Increase in Populations of *Rhizoctonia solani* and Wirestem of Collard with Velvet Bean Cover Crop Mulch

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ABSTRACT

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Velvet bean has been used traditionally as a summer cover crop in the southeastern United States. We investigated the use of killed velvet bean as a cover crop mulch left on the soil surface before collard was transplanted in the fall. Control treatments were weed-free fallow and velvet bean that was killed and disked into the soil before transplanting. Incidence of wirestem, caused by *Rhizoctonia solani*, reached a maximum of 25% in 2000 but only 4% in 2001 in cover crop mulch treatments. Nevertheless, in both years, the infection rate, area under the disease progress curve, and final incidence were significantly greater with cover crop mulch than in the fallow or disked treatments. Wirestem incidence did not differ between the disked and fallow treatments in either year. Populations of *R. solani* in soil were greater after cover crop mulch than in fallow plots in both years and greater in the disked treatment than in fallow soil in 2000 but not 2001. Velvet bean does not appear to be suitable as an organic mulch for fall collard production, but could be used as a summer cover crop if disked into the soil before transplanting collard.

Additional keywords: *Brassica oleracea* var. *acephala*, *Mucuna pruriens* var. *utilis*, *Pythium* spp.

Traditional nonchemical methods for managing soilborne pathogens are crop rotation and cover cropping. Velvet bean, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight), and several related *Mucuna* spp. are used as cover crops in sustainable agricultural systems in tropical regions, because they are valued for their ability to improve soil and suppress weeds (5,6). Weed suppression may be due to allelopathic properties (7). Because velvet bean also has been shown to reduce populations of plant-parasitic nematodes by over 85%, it is grown in rotation with vegetable crops in the Caribbean region to alleviate nematode problems (18). Velvet bean, however, like many other legumes, is susceptible to the soilborne pathogen *Macrophomina*

phaseolina, which was reported to reduce stands in Nigeria during the rainy season (5).

Increases in soilborne pathogens are potentially the most detrimental side effects of using legume cover crops in vegetable production systems. In a recent study in Georgia, legume cover crops killed with herbicide and incorporated into soil increased populations of *Rhizoctonia solani* Kühn and *Pythium irregulare*, severity of belly rot of cucumber fruit, and root rot on snap bean (25). Likewise, residue from a cowpea cover crop incorporated into soil reduced broccoli stand in Oklahoma (20). Populations of *R. solani* increased after incorporation of a spring snap bean crop in Maryland (17). However, in California, no increases in damping-off fungi or other pathogens were detected in soil planted to broccoli grown after the nonlegume cover crops phacelia (*Phacelia tanacetifolia*) and rye (30).

The soilborne fungal pathogen *R. solani* causes post-emergence damping-off known as wirestem on cole crops (*Brassica oleracea* L. varieties) (8,24). Wirestem is characterized by dark lesions of varying depth and breadth on the hypocotyl at or just above the soil line. When conditions are conducive for wirestem, the entire stem cortex decays, leaving only the thin, brittle

vascular cylinder intact (8). Cole crop plants, particularly heading cultivars such as cabbage and broccoli, that are slightly affected with wirestem, may survive and grow normally, but more severely affected plants are stunted and have a reduced yield (8,14,19).

To overcome the problems with incorporating cover crops (either green or killed), we investigated the use of cover crops as organic mulches left on the soil surface. Organic mulches have been used to reduce soil erosion and as a low-cost, biodegradable alternative to polyethylene mulches (1,2). We hypothesized that leaving killed cover crop residue on the soil surface would not increase levels of soil-borne fungal pathogens, while the beneficial effects of crop rotation, organic nitrogen inputs, and weed suppression would be realized. The objective of this study was to determine the effects of a killed velvet bean cover crop, either incorporated into soil or left on the soil surface, on growth of collard and populations of the soilborne pathogens *R. solani* and *Pythium* spp.

MATERIALS AND METHODS

Field plot establishment. Experiments were conducted at the Clemson University Coastal Research and Education Center, Charleston, SC. The soil was a Dupont very fine sandy loam (an Aquult) with pH 6.4 in 2000 and pH 6.9 in 2001. The experimental design was a 3-by-3 Latin square. The three cover crop treatments were weed-free fallow, a velvet bean cover crop that was disked into the soil, and a velvet bean cover crop that was killed with herbicide and left in place on the soil surface as an organic mulch to suppress weed growth.

In June 2000 and 2001, the fields were disked twice and raised beds were formed. Velvet bean was seeded in six of the nine plots on 27 June 2000 at 140 kg/ha in double rows spaced 0.38 m apart on 0.9-m-wide beds on 1.8-m centers. On 9 July 2001, velvet bean was seeded at 140 kg/ha in single rows on 0.45-m-wide beds on 0.9-m centers. The three remaining plots were left fallow. Metolachlor (Dual 8E) was applied to the entire field at 2.2 kg a.i./ha on 28 June 2000 and 9 July 2001.

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Overhead irrigation was applied so that plants received a total of approximately 2.5 cm of water per week from rainfall or irrigation. In 2001, benomyl (Benlate 50DF, 0.28 kg a.i./ha) was applied twice and chlorothalonil (Bravo Weather Stik, 2.5 kg a.i./ha) was applied once to control *Cercospora* leaf spot. On 15 August 2001, 224 kg of 10-10-10 N-P-K was applied per hectare to each plot to stimulate growth of velvet bean. Paraquat (Gramoxone Extra, 1.05 kg a.i./ha) was sprayed over the top of the velvet bean on 24 August and 7 September 2000 and 13 September 2001. Velvet bean was mowed on 1 September 2000 and 17 September 2001. Fallow plots and half of the velvet bean plots were disked three times, while the remaining three plots with killed velvet bean residue were not disturbed. On 27 September 2000 and 2001, raised beds were formed in the six freshly disked plots as described above, and metolachlor (Dual 8E) was applied at 2.2 kg a.i./ha. In 2000, 673 kg of 5-10-10 N-P-K per hectare was banded in the rows before beds were shaped. Because fertilizer was applied in August 2001, additional fertilizer was not applied before transplanting. Beds were not reshaped in the plots with killed velvet bean mulch.

Collard (*Brassica oleracea* var. *acephala* 'Champion') was seeded in a greenhouse on 28 August 2000 and 4 September 2001 in Metro-Mix 350 (50 to 60% vermiculite, 25 to 40% peat moss, 9 to 19% bark ash; Scotts-Sierra Horticultural Products Company, Marysville, OH). Seedlings were transplanted to the field on 28 September 2000 and 1 October 2001. In both years, plots were six rows wide and 30.4 m long. Row spacing was the same as described for velvet bean plots. Plots were surrounded by fallow borders 3.1 and 1.8 m wide along the width and length of plots, respectively. In 2000, two rows of plants spaced 0.38 m apart were placed on each 0.9-m-wide bed. In 2001, one row of collard plants was placed per 0.45-m-wide bed. Planting holes were made with a standard planting wheel in the fallow and disked plots, but were made by hand with a sharpened stick in the cover crop mulch plots. Plants were spaced 0.30 m apart within rows. Each year, 224 kg of 10-10-10 N-P-K was applied per hectare as a side dressing 3 and 6 weeks after transplanting.

Disease ratings and yields. A 15.2-m section in each of two rows in each plot (50 plants per row) was marked for determining the number of plants that showed symptoms of wirestem over time. The number of asymptomatic, symptomatic, and dead plants was determined six times in 2000 and eight times in 2001, beginning 4 days after transplanting. Plants were considered diseased if a purplish-black, sunken lesion was visible on the hypocotyl at the soil line or if leaves were wilted and the plant was stunted (13). Plants were considered dead when all leaves had se-

necsed. Wirestem incidence was calculated as the percentage of plants rated as diseased or dead. Area under the disease progress curve (AUDPC) was calculated from disease incidence data using a standard iterative method (21).

On 25 October 2000 and 13 November 2001, 10 plants in each of the rated sections were chosen using a random number scheme, dug, and washed. The lower stem and roots were rated for wirestem severity on a scale of 1 (symptomless) to 10 (dead and decayed) (13). Portions of necrotic lesions on stems of living plants were cut into 0.5-cm pieces, surface disinfested in 0.5% sodium hypochlorite for 1 min, and placed on 2% water agar amended with rifampicin at 10 mg/liter, ampicillin at 250 mg/liter, and the miticide fenpropathrin (Danitol; Valent Chemical Co., Walnut Creek, CA) at 0.5 µl/liter (12). Isolations were made from a total of 19 and 31 plants in 2000 and 2001, respectively. Plates were held at ambient temperature (23 to 25°C) and 16-h photoperiod. Fungi growing from the stem pieces were identified after 3 days of incubation. Hyphae of *Rhizoctonia* isolates were stained in situ with 3% KOH and alkaline safranin O and examined at ×200 to ×400 to determine the number of nuclei per cell (4).

Collards were harvested on 20 and 22 November 2000 and 20 and 27 November 2001. On the first harvest date, plants were cut from representative 3.1-m sections in each of two of the four center rows of each plot. On the second harvest date, plants were cut from the remaining two center rows. Plants were separated into marketable (≥20 cm tall) and unmarketable classes, after which marketable plants were weighed.

Pathogenicity tests. Two pathogenicity tests were performed with five isolates each of *Pythium* spp. and *R. solani* obtained from diseased collard plants in 2001. For each test, cornmeal sand medium (CMS) was prepared in 250-ml flasks with 100 g of fine sand, 3 g of yellow cornmeal, and 20 ml of deionized water (24). The CMS was sterilized by autoclaving for 1 h on 3 consecutive days at 125°C and 1 kg/cm² pressure. Each flask of CMS was seeded with five 0.3-cm agar plugs of an isolate cut from 7-day-old cultures growing on potato dextrose agar. The CMS was incubated at 24°C in the dark for 7 days. On alternating days, the flasks were shaken gently to mix the CMS and maximize fungal growth. Colonization of the CMS was verified by plating dilutions of 1.0 g of CMS in 9.0 ml of sterile deionized water on one-quarter-strength potato dextrose agar. On 13 December 2001 and 30 January 2002, 2-week-old collard plants cv. Champion were transplanted into a mixture of 1% vol/vol CMS and soilless mix (Metro-Mix 350, 50 to 60% vermiculite, 25 to 40% peat moss, 9 to 19% bark ash; Scotts-Sierra Horticultural

Products Company). CMS without fungi was used as the noninoculated control. There were four replications with eight plants per replication for each isolate in each test. Plants were evaluated for the presence of hypocotyl lesions on 20 and 28 December 2001 and 6 and 19 February 2002.

Recovery of *R. solani* and *Pythium* from soil. To estimate the population density of *R. solani* in soil, 10 2.5-cm-diameter soil cores were collected from each plot on 6 October and 3 November 2000 and 23 August, 25 September, 18 October, and 9 November 2001. Soil samples also were collected from the fallow and cover crop mulch treatments on 7 August and 22 September 2000. Cores were separated into 0- to 5- and 5- to 10-cm depths (17). Soil samples from each plot and depth combination were thoroughly mixed. Organic matter was recovered from 400 g of soil by wet sieving through a #18 (1-mm openings) mesh sieve and dried at ambient temperature (23 to 25°C) overnight. Dried organic matter from each sample was placed in 10 to 12 small heaps of equivalent size (27) on ethanol-potassium nitrate agar (26), prepared with 2% ethanol (28). After 1 and 2 days in the dark at 23 to 25°C, the total number of heaps with and without colonies of *R. solani* growing from them was recorded. The number of individual colonies of *R. solani* per heap also was recorded (25). Hyphae of selected colonies were stained in situ to determine the number of nuclei per cell as described previously.

To estimate the population density of *Pythium* spp. in soil, 10 additional soil cores were collected from each plot on 3 November 2000 and 23 August, 25 September, 18 October, and 9 November 2001. In 2000, cores were separated into 0- to 5-, 5- to 10-, and 10- to 15-cm depths, but in 2001, cores were not separated by depth. Soil from each depth in each plot was thoroughly mixed; then, 10.0 g of soil was added to 200 ml of 0.25% agar and stirred on a magnetic stir plate for 2 min. Five aliquots (0.5 ml each) were spread on plates of pimarin-ampicillin-rifampicin-pentachloronitrobenzene (PCNB) medium prepared with pimarin at 5 mg/liter (9). Plates were incubated at 23°C for 20 h; then, adhering soil was washed away and colonies of *Pythium* were counted at ×30 magnification.

Statistical analysis. Analysis of variance for a Latin square experimental design was performed with PROC GLM of SAS (version 6.12; SAS Institute, Inc., Cary, NC). In preliminary analyses, data were tested for homogeneity of variance and normality. The only variable that needed transformation was CFU of *R. solani*, which was transformed by square root. The Waller-Duncan *k*-ratio *t* test was used to compare treatment means. Repeated measures analysis was used to ex-

amine the effects of sampling time, treatment, and depth on populations of *R. solani* recovered from soil (16). Percentages of organic matter heaps colonized by *R. solani* were weighted by the inverse of the total number of heaps plated per plot (23). Weighted least-squares means at individual sampling times were compared using *t* tests. Disease progress curves and curves for recovery of *R. solani* over time were fit with regression equations that tested common versus separate intercepts and slopes for each treatment (3). Goodness of fit of models was checked with lack of fit tests and plots of residuals versus predicted values (3). Intercepts and slopes were compared among treatments with single-degree-of-freedom contrasts.

RESULTS

Due to an unidentified viral disease that stunted the growth of velvet bean in 2000, velvet bean in a second field was mowed on 14 September and distributed uniformly prior to disking in the plots planted to velvet bean in the experimental field. In 2001, velvet bean was affected with *Cercospora* leaf spot that reduced growth in the experimental field and with angular leaf spot (*Phaeoisariopsis griseola*) in a second field that had to be abandoned as a replicate experimental site. Consequently, residue in the cover crop mulch plots was approximately twice as thick in 2000 as in 2001. In 2000, residue at transplanting time completely covered the soil surface; in 2001, approximately 70% of the soil surface was covered at transplanting, but <40% was covered at harvest after velvet bean leaf residue decayed. In 2000, hyphae of *Rhizoctonia* were visible growing on cover crop residue around the stems of collard plants with wirestem lesions.

Disease progression and yield. In 2000, wirestem progressed as a typical simple-interest disease and plateaued at 25% incidence of symptomatic plants in the cover crop mulch treatment (Fig. 1). Wirestem in the other two treatments increased linearly, reaching less than 5% incidence at 27 days after transplanting. In 2001, overall disease levels were lower and disease increased linearly in all three treatments, reaching a maximum of 4% in the cover crop mulch treatment. In both years, the infection rate, AUDPC, and final wirestem incidence were greater in the cover crop mulch treatment than in the treatment left fallow or in which velvet bean was disked before transplanting (Table 1). The infection rate, AUDPC, and final incidence did not differ significantly between disked and fallow treatments in either year.

In both years, a linear model of the form $y = b_0 + b_1x$, where y = wirestem incidence (%), x = days after transplanting, and t = treatment, with a common intercept (b_0) but separate slopes for each treatment (b_1), gave the best fit to the disease progress

curves. A quadratic term for time was not needed (F for lack of fit tests not significant, $P = 0.075$ in 2000 and $P = 0.77$ in 2001). Slopes for all three treatments in both years were significantly different from 0 (t tests, $P \leq 0.05$ in 2000 and $P \leq 0.01$ in 2001). In 2000, wirestem incidence in the cover crop mulch treatment increased by 1 plant per 100 each day; whereas, in the other two treatments, the infection rate was only 1 in 100 plants every 5 days (Table 1). In 2001, the infection rate again was greater in the cover crop mulch than the other two

treatments. However, the infection rates in all treatments were only 1/10 of the rates in 2000 (Table 1).

In 2000, 135 stems on 180 plants dug from the field 4 weeks after transplanting were symptomless (75%) and 26 plants were dead and decayed (14%). In 2001, 147 of the 180 plants were symptomless (82%) and only two were dead (1.1%). *R. solani* and *Pythium* spp. were isolated from living plants showing symptoms of wirestem in both years (Table 2). In 2000, *R. solani* was isolated, either alone or to-

Fig. 1. Wirestem progress curves for collard grown in fall 2000 and 2001 in plots left fallow or previously cropped to velvet bean that was either killed and left on the soil surface as mulch or disked into the soil. Each data point is the mean of three replications and error bars show one standard error of each mean.

Table 1. Infection rates, areas under disease progress curves (AUDPC), and final disease incidences for wirestem on collard grown with or without a velvet bean cover crop in fall 2000 and 2001^x

Year, treatment	Infection rate ^y	AUDPC ^z	Final wirestem incidence (%) ^z
2000			
Fallow	0.18 b	49.5 b	4.1 b
Disked cover crop	0.20 b	66.3 b	3.3 b
Cover crop mulch	1.08 a	384.2 a	24.6 a
2001			
Fallow	0.029 b	7.9 b	0.7 b
Disked cover crop	0.023 b	3.8 b	1.0 b
Cover crop mulch	0.109 a	67.5 a	4.1 a

^x Plots were planted to velvet bean or left fallow during the summer, then velvet bean was killed with herbicide and disked into the soil or left on the soil surface as killed cover crop mulch, before collard was transplanted.

^y Infection rates (b_{1t}) are the slope of the linear equations $y = -0.52 + b_{1t}x$ for 2000 and $y = -0.29 + b_{1t}x$ for 2001, where y = wirestem incidence (%), x = days after transplanting, and t = treatment. Infection rates followed by the same letter within years do not differ significantly, based on single-degree-of-freedom contrasts, $P \leq 0.0001$.

^z Means followed by the same letter within years do not differ significantly according to the Waller-Duncan k -ratio t test, $k = 500$ and 100 in 2000 and 2001, respectively (approximates $P = 0.01$ and 0.05 , respectively).

Table 2. Number of living collard plants from which *Rhizoctonia solani* and *Pythium* spp. were recovered 4 weeks after transplanting^z

Year	<i>Pythium</i> spp. alone	<i>R. solani</i> alone	<i>Pythium</i> spp. and <i>R. solani</i>	Neither
2000	4	6	5	4
2001	8	9	0	14
Total	12	15	5	18

^z Portions of necrotic lesions on stems of living plants ($n = 19$ in 2000 and $n = 31$ in 2001) were surface disinfested and cultured on water agar plus antibiotics. Fungi growing from the tissues were identified after 3 days of incubation. All isolates identified as *R. solani*, 11 in 2000 and 9 in 2001, were multinucleate.

gether with *Pythium* spp., from 58% of the plants cultured ($n = 19$); in 2001, *R. solani* was isolated from 29% of the plants cultured ($n = 31$). All isolates identified as *R. solani*, 11 in 2000 and 9 in 2001, were multinucleate. In addition, one binucleate isolate (*Rhizoctonia* sp.) was recovered along with *R. solani* and *Pythium* spp. from one plant in 2000. When the mean percentage of dead plants and plants from which *R. solani* was isolated was calculated, 42 ± 12 (standard error), 7.6 ± 4.4 , and $3.3 \pm 1.7\%$ of the plants were infected by *R. solani* in the cover crop mulch, disked, and fallow treatments, respectively, in 2000. In 2001, the corresponding values were 8.3 ± 4.5 , 8.3 ± 4.5 , and $1.7 \pm 1.7\%$, respectively. The percentage of infected plants in the cover crop mulch treatment was significantly greater than in the other two treatments in 2000 (Waller-Duncan k -ratio t test, $k = 100$) but not in 2001.

Five isolates of *R. solani* and five isolates of *Pythium* recovered from diseased plants in 2001 were tested for pathogenicity to collard plants. Two of the *R. solani* isolates were pathogenic, with mean disease incidences across two tests of 32.1 and 62.7%; the other three isolates were nonpathogenic at the level of inoculum (1% vol/vol) tested. Four of the *Pythium* isolates caused no disease; one isolate affected one plant (3.1% disease incidence) in only one of the two tests.

Numbers of marketable-sized collard plants were significantly lower in the cover crop mulch treatment than in the disked or fallow treatments in 2000 (Table 3). Per plant weight in the cover crop mulch treatment was almost 0.1 kg (or 16%) greater than in the other two treatments; hence, overall marketable weights did not differ among the three treatments. In 2001, there were no significant differences in yields among the three treatments (Table 3).

Populations of *R. solani* and *Pythium* spp. At the 28 September 2001 sampling, nuclei of one to eight *Rhizoctonia* colonies from each plot and depth were stained and examined. All 60 colonies examined were multinucleate (i.e., were *R. solani*).

In both years, populations of *R. solani*, measured as the percentage of organic matter heaps colonized by the fungus, were significantly greater in the cover crop mulch plots than in the fallow plots (Table 4; Fig. 2). There was no significant effect of depth and no significant interactions among treatment, depth, or time in either year (Table 4). In 2000, the percentage of organic matter heaps colonized by *R. solani* did not change significantly over time, but it was consistently higher in the cover crop mulch and disked treatments than in the fallow treatment at each sampling (treatment F significant at $P = 0.04$, 0.02, 0.003, and 0.02 for the four samplings). Averaged across the two soil samplings

taken after collard was transplanted, 76 ± 6 (standard error), 72 ± 6 , and $38 \pm 4\%$ of the organic matter heaps had at least one colony of *R. solani* growing from them in the cover crop mulch, disked, and fallow treatments, respectively. In 2001, the percentage of organic matter heaps colonized by *R. solani* increased significantly between the first and third samplings for all treatments, then decreased at the last sampling (Table 4, Fig. 2). Differences among treatments were most pronounced at the last sampling (treatment F significant at $P = 0.01$), when the percentage of organic matter heaps colonized in the cover crop mulch treatment ($81 \pm 6\%$) was greater than in the fallow ($50 \pm 4\%$) or disked ($59 \pm 5\%$) treatments.

Population density of *R. solani* also was estimated by counting the number of CFU associated with heaps of organic matter placed on semiselective agar. In both years, there was a significant time-treatment interaction with repeated measures analysis of variance (Table 4). In 2000, there was no significant change in CFU recovered over time (slopes not significantly different from 0), but intercepts (means calculated across replications, depths, and times) for the cover crop mulch and fallow treatments differed significantly from each other (treatment F significant at $P = 0.0001$) (Fig. 3). At the final sampling, there were 61.9 and 50.9 CFU of *R. solani* per 400 g

Table 3. Mean marketable yields of collard grown with or without a velvet bean cover crop in fall 2000 and 2001^y

Treatment	2000			2001		
	Number/3.1 m	Weight (kg/3.1 m)	Weight/plant (kg)	Number/3.1 m	Weight (kg/3.1 m)	Weight/plant (kg)
Fallow	9.1	4.64	0.51	5.3	1.66	0.26
Disked cover crop	9.2	4.98	0.55	6.2	2.26	0.32
Cover crop mulch	7.5	4.69	0.63	7.0	1.85	0.25
MSD ^z	1.3	NS	0.06	NS	NS	NS

^y Plants ≥ 20 cm tall were considered marketable. Plants were harvested from one 3.1-m section in each of two rows per plot on 20 and 22 November 2000 and 20 and 27 November 2001. NS = not significantly different.

^z MSD = minimum significant difference, Waller-Duncan k -ratio t test, $k = 100$ (approximates $P = 0.05$).

Table 4. Repeated measures analysis of variance for two measurements of populations of *Rhizoctonia solani* on organic matter recovered from soil with or without a velvet bean cover crop in 2000 and 2001

Source ^w	df	2000				2001			
		CFU/400 g of soil ^a		Organic matter (%) ^y		CFU/400 g of soil ^a		Organic matter (%) ^y	
		SS ^x	Pr > F	SS ^x	Pr > F	SS ^x	Pr > F	SS ^x	Pr > F
Treatment (Tmt)	1 ^y	19967.52	0.01	0.0440	0.005	2 ^z	94.31	0.001	0.0068
Depth	1	3451.02	0.13	0.00001	0.94	1	36.94	0.006	0.0017
Tmt-Depth	1	1291.69	0.30	0.00006	0.84	2	16.02	0.11	0.0009
Error	4	3724.67	...	0.0057	...	8	21.47	...	0.0052
Time	3	3321.89	0.07	0.0052	0.19	3	260.19	0.0001	0.0461
Time-Tmt	3	3800.40	0.05	0.0026	0.38	6	58.30	0.01	0.0043
Time-Depth	3	2561.56	0.11	0.0025	0.38	3	15.07	0.12	0.0016
Time-Tmt-Depth	3	1692.23	0.20	0.0068	0.14	6	18.57	0.26	0.0024
Error (time)	12	3346.33	...	0.0096	...	24	50.54	...	0.0104

^a CFU of *R. solani* transformed by square root before analysis.

^y Percentage of organic matter heaps colonized by *R. solani* was weighted by the inverse of the total number of heaps plated per plot averaged over all four samplings; hence, the sums of squares are much lower than for CFU of *R. solani*.

^w Row and column variables for the Latin square experimental design were included in the models, but are not shown here.

^x SS = type III sums of squares.

^y Fallow and cover crop mulch treatments were sampled four times between August to November; disked plots were sampled only in October and November and, therefore, could not be included in repeated measures analysis of variance.

^z All three treatments were sampled four times between August and November.

of soil averaged over 0 to 10 cm in the cover crop mulch and disked treatments, respectively, but only 6.9 CFU in the fallow treatment. In 2001, CFU averaged over the 0- to 5- and the 5- to 10-cm samples in the cover crop mulch treatment were greater than in the fallow and disked treatments (Fig. 3), and both intercepts and slopes differed among treatments (F values significant at $P \leq 0.0006$). In 2001, but not 2000, there was an overall significant effect of depth (Table 4), because more CFU were recovered at 0 to 5 cm than at 5 to 10 cm (Fig. 3).

In 2000, *Pythium* populations in soil were sampled only on 6 November, approximately 2 months after the velvet bean was killed. Population densities of *Pythium* spp. were slightly greater ($P = 0.05$) in the cover crop and disked treatments (both 1.3×10^3 CFU/g soil) than in the fallow treatment (0.89×10^3 CFU/g soil). There were no significant differences between 0- to 5-, 5- to 10-, or 10- to 15-cm depths. In 2001, populations of *Pythium* spp. increased quadratically in all three treatments over the four sampling times, but there were no significant differences among treatments (*data not shown*).

DISCUSSION

Killed velvet bean residue used as organic mulch increased wirestem incidence on collard over weed-free fallow. The percentage of plants infected by *R. solani* was greater with cover crop mulch than in the disked and fallow treatments and was greater in 2000 than in 2001. A similar disease progress curve, an asymptotic growth curve typical of a monocyclic pathogen and disease (16), was seen in the cover crop mulch treatment both years, although the asymptote was about six times greater in 2000 than in 2001. One difference between the 2 years of this study was that the killed velvet bean mulch layer was thicker in 2000, when it was supplemented with velvet bean residue from a second field, than in 2001. Virulence of *R. solani* increases as the concentration of nitrogen in the soil environment increases (29). The larger amount of velvet bean biomass in 2000, particularly the succulent leaves, may have increased the amount of nitrogen available to *R. solani* at and near the soil surface, directly in the infection court of the collard hypocotyls. In addition, a thicker layer of mulch covered more of the hypocotyls, effectively increasing the size of the infection court. Similarly, residue from a summer cover crop of another legume, cowpea, significantly reduced stands of fall-transplanted broccoli in two of four experiments in Oklahoma (20). Stand loss was not related to herbicide or nitrogen applications also examined in that study. Contrary to these results, soybean or soybean plus millet used as killed cover crop mulches did not reduce yield of broccoli in Maryland, al-

though data on the survival of transplants was not reported (1).

The effect of using velvet bean as a cover crop when it was disked before transplanting collard varied between the two experiments. In 2000, with a high amount of biomass, velvet bean residue incorporated into the soil increased populations of *R. solani* compared with no velvet bean and the increase was as great as when the residue was left on the soil surface. With lower biomass in 2001,

populations of *R. solani* were not higher with incorporated velvet bean residue than without velvet bean in the fallow treatment. In both years, despite the higher level of *R. solani* in soil in 2000, wirestem incidence in the disked treatment was as low as in the fallow treatment. Apparently, few propagules of *R. solani* were in or near the infection court after disked the cover crop into the soil. Therefore, velvet bean could be used as a summer cover crop for fall-transplanted collard, provided it is

Fig. 2. Percentage of soil organic matter recovered from 400 g of soil and placed in heaps on semiselective agar that was colonized by *Rhizoctonia solani*. Soil was collected to 10-cm depth in plots left fallow or cropped to velvet bean that was either killed and left on the soil surface as mulch or disked into the soil in summer and fall 2000 and 2001. Each data point is the mean of six replicate samples. Error bars show one standard error of the mean.

Fig. 3. Mean number of CFU of *Rhizoctonia solani* associated with soil organic matter in 400 g of soil recovered from 0- to 5-cm and 5- to 10-cm depths in plots left fallow or cropped to velvet bean that was either killed and left on the soil surface as mulch or disked into the soil in summer and fall 2000 and 2001. Each data point is the mean of three replications. Error bars show one standard error of the mean.

disked into soil several weeks before transplanting. Tillage has been shown to reduce vegetable diseases caused by *R. solani*, such as belly rot on cucumber (15).

Levels of *R. solani*, both as percentages of organic matter heaps colonized and as CFU, increased over time in 2001 in all treatments, including fallow, but not in 2000. However, in 2000, the percentage of organic matter heaps colonized at the first sampling was already relatively high (55%) in the velvet bean treatment, twice as high as the initial level in 2001 (28.5%). Perhaps this level was close to the maximum amount of organic matter available for *R. solani* to occupy, because the percentage of organic matter heaps colonized by *R. solani* in the cover crop mulch treatment was essentially identical at the end of the sampling period each year in two different fields, 76% in 2000 and 80% in 2001. This final level of colonization of organic matter is higher than levels reported previously: 53% of organic matter recovered from soil in South Carolina (11) and 44% of beet seed used as a soil bait in Maryland (17).

Pythium spp. were not important pathogens in this cover crop system. There was little response of *Pythium* spp. to velvet bean. Isolates of *Pythium* recovered from collard stems or wirestem lesions were not pathogenic on collard. Likewise, Sumner (24) found that *R. solani* was much more virulent than *Pythium* spp. on several different cole crop vegetables. Wirestem lesions generally must encompass >75 to 100% of the stem circumference and reach ≥1 mm deep into the cortex (a rating of 6 on a 1-to-10 scale), before foliar symptoms are evident (13,14). *Pythium* spp. were isolated alone from only 3 of 36 plants (8.3%) that were rated 6 or greater. Hence, approximately 8% of the plants rated as having wirestem in the field may have been infected with *Pythium* spp. Because disease did not appear on collard inoculated with *Pythium* spp. in the greenhouse, *Pythium* spp. co-isolated with *R. solani* appear to have been secondary invaders after initial infection of collard stems by *R. solani* or some other injury to the stem.

Recovery of *R. solani* from lesions on stems of living collard plants averaged 30 to 60% in the 2 years. This percentage recovery, although somewhat low, was within the range previously observed (20 to 72%) in experiments with field-grown cabbage seedlings (10; A. P. Keinath, unpublished). In this previous study, percentage of recovery increased as the inoculum density (number of sclerotia added per kilogram of soil) increased. Recovery also was greater in the fall, when overall levels of infection were higher, than in the spring. Similarly, recovery of *R. solani* from wirestem lesions on collard was greater in 2000, when overall disease incidence was greater, than in 2001, even when the most se-

verely affected plants were not cultured in 2000 because they were already dead.

Three of the five isolates of *R. solani* obtained from collard in 2001 were not pathogenic on collard in the greenhouse. Although the level of CMS inoculum used (1%) gave reproducible levels of wirestem in previous studies (10,13), it is possible that these isolates were of such low virulence that they did not cause symptoms at this inoculum density. In addition, nonpathogenic isolates of *R. solani* have been found in several different anastomosis groups (AGs) (22), including AG 2 and 4, the most common AGs infecting *Brassica* spp. (13).

Plants surviving in the cover crop mulch treatment in 2000 compensated for stand loss by growing larger, as demonstrated by a significant increase in mean weight of individual plants, so that the total harvested weight of this treatment was similar to that of the other two treatments. However, collard generally is marketed by the bunch with two or three plants per bunch. Therefore, a reduction in the number of plants per hectare due to wirestem, as occurred in 2000, would reduce marketable yields and possibly increase harvest costs, because harvesters would take more time to cover more area to get the required number of plants for a bunch.

Growth of the velvet bean cover crop was reduced by a viral disease in 2000 and by foliar fungal diseases in 2001. Consequently, the biomass obtained was less than expected in both years. In addition, velvet bean is very susceptible to *M. phaseolina* (5), the causal agent of charcoal rot, which is present in South Carolina soils. Because several diseases appeared during the 2 years of this study, it may be difficult to grow velvet bean without disease control measures. Velvet bean could be used as a summer cover crop for fall-transplanted collard, provided it is disked into soil several weeks before transplanting to reduce the potential for an epidemic of wirestem. However, we found no beneficial effects of velvet bean on growth of collard in a conventional production system.

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