 sieve and cut into 100 1-cm sections which were plated onto NP10 medium (48). Plates were incubated in the dark at room temperature for at least 10 days, after which the number of *V. dahliae* colonies emerging from plated root pieces was counted and expressed as CFU per meter of 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 weeks.

At harvest, the middle two rows of each plot were machine excavated. Tubers from the middle 2.1 m (2002) or 2.4 m (2003) of each row were hand harvested, counted, weighed, and expressed as kilograms per hectare.

Statistical analyses. Area under the senescence progress curve (AUSPC) was calculated based on the weekly disease reading (45) and divided by the number of growing degree-days (base = 10°C, maximum = 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 root infection (RI) were square-root transformed and RAUSPC was log-transformed after addition of one [ln(x + 1)] to approximate normal distribution for these variables. Unless otherwise indicated, all statistical analyses were conducted on transformed data and back-transformed numbers 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 noninfested plots and clearly lower than in the infested plots; therefore, analyses of variance of these three response variables were conducted using only data from plots artificially infested with *V. dahliae*. Mixed-model analysis of variance (PROC GLM; SAS release 8.02; SAS Institute, Inc., Cary, NC) was conducted, including main effect terms for experiment, block (nested in experiment), green manure type, amendment rate, and terms for all interactions of experiment, type, and rate. Because there were no significant experiment-treatment interactions for RI or RAUSPC, data from the 2002 and 2003 experiments were pooled for comparison of means. Although there was no significant experiment-treatment interaction for tuber yield, separate analyses of data from 2002 and 2003 experiments was warranted based on significant differences in experimental conditions between studies. Mean separation was conducted using the Tukey-Kramer procedure for (i) amendment rate, averaged across green manures, and (ii) green manure type, averaged across amendment rates (LSMEANS statement in PROC GLM). For the green manure type mean separation, a nonamended infested control was included as a fourth green manure type. Mean separation for individual green manure treatments was conducted using Fisher's protected

least significant difference (MEANS statement in PROC GLM) after confirming significance of the *F* test for difference of means. Correlations of green manure amendment rate, soil chemical and biological properties (soil pH, Ca, K, FDA hydrolysis, MR, and MBC), ID, RAUSPC, and tuber yield were generated using PROC CORR.

RESULTS

Effects of experiment, green manure type, and amendment rate. Inoculum density was, on average, 2.8 CFU g⁻¹ of soil higher in 2003 than 2002 (Table 1). Root infection was, on average, 3.8 CFU m⁻¹ root lower in 2003 than in 2002; however, because there was no significant interaction between experiment and green manure type or amendment rate, statistical analyses were performed on pooled data. Verticillium wilt severity (RAUSPC) was estimated to be 55% higher, on average, in 2003 than in 2002. For response variables ID, RI, and RAUSPC, statistical analyses were performed on pooled data because there was no significant interaction between experiment and green manure type or amendment rate. Mean total tuber yield was approximately 23 kg ha⁻¹ lower in 2003 than in 2002, due to an earlier harvest in 2003. We determined that difference in harvest date constituted a significant difference between experimental conditions, which warranted separate analyses for the 2 years' data.

After accounting for differences between experiments, green manure rate and type had a significant effect on mean ID (Table 1; Fig. 1A and B). Although no significant differences were observed among amendment rates, the intermediate amendment rate (12 Mg ha⁻¹) averaged across all green manure types reduced mean ID by 50% (*P* < 0.01) relative to the nonamended control (Fig. 1A). Although no significant differences were observed among green manure types, broccoli green manures averaged across application rates reduced mean ID by 50% (*P* < 0.01) relative to the nonamended control (Fig. 1B).

Green manure type, but not amendment rate, had an effect on RI (Table 1). No green manure type averaged across application rates reduced RI relative to the nonamended control; however, broccoli green manures averaged across all application rates resulted in 50% (*P* < 0.05) lower RI than the Austrian winter pea green manures (Table 2). Both green manure type and amendment rate had a significant effect on RAUSPC (Table 1; Fig. 1C and D). Although no differences in mean RAUSPC were observed between green manure types, Austrian winter pea green manures averaged across application rates reduced mean disease severity by 61% (*P* < 0.05) relative to the nonamended control (Fig. 1C). The two highest amendment rates (12 and 24 Mg ha⁻¹) reduced disease severity relative to the nonamended control by 60 and 69% (*P* < 0.05 and 0.001), respectively (Fig. 1D). The 24 Mg ha⁻¹ amendment rate resulted in 60% (*P* < 0.01) lower disease severity than the 6 Mg ha⁻¹ rate. Neither green manure type nor amendment rate had a significant effect on mean tuber yield in 2002 or 2003 (Table 1).

Effects of individual green manure treatments. Six of the individual green manure treatments resulted in reduction of ID relative to the nonamended control (Table 3). After accounting for differences between experiments, green manures of Austrian winter pea applied at 12 and 24 Mg ha⁻¹ reduced ID by 35 and 38% (*P* < 0.05 and 0.033), respectively; broccoli applied at 6, 12, and 24 Mg ha⁻¹ by 44, 63, and 44% (*P* < 0.05, 0.001, and 0.05), respectively; and Sudan grass applied at 12 Mg ha⁻¹ by 51% (*P* < 0.01). Green manure of broccoli applied at 12 Mg ha⁻¹ resulted in significantly lower ID than Austrian winter pea applied at 6 Mg ha⁻¹ (*P* < 0.01) or Sudan grass applied at 6 Mg ha⁻¹ (*P* < 0.05) or 24 Mg ha⁻¹ (*P* < 0.05). Green manure of Sudan grass resulted in lower ID than Austrian winter pea applied at 6 Mg ha⁻¹ (*P* < 0.05). Although differences in mean RI were observed between green manure treatments, no green manure

treatment resulted in RI significantly different from that of the nonamended control, which fell near the center of the range of observed RI means (Table 2).

After accounting for differences between experiments compared with the nonamended control, green manure of Austrian winter pea applied at the two higher rates (12 and 24 Mg ha⁻¹) reduced RAUSPC by 74 and 68% (*P* < 0.01 and 0.01), respectively (Fig. 2). In addition, Austrian winter pea applied at 12 Mg ha⁻¹ resulted in lower RAUSPC than all green manure types applied 6 Mg ha⁻¹. Green manure of broccoli or Sudan grass applied at the 24 Mg ha⁻¹ reduced RAUSPC by 69 and 70% (*P* < 0.01), respectively. Broccoli and Sudan grass applied at 24 Mg ha⁻¹ resulted in lower RAUSPC than broccoli or Sudan grass applied at 6 Mg ha⁻¹.

Total tuber yield was significantly lower in 2003 than in 2002, as evidenced by the difference in mean yields of the noninfested controls (Table 4). Among the noninfested controls in either experiment, amendment with green manure had no effect on total tuber yield. For both experiments, mean tuber yield was 20% lower in nonamended plots infested with *V. dahliae* compared with the noninfested control plots. For both experiments, no green manure treatment significantly increased mean yield relative to the nonamended infested control. In 2002, green manures of Austrian winter pea applied at all rates, broccoli applied at 24 Mg ha⁻¹, and Sudan grass applied at 12 or 24 Mg ha⁻¹ resulted in mean yields that were not significantly different from that of the noninfested controls. In 2003, green manures of broccoli applied at 6 Mg ha⁻¹ and Sudan grass applied at 24 Mg ha⁻¹ resulted in mean tuber yields that were not significantly different from that of the noninfested controls.

Correlation of soil properties with ID, Verticillium wilt severity, and yield. Several soil chemical and biological properties were correlated with ID, Verticillium wilt severity, and yield (Table 5). Inoculum density was moderately, positively corre-

Table 1. Effect of experiment, green manure type (Austrian winter pea, broccoli, or Sudan grass), and amendment rate (0, 6, 12, or 24 Mg ha⁻¹) on inoculum density (CFU g⁻¹ soil) and root infection (CFU m⁻¹ root) by *Verticillium dahliae* and Verticillium wilt severity of potato as measured by the relative area under the senescence progress curve (RAUSPC) and tuber yield in 2002 and 2003^a

Source of variation	df	Inoculum density		Root infection		RAUSPC		Yield	
		MS	<i>P</i> > <i>F</i>	MS	<i>P</i> > <i>F</i>	MS	<i>P</i> > <i>F</i>	MS	<i>P</i> > <i>F</i>
Experiment	1	7.666	0.001	11.144	0.003	3.018	0.024	10,984.0	<0.001
Block in experiment	6	1.309	0.073	1.438	0.305	0.583	0.410	608.0	0.342
Treatment									
Amendment rate	3	3.756	0.001	0.105	0.965	3.433	0.001	260.1	0.687
Green manure type	2	2.033	0.049	4.480	0.028	0.317	0.571	27.2	0.950
Rate × type	4	0.330	0.721	1.042	0.475	0.262	0.760	309.1	0.672
Experiment × treatment									
Experiment × rate	3	0.961	0.222	0.101	0.967	0.175	0.816	296.9	0.640
Experiment × type	2	0.592	0.400	2.222	0.159	0.128	0.796	590.0	0.333
Experiment × rate × type	4	1.167	0.135	0.970	0.512	0.493	0.482	48.8	0.984
Pooled error	54	...	...	...	...	...	...	...	...

^a MS = mean square.

lated with calcium ($P < 0.01$) and moderately, negatively correlated with MR ($P < 0.01$) and MBC ($P < 0.05$). Verticillium wilt severity was positively correlated with soil pH ($P < 0.001$) and ID ($P < 0.05$) and negatively correlated with amendment rate ($P < 0.001$), potassium ($P < 0.05$), TOC ($P < 0.01$), FDA hydrolysis ($P < 0.001$), MR ($P < 0.05$), and MBC ($P < 0.05$). Yield was positively correlated with TOC ($P < 0.05$) and FDA ($P < 0.001$) and negatively correlated with soil pH ($P < 0.01$) and wilt severity ($P < 0.001$). TOC, FDA hydrolysis, MR, and MBC were positively correlated with amendment rate. Soil pH, calcium, and potassium were intercorrelated, reflecting a preexisting spatial gradient of these three properties in the field used in 2002. Both measures of microbial activity (FDA hydrolysis and MR) were correlated with MBC and TOC, although they were not correlated with each other.

DISCUSSION

The ultimate goal of using green manures to manage soilborne diseases is to reduce yield losses caused by the pathogens. In this study, no green manure treatment resulted in statistically significant improvement of potato yield compared with the nonamended control. All three

green manure types, however, when applied at the highest rate (24 Mg ha⁻¹) and Austrian winter pea applied at the intermediate rate (12 Mg ha⁻¹) significantly reduced severity of Verticillium wilt relative to the nonamended control. Furthermore, the two highest amendment rates (24 and 12 Mg ha⁻¹), averaged across green ma-

Table 2. Effect of green manure amendments on infection of potato roots by *Verticillium dahliae* (CFU m⁻¹ root)^y

Green manure type	Root infection by <i>V. dahliae</i>				Treatment mean ^z
	Green manure amendment rate (Mg ha ⁻¹)				
	0	6	12	24	
Nonamended control	7.3	...	...	...	7.3 ab
Austrian winter pea	...	8.0	7.4	11.4	8.9 a
Broccoli	...	4.4	5.4	3.7	4.5 b
Sudan grass	...	7.4	7.6	4.8	6.6 ab

^y Pooled data from 2002 and 2003 experiments.

^z Within the treatment means column, means followed by the same letter are not significantly different according to the Tukey-Kramer procedure ($P \leq 0.05$).

Fig. 1. Effect of green manure type, averaged across amendment rates, and green manure amendment rate, averaged across green manure types, on inoculum density of **A** and **B**, *Verticillium dahliae* and **C** and **D**, wilt severity for pooled data from 2002 and 2003 experiments. Within figures, means with different letters indicate significant differences between mean inoculum densities ($P \leq 0.05$) according to the Tukey-Kramer multiple comparison procedure.

nure types, resulted in significant reduction of wilt severity compared with the non-amended control.

The lack of significant impact of green manures on tuber yield even with the highest amendment rates, which is inconsistent with work by others (11,27), is somewhat unexpected and suggests three possible interpretations: that the green manure treatments (i) only impact factors related to disease symptoms and not disease factors related to yield reduction; (ii) reduced wilt symptoms, but not enough to mitigate detrimental effect on yield; or (iii) affected yield, but the magnitude of impact was such that it was not statistically discernible. The former interpretation is not consistent with current understanding of how *V. dahliae* infection causes reduction of tuber yield. Briefly, *V. dahliae* infection is believed to reduce leaf surface area (19) as well as photosynthetic efficiency (2,40) of the plant, which in turn reduces tuber bulking rate and ultimately lowers yield (24). Therefore, treatments which reduce wilt severity theoretically also should increase yield. The latter two interpretations are more consistent with theoretical understanding of the relationship between Verticillium wilt and tuber development, but are difficult to verify.

The negative correlation between disease severity and yield, taken together with the fact that several of the green manure treatments significantly reduced wilt severity, suggest that green manures had an indirect impact on yield. It is worth noting that seven of nine green manure treatments in 2002 resulted in mean yields that were nominally, although not significantly ($P > 0.05$), higher than that of the nonamended infested control. Of these seven treatments, six resulted in mean yields that were lower than, but not significantly different from, that of the noninfested controls. Yield data

from 2003, however, revealed fewer differences in mean yield between the non-amended infested control and green manure treatments. In part, this was due to the earlier harvest in 2003, which occurred in September, approximately 4 weeks earlier than in 2002. As a result, mean yield across all treatments was lower in 2003 than in 2002, reflecting the shorter period of tuber bulking over which differences in bulking rate could be expressed. The lack of clear effect of green manure treatments on yield indicates the need for further investigation of the relationship between suppression of disease and impacts on yield.

Although there was not a statistically significant yield response to green manures, several of the green manure treatments clearly reduced wilt severity. Disease suppression likely resulted in part from reduced soil populations of *V. dahliae*, as evidenced by positive correlation between ID and wilt severity in both 2002 and 2003. Some reduction in soil populations of *V. dahliae* was achieved by all three green manure types, but did not appear to be a function of amendment rate. Based on previous studies (1,26,50,51,57) and knowledge of the biofumigant effect of *Brassica* spp. residues (2,34,41), it is not surprising that broccoli green manures reduced soil populations of *V. dahliae*. Suppression of Verticillium wilt by green manures of Austrian winter pea and Sudan grass, however, have not previously been demonstrated to rely on reduction of soil populations of *V. dahliae* (10,11,36). Although there is speculation that Sudan grass residue also may act as a biofumigant by release of p-hydroxybenzaldehyde

and hydrogen cyanide (HCN) (53), this has not been shown to be relevant for reduction of soil populations of *V. dahliae*. Reduction of ID by Austrian winter pea or Sudan grass green manure was reported by Davis et al. (11), but only after five or four consecutive years, respectively, of incorporation. The slight, negative correlation between MR and biomass with ID observed in our studies support the possibility that reduction of *V. dahliae* soil populations by all three green manure types, including broccoli (13,46), may be due to an increase in general soil microbial populations and activity stimulated by addition of organic material.

Factors other than reduction of *V. dahliae* soil populations also likely contributed to lowering of disease severity. Not all treatments which significantly lowered disease severity resulted in reduced ID and not all treatments which significantly reduced ID resulted in lowered disease severity. The two application rates of Austrian winter pea green manures which reduced disease severity (12 and 24 Mg ha⁻¹) also reduced ID. However, although broccoli applied at all three rates reduced ID, only the highest application rate lowered disease severity. Although green manure of Sudan grass applied at the intermediate rate reduced soil populations of *V. dahliae*, this treatment did not result in reduced wilt severity. Finally, although the highest application rate of Sudan grass green manure reduced wilt severity it did not significantly lower soil ID. These results, along with findings by Davis et al. (11), indicate that reduction of ID is not a necessary condition for lowering of wilt severity.

Table 3. Effect of individual green manure treatments on inoculum density of *Verticillium dahliae* (CFU g⁻¹ soil) using pooled data from 2002 and 2003 experiments

Green manure amendment rate (Mg ha ⁻¹)	Inoculum density
Control	
Nonamended infested	19.8 a
Austrian winter pea	
6	15.8 ab
12	12.8 bcd
24	12.4 bcd
Broccoli	
6	11.1 bcd
12	7.4 d
24	11.2 bcd
Sudan grass	
6	14.7 abc
12	9.6 cd
24	14.1 abc

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P \leq 0.05$).

Fig. 2. Effect of individual green manure treatments on severity of Verticillium wilt of potato, measured as relative area under the senescence property curve (RAUSPC). Means with different letters indicate significant differences between mean RAUSPC ($P \leq 0.05$) according to the Fisher's protected least squared difference procedure.

Disease reduction by green manures was dependent on amendment rate as well as green manure type. When applied at the lowest rate (6 Mg ha⁻¹), none of the green manures significantly reduced disease severity relative to the nonamended infested control. When applied at the highest rate (24 Mg ha⁻¹), however, all green manures reduced disease severity to a similar degree. At the intermediate amendment rate (12 Mg ha⁻¹), only the Austrian winter pea green manure significantly reduced RAUSPC, indicating a difference in efficacy among green manure types. Effect of amendment rate on suppression of *Verticillium* wilt has been reported in only one study (10), although rate dependence of OM-mediated disease suppression has been found for other soilborne pathogens (7,8,14,21,33,49). This rate dependence is consistent with our assumption that amendments alter soil physicochemical (29) or biological properties (43,44), resulting in conditions unfavorable to pathogen survival or activity (6,22). However, due to complex effects that OM amendments have on soil properties and host plants, identification of specific suppres-

sive mechanisms not involving reduction in ID poses a substantial challenge. In 2002, abiotic factors related to soil pH, which were preexisting and not affected by the green manure amendments, significantly contributed to the variability in wilt severity and yield. In both experiments, reduced wilt severity was correlated with increasing amendment rate, TOC, and increasing microbial biomass and activity. These observations are consistent with reports by others (5,11,12,46,54,55) and suggest, although do not prove, that green manure-mediated suppression of *Verticillium* wilt is microbiological in nature.

In summary, these studies demonstrated consistent reduction of *Verticillium* wilt of potato by all three green manure types applied at the highest amendment rate (24 Mg ha⁻¹) and indicated a positive relationship between amendment rate and degree of disease reduction. Austrian winter pea applied at 12 Mg ha⁻¹ also resulted in significant reduction of disease severity, revealing a difference in efficacy among the green manure types. Although several green manure treatments lowered soil populations of *V. dahliae*, reduction of

disease severity was not always linked to reduced ID. Thus, other mechanisms, in addition to inoculum reduction, likely contributed to green manure-mediated reduction of *Verticillium* wilt severity. Significant correlation of disease severity with soil chemical factors related to soil pH in 2002 demonstrated abiotic disease suppression unrelated to green manure amendments. Strong negative correlation of disease severity with microbial biomass and activity measures in 2003 suggests a microbiological nature to green manure-mediated suppression of *Verticillium* wilt. Green manure treatments did not have a clear effect on reducing yield loss in either study.

Critical issues remain which must be addressed before green manures can be considered a viable option for management of *Verticillium* wilt of potato (39). First, green manures must be shown to not only reduce wilt severity but also consistently reduce tuber yield losses. Second, given that the high amendment rates used in these studies (24 Mg ha⁻¹) would be impractical for most growers, it is necessary to develop realistic green manure management practices to achieve the desired disease suppression. This may be accomplished by improving disease suppression efficiency of green manures, which may be related to a number of factors other than rate, including (i) quality or composition of plant material (13,21), (ii) parts of plant incorporated (13), (iii) method of incorporation (25), or (iv) timing of amendment. Alternatively, disease suppressiveness may be developed after successive incorporation of lower rates of green manure over several years, as reported by Davis et al. (11). A clearer understanding of how different green manure factors impact disease suppression over time will enable consistent and optimized use of green manures for management of *Verticillium* wilt.

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Table 4. Effect of individual green manure treatments on total tuber yield in 2002 and 2003

Green manure amendment rate (Mg ha ⁻¹)	Total tuber yield (kg ha ⁻¹) ^y	
	2002	2003
Control		
Nonamended noninfested	178 a	151 a
Amended noninfested ^z	174 a	153 a
Nonamended infested	140 b	122 b
Austrian winter pea		
6	150 ab	120 b
12	154 ab	111 b
24	152 ab	120 b
Broccoli		
6	139 b	134 ab
12	147 b	118 b
24	151 ab	134 ab
Sudan grass		
6	131 b	118 b
12	152 ab	128 b
24	152 ab	118 b

^y Within experiments (year), means followed by the same letter are not significantly different according to Fisher's protected least significant difference ($P \leq 0.05$).

^z Plots not artificially infested with *Verticillium dahliae* but amended with Austrian winter pea, broccoli, or Sudan grass green manure at 12 Mg ha⁻¹.

Table 5. Pearson's correlation coefficients r for green manure amendment rate, selected soil chemical and biological properties, inoculum density of *Verticillium dahliae*, *Verticillium* wilt severity, and tuber yield using pooled data from 2002 and 2003 experiments^z

Variables	Rate	pH	Ca	K	TOC	FDA	MR	MBC	ID	WS
Soil pH	0.10	...	...	...	...	...	...	...	...	...
Ca (ppm)	0.10	0.49****	...	...	...	...	...	...	...	...
K (ppm)	0.23*	-0.36****	-0.30***	...	...	...	...	...	...	...
TOC	0.51****	-0.12	0.00	0.26*	...	...	...	...	...	...
FDA	0.39****	-0.09	0.53****	0.01	0.28*	...	...	...	...	...
MR	0.69****	0.13	-0.19	0.16	0.52****	0.07	...	...	...	...
MBC	0.46****	0.15	0.07	-0.03	0.41****	0.32****	0.72****	...	...	...
ID	-0.18	0.02	0.29***	-0.04	-0.15	0.20	-0.38****	-0.28*	...	...
WS	-0.42****	0.43****	0.22	-0.26*	-0.40****	-0.40****	-0.27*	-0.27*	0.28*	...
Tuber yield	0.13	-0.36***	0.05	0.13	0.28*	0.46****	0.04	0.20	0.02	-0.53****

^z Variables = Soil property, pathogen, disease, and yield; Rate = green manure amendment rate; TOC = total organic carbon; FDA = fluorescein diacetate hydrolysis; MR = microbial respiration; MBC = microbial biomass carbon; ID = inoculum density; WS = Wilt severity; and *, **, ***, and **** indicate significance at $P \leq 0.05$, 0.01, 0.001, and 0.0001, respectively.

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