Impact of Five Cover Crop Green Manures and Actinovate on Fusarium Wilt of Watermelon

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Abstract

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Triploid watermelon cultivars are grown on more than 2,023 ha in Maryland and in Delaware. Triploid watermelon cultivars have little host resistance to Fusarium wilt of watermelon (*Fusarium oxysporum* f. sp. *niveum*). The effects of four different fall-planted cover crops (*Vicia villosa*, *Trifolium incarnatum*, *Secale cereale*, and *Brassica juncea*) that were tilled in the spring as green manures, and bare ground, were evaluated alone and in combination with the biocontrol product Actinovate (*Streptomyces lydicus*) on Fusarium wilt severity and watermelon fruit yield and quality. Six field experiments were conducted over 3 years in Beltsville and Salisbury, MD and Georgetown, DE. Both *V. villosa* and *T. incarnatum* significantly suppressed Fusarium wilt of watermelon as much as 21% compared with watermelon in nonamended plots. However, no suppression of

Fusarium wilt occurred at low disease levels or where low cover crop biomass was present. In general, Beltsville, MD had lower disease levels than Salisbury, MD and Georgetown, DE. *T. incarnatum* was the only cover crop that yielded significantly more fruit than nonamended treatments (129% more fruit per hectare) but only for one field trial. The Actinovate product either did not reduce Fusarium wilt or the magnitude of the reduction was nominal. Actinovate significantly reduced Fusarium wilt by 2% in 2009 and as much as 7% in 2010, and increased Fusarium wilt severity by 2.5% in 2011. Actinovate significantly increased yield for one field trial but only when applied to non-amended or *Secale cereal*-amended plots. This is the first report of a reduction in Fusarium wilt following a *T. incarnatum* cover crop incorporated as a green manure.

Fusarium oxysporum f. sp. *niveum* W.C. Snyder & H.N. Hansen is the cause of Fusarium wilt of watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai). Watermelon growers on the eastern shore of Maryland and in Delaware experience significant losses in yield due to the prevalence of Fusarium wilt in their fields (45). Losses can approach 100% if susceptible cultivars are planted in heavily infested fields (47). Yield loss is especially high in fields planted with triploid watermelon, which currently lacks resistance to Fusarium wilt (47). Consumer demand for triploid (seedless) watermelon has increased dramatically in recent years and triploid cultivars are now grown on more than 90% of watermelon production acreage. As a result, the need for management options for Fusarium wilt have also increased (25,36).

Management options for Fusarium wilt of watermelon vary in effectiveness and cost. Grafting is an effective disease management tool; however, it requires high labor and seed costs and, therefore, is not currently practiced in the United States (17). Methyl bromide (MeBr), a soil fumigant that was historically used to manage Fusarium wilt of watermelon (45), has been phased out due to its destructive effects on stratospheric ozone (12). Other soil fumigants such as metam sodium are available for management of Fusarium wilt but are generally not as effective as MeBr at reducing Fusarium wilt, are costly, and also have detrimental environmental effects (31). Alternative biological and cultural control methods are preferred.

Green manures are known to suppress several plant diseases (20,21). *Secale cereale* residues decreased Fusarium root rot on beans by inhibiting chlamydospore germination of *F. solani* f. sp. *phaseoli* (21). *Vicia villosa* Roth (hairy vetch) cover crop amend-

ments reduced inoculum density and chlamydospore viability of *Thielaviopsis basicola*, a pathogen of cotton seedlings (1,4). In 2004, Zhou and Everts found that a *V. villosa* green manure decreased the incidence of Fusarium wilt on watermelon as much as 42 to 48% (45). In additional studies, a *V. villosa* cover crop was similarly effective against Fusarium wilt of watermelon, reducing it as much as 63% (16,43,46,47). Both *V. villosa* and *T. incarnatum* also increased watermelon fruit yield (16,32). These yield increases were likely due to a decrease in Fusarium wilt and improved soil health. Despite these production benefits, the mechanism of *V. villosa* disease suppression of Fusarium wilt is still unknown.

There are a limited number of biocontrol products available for management of Fusarium wilt of watermelon and no one product is widely used (22,23,39). *Streptomyces* spp. have been used as the active ingredient in disease management products (9). Actinovate AG (Natural Industries Inc.) is an Organic Materials Review Institute-approved biocontrol product whose active ingredient is *Streptomyces lydicus* strain WYEC 108. Actinovate is labeled for protection of watermelon from Fusarium wilt and is supposed to increase plant yields. Actinovate was effective at reducing disease in other pathosystems, such as Fusarium surface rot (*Fusarium* spp.) of postharvest sweet potato and dollar spot (*Sclerotinia homoeocarpa*) of warm- and cool-season grasses (13,35). However, few studies have been conducted to evaluate Actinovate product effects on Fusarium wilt of watermelon under field conditions. Likewise, there are no studies that evaluate whether Actinovate in combination with a *V. villosa* cover crop will enhance disease suppression.

A combination of multiple biocontrol methods may achieve greater disease suppression than a single method. The dual application of an organic fertilizer amendment and antagonistic bacteria (*Paenibacillus polymyxa* SQR-1 and *Bacillus subtilis* SQR-5) significantly decreased Fusarium wilt of cucumber (*F. oxysporum* f. sp. *cucumerinum*; 42). A complement of wood chip amendments and *Streptomyces* spp. synergistically decreased Verticillium wilt (*Verticillium dahliae*) of potato (10). Actinovate was significantly

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more effective at reducing powdery mildew of summer squash (*Cucurbita pepo*) and cantaloupe (*Cucumis melo*) when applied with the fungicide Procure 480SC than when applied alone (41).

The objective of this study was to evaluate the efficacy of four cover crops—*Vicia villosa*, *T. incarnatum*, *Brassica juncea*, and *Secale cereal*—with and without an Actinovate biocontrol product on Fusarium wilt of field-grown triploid watermelon.

Materials and Methods

Experimental design. An experiment was conducted six times as randomized split plot block designs in three different locations over a period of 3 years (2009 to 2011; Table 1). Field locations included the United States Department of Agriculture (USDA) Henry A. Wallace Beltsville Agricultural Research Center (USDA-BARC) in Beltsville, MD (2009 and 2010); the University of Maryland Lower Eastern Shore Research and Education Center (UM-LESREC) in Salisbury (2009, 2010, and 2011); and the University of Delaware Carvel Research and Education Center (UD-REC) in Georgetown (2011). The main plot treatments were cover crops and the subplot treatments were Actinovate and, in fields that were not infested, *F. oxysporum* f. sp. *niveum* inoculation (Table 1). Noninfested fields were inoculated with *F. oxysporum* f. sp. *niveum* race 1 at USDA-BARC in 2009 and 2010 and at UM-LESREC in 2009. In these manually inoculated fields, the inoculum did not become adequately established and little to no disease was observed. Therefore, for other years and locations, trials were conducted in *F. oxysporum* f. sp. *niveum*-infested fields (Table 1).

At the USDA-BARC location, field soil was an amalgam of a Russett-Christiana and Downer-Hammon complex of sandy loam. At UM-LESREC, field soil was Fort Mott loamy sand and, at UD-REC, Rosedale loamy sand. Cover crops were incorporated in the spring as a green manure. Cover crop treatments were *V. villosa*, *T. incarnatum*, *B. juncea*, and *S. cereale* but varied by year and location (Table 1).

Cover crops were seeded in late fall according to local seeding rates (Table 1). In 2011, due to low germination or winter kill the previous season, cover crops were overseeded at UM-LESREC and UD-REC, respectively, to ensure sufficient aboveground biomass (Table 1). Prior to planting, the seed of *V. villosa* and *T. incarnatum*

were inoculated with a commercial *Rhizobium* sp., N-DURE (IN TX Microbials, LLC). At UM-LESREC in 2009, the cover crops were flattened with a culti-packer, mowed, and tilled. In all other locations, the cover crops were killed by a paraquat treatment (Gramoxone Extra 2.5SC, 1.2 kg a.i./ha, 1.75 liters/ha) in early spring (Table 1) and a tractor-mounted rototiller was used to disk the soil three times to a depth of approximately 15 to 20 cm. Control plots were cultivated in the same manner.

In each plot, three 1-m² quadrats of aboveground plant biomass were collected, dried, and weighed prior to green manure incorporation. Biomass sample weights were used to estimate the amount of N the various cover crops contributed to their respective plots, and subsequent fertilization was based on these calculations. The fertilizer for each treatment was applied all at once, immediately prior to the laying of the black polyethylene mulch. All beds were formed by a bed shaper and a single drip irrigation tube was laid under black polyethylene mulch. Beds were laid on a 1.83-m center in fields in 2009 and a 2.13-m center in 2010 and 2011.

Seed of 'Sugar Heart' watermelon were planted into trays in a potting mix (Sun Gro Redi-earth Plug and Seedling Mix; Sun Gro Horticulture). After 4 to 5 weeks of growth, watermelon seedlings were planted 0.91 m apart in each bed (Table 1). The pollinizer 'SP4,' which is resistant to Fusarium wilt race 1, was transplanted between every third seedless watermelon at all locations, except LESREC-UM in 2009 and USDA-BARC in 2010, when the diploid watermelon 'Royal Majesty' was used as a pollinizer. Insecticides and foliar fungicides were applied as needed according to extension recommendations for the mid-Atlantic. At USDA-BARC, two beehives were maintained centrally in the experimental site. On-farm bee hives at UD-REC and UM-LESREC were adjacent to the experimental fields. All fields were managed conventionally, except for the UM-LESREC field in 2009, which was managed with organic practices on organic-certified land.

The Natural Industries recommended rate of Actinovate AG foliar spray is 850.48 g of Actinovate AG per 946.25 liters of H₂O per hectare. Therefore, 0.011 g of Actinovate AG per 12.2 ml of H₂O per 0.156-m² transplant tray was applied as a suspension to watermelon seedlings 1 to 2 weeks prior to transplanting (Table 1). Actinovate was also applied within the week following transplanting as a soil drench around the base of the plant at 255.14 g of

Table 1. Management and design of field experiments to evaluate tilled cover crop and Actinovate biocontrol application on Fusarium wilt severity (caused by *Fusarium oxysporum* f. sp. *niveum* [FON]) and watermelon yield for six different field trials in Maryland and in Delaware^y

Parameters	USDA-BARC 2009	USDA-BARC 2010	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	UD-REC 2011
Main plot treatment	<i>V. villosa</i> , <i>T. incarnatum</i> , <i>S. cereale</i> , bare ground	<i>V. villosa</i> , <i>T. incarnatum</i> , <i>B. juncea</i> , <i>S. cereale</i> , bare ground	<i>V. villosa</i> , <i>S. cereale</i> , bare ground	<i>V. villosa</i> , <i>T. incarnatum</i> , <i>B. juncea</i> , <i>S. cereale</i> , bare ground	<i>V. villosa</i> , <i>T. incarnatum</i> , <i>B. juncea</i> , <i>S. cereale</i> , bare ground	<i>V. villosa</i> , <i>T. incarnatum</i> , <i>B. juncea</i> , <i>S. cereale</i> , bare ground
Subplot treatment	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate, No FON	FON, FON + Actinovate	FON, FON + Actinovate	FON, FON + Actinovate
Cover crop seeding rates (kg/ha)	<i>V. villosa</i> 44.83, <i>T. incarnatum</i> 28.02, <i>S. cereale</i> 134.50	<i>V. villosa</i> 44.83, <i>T. incarnatum</i> 28.02, <i>S. cereale</i> 134.50, <i>B. juncea</i> 6.73	<i>V. villosa</i> 50.44, <i>S. cereale</i> 134.50	<i>V. villosa</i> 50.44, <i>T. incarnatum</i> 11.21, <i>S. cereale</i> 125.54, <i>B. juncea</i> 11.21	<i>V. villosa</i> 50.44, <i>T. incarnatum</i> 11.21, <i>S. cereale</i> 125.54, <i>B. juncea</i> 11.21	<i>V. villosa</i> 50.44, <i>T. incarnatum</i> 11.21, <i>S. cereale</i> 125.54, <i>B. juncea</i> 11.21
Cover crop seeding dates	9/24/2008	9/22/2009	10/16/2008	9/25/2009	3/9/2011 ^z	3/15/2011
Cover crop tillage	5/23/2009	5/23/2010	5/15/2009	5/24/2010	5/19/2011	5/25/2011
Dates of Actinovate applications	Foliar 6/03/2009; Soil drench 6/29/2009	Foliar 6/16/2010; Soil drench 6/29/2010	Foliar 6/02/2009; Soil drench 6/29/2009	Foliar 6/08/2010; Soil drench 6/15/2010	Foliar 5/20/2011; Soil drench 6/03/2011	Foliar 5/26/2011; Soil drench 6/07/2011
Watermelon transplant date	6/17/2009	6/18/2010	6/19/2009	6/12/2010	6/03/2011	6/07/2011

^y USDA-BARC = United States Department of Agriculture Beltsville Agricultural Research Center located in Beltsville, MD; UM-LESREC = University of Maryland Lower Eastern Shore Research and Education Center located in Salisbury; and UD-REC = University of Delaware's Carvel Research and Education Center, located in Georgetown. Cover crops were *Vicia villosa*, *Trifolium incarnatum*, *Secale cereal*, and *Brassica juncea*. Actinovate AG is a biocontrol product (Natural Industries Inc.) which is labeled for management of Fusarium wilt of watermelon.

^z In 2011, cover crops were overseeded in the early spring to ensure that plots had sufficient cover crop biomass.

Actinovate AG per 378.5 liters of H₂O per 4,046.86 m²; approximately 0.115 g per 172 ml of H₂O per 1.89 m² per plant.

A race 1 isolate (F-030-1) of *F. oxysporum* f. sp. *niveum*, which was acquired from a wilted watermelon plant in Wicomico, MD in a previous study (44), was used for disease inoculations. Strains were maintained in a mixture of sandy soil and perlite (1:2, wt/wt) at 4°C. Inoculum was prepared by transferring agar pieces containing *F. oxysporum* f. sp. *niveum* (F-030-1) mycelium and spores grown on Komadas media (19) into a liquid mineral salts medium (27). Each 2-liter flask received approximately half of an 80-mm plate. The culture was incubated on an orbital shaker at 128 rpm at room temperature for approximately 2 weeks before filtering through eight layers of cheesecloth. A Spencer hemacytometer was used to adjust the spore suspension to the desired concentration.

In 2009 at both USDA-BARC and UM-LESREC, seedlings were inoculated immediately after transplanting by pipetting 6 ml of *F. oxysporum* f. sp. *niveum* inoculum at a concentration of 2.45 × 10⁶ CFU (primarily micronidia) per milliliter into a hole (roughly 3 cm in diameter and 8 cm deep) 7 cm away from the crown of the watermelon plant (45). In 2010 at USDA-BARC, the *F. oxysporum* f. sp. *niveum* inoculation method was modified to increase disease incidence. Two days after cover crop incorporation and a week prior to the laying of black plastic, 60 ml of *F. oxysporum* f. sp. *niveum* inoculum at 2.85 × 10⁸ CFU/ml was mixed into 3.79 liters of H₂O and evenly banded across the center of each 36.92-m² bed with a watering can. A second application of 11 ml of *F. oxysporum* f. sp. *niveum* inoculum at a concentration of 2.33 × 10⁶ CFU/ml was pipetted next to each watermelon plant using the same method employed in 2009. Inoculum was applied at concentrations that were previously calculated to result in approximately 60% wilt on a highly susceptible cultivar (44).

Fusarium wilt ratings. Watermelon were planted 0.91 m apart in a bed but the total number of watermelon planted per treatment bed varied depending on year and location (*n* = 10 to 16 watermelon plants/treatment bed). Wilt severity was rated as the percentage of wilted watermelon foliage for each plant and averaged for each bed (20). Fusarium wilt severity was evaluated weekly after transplanting in 2009. In 2009, additional symptoms of stunting

and chlorosis were present and included in the wilt rating, as follows. For 2009 only, if a watermelon plant was chlorotic, the wilt rating was increased by 10% for that plant and, if a watermelon was stunted, the wilt rating was increased by 25%. In 2010, Fusarium wilt severity was measured three times at UM-LESREC and once at USDA-BARC. In 2011, Fusarium wilt severity was evaluated weekly following transplanting. The area under the disease progress curve (AUDPC) was calculated for each field trial except at USDA-BARC in 2010 because only one wilt rating was taken. The AUDPC was calculated as $AUDPC = \sum [(y_i + y_{i+1})/2 \times (t_{i+1} - t_i)]$. Stems of wilted watermelon plants were plated on Komadas media to verify the presence of *F. oxysporum* f. sp. *niveum* (19).

Plant vigor and yield. In 2010, watermelon vine length was measured approximately 3 weeks following transplanting at both locations. In 2011, vines were measured twice at UM-LESREC: once 7 days after transplanting and again the following week. At UD-REC, vines were measured three times at weekly intervals beginning 7 days after transplanting.

All fruit were individually weighed and counted at harvest, percent Brix (sugar content) was assessed for three watermelon fruit per bed (*n* = 144 to 180 fruit sampled/field) with a hand held refractometer, and the numbers of sunburned fruit were counted. Treatment effects were evaluated for marketable watermelon yield and total watermelon weight. Marketable-sized fruit were defined as watermelon that weighed >3.18 kg.

Streptomyces lydicus in the rhizosphere. In 2010, directly after fruit harvest, seminal watermelon roots from each plot in four of the replicate blocks at UM-LESREC and USDA-BARC (*n* = 40 to 60, respectively) were plated on arginine-glycerol-salt agar to observe any growth of *Streptomyces* spp. from the roots, to determine whether *Streptomyces lydicus* successfully colonized the watermelon roots (7). Because no *S. lydicus* colonies were observed in 2010, sampling was instead done 2 weeks after watermelon were transplanted to the field in 2011 at both UM-LESREC and UD-REC. Seminal roots of destructively harvested watermelon plants, one for each plot (*n* = 60), were cut into three 3-cm root sections, put into test tubes with 5 ml of sterile distilled water, soaked for 5

Table 2. Variance analysis (*P* values) for the effects of cover crop and Actinovate AG application main effects on watermelon Fusarium wilt severity and the area under the disease progress curve (AUDPC) in field experiments conducted at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture at the Beltsville Agricultural Research Center (USDA-BARC), and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010, and 2011^y

Location, year ^z	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	AUDPC
2009									
UM-LESREC									
Cover Crop	–	0.0016	0.6832	0.6743	0.8041	0.4853	0.5593	–	0.6459
Actinovate	–	0.7363	0.1392	0.1396	0.3596	0.0096	0.7203	–	0.1533
USDA-BARC									
Cover Crop	–	–	0.3475	0.7066	0.4363	0.5578	0.3569	–	0.3858
Actinovate	–	–	0.9085	0.5346	0.3829	0.4297	1.0000	–	0.8924
2010									
UM-LESREC									
Cover Crop	0.5496	–	–	0.1619	–	0.0887	–	–	0.2024
Actinovate	0.0670	–	–	0.0379	–	0.0603	–	–	0.0373
USDA-BARC									
Cover Crop	–	–	–	–	–	–	–	0.1386	–
Actinovate	–	–	–	–	–	–	–	0.3707	–
2011									
UM-LESREC									
Cover Crop	0.4118	0.1152	0.1445	0.0013	0.0056	<0.0001	0.0458	–	<0.0001
Actinovate	0.0924	0.7092	0.6713	0.9771	0.5115	0.6459	0.5030	–	0.5247
UD-REC									
Cover Crop	0.3888	0.4779	0.2418	0.4201	0.1641	0.0175	0.0535	–	0.0251
Actinovate	0.1319	0.1898	0.0206	0.7908	0.6214	0.6861	0.3333	–	0.8020

^y Number of weeks after watermelons were transplanted to the field; – indicates that the measurement was not taken for that date and location or, for USDA-BARC in 2010, that no wilt was observed.

^z Cover crop seeding rates at UM-LESREC and UD-REC were *Vicia villosa* at 50.44 kg/ha, *Trifolium incarnatum* at 11.21 kg/ha, *Secale cereale* at 125.54–134.50 kg/ha, and *Brassica juncea* at 11.21 kg/ha; and, at USDA-BARC, the seeding rates were *V. villosa* at 44.83 kg/ha, *T. incarnatum* at 28.02 kg/ha, *S. cereale* at 134.50 kg/ha, and *B. juncea* at 6.73 kg/ha. Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants 2 weeks after seeding at a rate of 0.011 g per 12.2 ml of H₂O per 0.156-m² transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g per 172 ml of H₂O per 1.89 m² per plant.

min, and vortexed. The root segments were removed and the resulting soil–water mixture was serially diluted onto sporulation agar amended with nyastatin, carbenicillin, and cycloheximide and casein agar to observe if *Streptomyces* spp. were present in the soil rhizosphere (40).

Nutrient analyses. Treatment effects on the nutrient content of soil, dried cover crop foliage, and dried watermelon foliage were analyzed in 2011 to determine whether nutritional differences impacted disease suppression (29). In 2011, three 6-in. (15 cm) soil cores were collected from each treatment plot following tillage (25 May at UD-REC and 20 June at UM-LESREC) and analyzed at the University of Delaware's soil testing lab for pH; buffer pH; percent organic matter; P, K, Ca, Mg, Mn, Zn, Cu, Fe, B, S, and Al in milligrams per kilogram; CEC (meq/100 g); percent base saturation; and P saturation ratio. Cover crop biomass was collected three times from 2 m² in each plot, at both locations, approximately a week prior to soil incorporation.

Statistical analyses. Data were analyzed using the MIXED procedure with the Statistical Analysis System (version 9.2; SAS Institute, Cary, NC), which integrates random effects in the statistical model and performs covariance structure modeling (24). Treatment means were separated using a Fisher's protected least significant difference test at $P \leq 0.05$. When disease was low, the proportional wilt data was usually not normally distributed. However, because of the large sample size ($n > 30$), this data was considered robust enough to be analyzed without transformation (30). This is supported by the Central Limit Theorem, which states that the sample

mean of a population same size of $n > 30$ will converge to a standard normal distribution (3).

Results

Fusarium wilt severity. Fusarium wilt severity varied by location and year. Fusarium wilt severity was low at USDA-BARC while it was high in fields on the eastern shore of Maryland and in Delaware, and significant differences in disease suppression across locations were observed.

Cover crop biomass also varied across locations and, at UM-LESREC in 2010, heavy winter snowfall reduced cover crop biomass in many of the plots. Therefore, wilt severity data from the UM-LESREC 2010 field season are excluded from these results. In 2011 at UD-REC and UM-LESREC, cover crops were overseeded in the early spring to ensure sufficient biomass coverage, the weather was moderate, and watermelon crops were planted in fields with soil conditions conducive to Fusarium wilt and which already had high levels of *F. oxysporum* f. sp. *niveum* (Table 1).

There was low disease pressure at UM-LESREC in 2009, which resulted in no or very low wilt severity (less than a percent difference) among treatments (Tables 2 and 3). *V. villosa* and *T. incarnatum* green manures suppressed Fusarium wilt of watermelon at both UM-LESREC and UD-REC in 2011 (Table 3). For the majority of the watermelon growing season, watermelon plants in *V. villosa*-amended plots and *T. incarnatum*-amended plots had significantly less disease than plants in nonamended plots or in plots amended with any other cover crop treatment at UM-LESREC in

Table 3. Percent watermelon wilt severity following tilled cover crop or bare ground at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture Beltsville Agricultural Research Center (USDA-BARC), and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010, and 2011

	Weeks after transplanting ^y								
Location, year, crop ^z	1	2	3	4	5	6	7	8	AUDPC
USDA-BARC, 2009									
<i>V. villosa</i>	—	—	1.30 a	0.00 a	0.42 a	0.42 a	0.00 a	—	1.50 a
<i>T. incarnatum</i>	—	—	0.42 a	0.42 a	0.00 a	0.42 a	0.42 a	—	1.20 a
<i>S. cereale</i>	—	—	0.71 a	0.21 a	0.00 a	1.25 a	0.83 a	—	2.22 a
Bare ground	—	—	2.92 a	0.63 a	0.00 a	0.21 a	0.00 a	—	4.17 a
<i>P</i> > <i>F</i>	...	...	0.3475	0.7066	0.4363	0.5578	0.3569	...	0.3858
UM-LESREC, 2009									
<i>V. villosa</i>	—	0.97 b	0.92 a	0.053 a	1.10 a	0.28 a	1.40 a	—	3.50 a
<i>S. cereale</i>	—	0.49 a	6.20 a	0.38 a	1.10 a	1.10 a	1.70 a	—	12.10 a
Bare ground	—	0.97 b	6.80 a	0.40 a	0.56 a	0.56 a	2.50 a	—	10.00 a
<i>P</i> > <i>F</i>	...	0.0016	0.6832	0.6743	0.8041	0.4853	0.5593	...	0.6459
USDA-BARC, 2010									
<i>V. villosa</i>	—	—	—	—	—	—	—	12.12 a	—
<i>T. incarnatum</i>	—	—	—	—	—	—	—	13.82 a	—
<i>S. cereale</i>	—	—	—	—	—	—	—	18.50 a	—
<i>B. juncea</i>	—	—	—	—	—	—	—	26.91 a	—
Bare ground	—	—	—	—	—	—	—	20.64 a	—
<i>P</i> > <i>F</i>	...	...	...	...	...	...	...	0.1386	
UM-LESREC, 2011									
<i>V. villosa</i>	0.64 a	5.63 a	8.71 a	27.58 b	41.86 b	40.68 b	35.90 b	—	124.55 b
<i>T. incarnatum</i>	0.00 a	2.08 a	4.92 a	31.36 b	47.20 b	31.14 b	43.48 ab	—	119.16 b
<i>S. cereale</i>	1.12 a	4.38 a	13.83 a	46.44 a	60.95 a	54.81 a	51.02 a	—	189.43 a
<i>B. juncea</i>	0.32 a	6.25 a	14.48 a	48.56 a	66.10 a	56.97 a	54.62 a	—	196.66 a
Bare ground	1.44 a	6.46 a	7.01 a	48.26 a	57.31 a	52.05 a	52.61 a	—	184.09 a
<i>P</i> > <i>F</i>	0.4180	0.1152	0.1445	0.0056	0.0056	<0.0001	0.0458		<0.0001
UD-REC, 2011									
<i>V. villosa</i>	0.60 a	1.49 a	14.00 a	13.74 a	11.50 a	6.68 c	12.12 a	—	56.27 b
<i>T. incarnatum</i>	0.60 a	1.49 a	16.03 a	15.97 a	15.19 a	9.49 bc	15.61 a	—	68.04 ab
<i>S. cereale</i>	1.79 a	8.93 a	15.18 a	16.42 a	13.53 a	12.91 ab	19.35 a	—	72.91 ab
<i>B. juncea</i>	3.57 a	13.39 a	16.69 a	18.16 a	16.34 a	12.98 ab	22.46 a	—	81.82 a
Bare ground	2.38 a	8.93 a	18.18 a	19.97 a	17.34 a	15.19 a	23.53 a	—	84.57 a
<i>P</i> > <i>F</i>	0.3888	0.4779	0.2418	0.4201	0.1641	0.0175	0.0535	...	0.0251

³ Means in a column followed by the same letter are not significantly different at $\alpha = 0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure; – indicates that the measurement was not taken for that date and location or, for USDA-BARC in 2010, that no wilt was observed; AUDPC = area under the disease progress curve.

² Due to low levels of aboveground cover crop, biomass results from UM-LESREC 2010 are omitted. Cover crop seeding rates at UM-LESREC and UD-REC were *Vicia villosa* at 50.44 kg/ha, *Trifolium incarnatum* at 11.21 kg/ha, *Secale cereale* at 125.54–134.50 kg/ha, and *Brassica juncea* at 11.21 kg/ha; and, at USDA-BARC, the seeding rates were *V. villosa* at 44.83 kg/ha, *T. incarnatum* at 28.02 kg/ha, *S. cereale* at 134.50 kg/ha, and *B. juncea* at 6.73 kg/ha.

2011 (Table 3). Watermelon in *V. villosa* and *T. incarnatum*-amended plots at UM-LESREC in 2011 also had a lower AUDPC (124.55 and 119.16, respectively) compared with watermelon planted in *B. juncea*-amended (196.66) or bare ground (184.09) plots (Table 3). Fusarium wilt severity of watermelon in *B. juncea*-amended plots was consistently the highest or second highest numerically, although never significantly higher than on watermelon in nonamended or *Secale cereale*-amended plots (Table 3).

Both leguminous cover crops also suppressed Fusarium wilt at UD-REC in 2011. However, *T. incarnatum* did not achieve the same magnitude of disease suppression as *V. villosa*. Six weeks after transplanting to the field, the watermelon grown in *V. villosa*-amended plots had significantly less disease than watermelon in any other treatment plots except for that of *T. incarnatum*-amended plots. Watermelon in *T. incarnatum* plots also had significantly less Fusarium wilt than plants in bare ground plots (Table 3). Watermelon wilt severity was similarly lowest in *V. villosa*- and *T. incarnatum*-amended plots the following week, although the *P* value was marginally significant at 0.0535 (Table 3). The AUDPC of watermelon in *V. villosa*-amended plots was significantly less (56.27) than watermelon in *B. juncea*- (81.82) and bare ground- (84.57) treated plots (Table 3). Cover crop treatments did not significantly influence Fusarium wilt at USDA-BARC, where disease was low, but the same trend in *V. villosa* and *T. incarnatum* disease suppression was still numerically apparent in 2009 and 2010 (Table 3).

Actinovate treatments nominally reduced Fusarium wilt for two field trials and increased Fusarium wilt for one field trial. Six weeks after transplanting, at UM-LESREC in 2009, watermelon treated with Actinovate had 1.94% less Fusarium wilt than watermelon in untreated plots (Table 4). At UM-LESREC in 2010, the AUDPC and Fusarium wilt severity measured 4 weeks after transplanting was 7.07% lower for watermelon in Actinovate-treated plots than for watermelon in control plots. In contrast, Actinovate biocontrol applications increased Fusarium wilt severity 2.45% for the wilt reading taken 3 weeks after transplanting at UD-REC in 2011 (Table 4). Ultimately, Actinovate treatment effects were minimal and inconsistent. For all field trials, there were no significant interactions between the cover crop and Actinovate biocontrol treatments for their effects on Fusarium wilt severity ($P \geq 0.1340$).

Plant vigor and yield. Significant cover crop treatment effects on marketable watermelon yield were only observed at UM-LESREC in 2011, where *T. incarnatum*-amended plots had marketable watermelon yield that was higher (14.67×10^2 to 20.68×10^2 more fruit per hectare) than that of all other amended or nonamended plots (Table 5).

There was a significant Actinovate–cover crop treatment interaction for marketable watermelon number for only one field trial. Beds amended with *S. cereale* or bare ground and treated with Actinovate had significantly higher marketable yield compared with their respective untreated or uninoculated beds at USDA-BARC in 2009 ($P = 0.0109$). The only other field trial where Actinovate demonstrated significant positive effects on marketable watermelon yield was at UM-LESREC in 2011, when Actinovate significantly increased marketable watermelon yield (933 more fruit per hectare) compared with beds that were not treated with Actinovate (Table 5).

No individual cover crop or Actinovate treatment affected marketable watermelon weight for any field trial ($P \geq 0.1314$). In addition, no cover crop or Actinovate treatment effects were seen in more than one field trial for sugar content, watermelon vine length, or the number of sunburned watermelon (*data not shown*).

***Streptomyces lydicus* isolation.** *Streptomyces lydicus* was never isolated from watermelon roots in any of the sampled locations (UM-LESREC and USDA-BARC in 2010 and UM-LESREC and UD-REC in 2011). Therefore, the establishment of *S. lydicus* was never confirmed.

Nutrient analyses. Differences in cover crop and soil nutrient concentrations were not consistent across field locations and there were no obvious trends that linked these variations to Fusarium wilt disease suppression (*data not shown*). No other significant differences were seen for the soil analysis conducted at UM-LESREC and UD-REC.

Discussion

V. villosa and *T. incarnatum* reduced Fusarium wilt of watermelon at UD-REC and UM-LESREC in 2011. The ability of a *V. villosa* cover crop to suppress Fusarium wilt of watermelon has been observed in previous studies done on the eastern shore of Maryland (44–46); however, no previous studies have reported the disease suppressive effects of a *T. incarnatum* green manure on Fusarium wilt of watermelon or, to our knowledge, for any other plant disease.

The efficacy of *T. incarnatum* was tested in these trials because *V. villosa* and *T. incarnatum* cover crops have similar growth characteristics and confer comparable soil-improving benefits. Both cover crops rapidly produce large amounts of aboveground biomass in early spring, serving as effective weed suppressants (2). Their low C:N ratios result in a fast, extensive breakdown of foliage, stimulating substantial soil microbial activity. They are both flowering legumes which encourage beneficial insects and fix a corresponding amount of nitrogen per acre (2).

Table 4. Effect of Actinovate AG on percent Fusarium wilt severity of watermelon, caused by the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON), at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC) in 2009 and 2010 and the University of Delaware's Carvel Research and Education Center (UD-REC) in 2011

Location, year, treatment	Week after transplanting ^a								AUDPC
	1	2	3	4	5	6	7	8	
UM-LESREC, 2009									
Nontreated	—	2.25 a	0.28 a	0.01 a	0.83 a	0.00 b	2.22 a	—	3.36 a
FON	—	1.94 a	13.25 a	0.84 a	1.67 a	1.94 a	1.39 a	—	19.37 a
FON + Actinovate	—	2.64 a	0.40 a	0.02 a	0.28 a	0.00 b	1.94 a	—	2.99 a
<i>P</i> > <i>F</i>	...	0.7363	0.1392	0.1396	0.3596	0.0096	0.7203	...	0.1533
UM-LESREC, 2010									
FON	4.88 a	—	—	9.75 a	—	10.31 a	—	—	28.07 a
FON+ Actinovate	2.44 a	—	—	2.68 b	—	3.81 a	—	—	9.95 b
<i>P</i> > <i>F</i>	0.067	...	...	0.0379	...	0.0603	...	...	0.0373
UD-REC, 2011									
FON	2.62 a	1.01 a	14.80 b	16.26 a	15.12 a	11.17 a	19.46 a	—	72.09 a
FON + Actinovate	0.95 a	0.36 a	17.25 a	16.64 a	14.44 a	11.74 a	17.79 a	—	73.32 a
<i>P</i> > <i>F</i>	0.1319	0.1898	0.0206	0.7908	0.6214	0.6861	0.3333	...	0.8026

^a Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants 2 weeks after seeding at 0.011 g per 12.2 ml of H₂O per 0.156-m² transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g per 172 ml of H₂O per 1.89 m² per plant. Means in a column followed by the same letter are not significantly different at $\alpha = 0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure; — indicates that the measurement was not taken for that date and location; AUDPC = area under the disease progress curve.

The extent of the disease suppression by *T. incarnatum* was comparable with that of *V. villosa* at UM-LESREC in 2011 but was lower at UD-REC in 2011. However, *T. incarnatum* significantly improved marketable watermelon yield compared with all other amended and nonamended plots at UM-LESREC in 2011, whereas *V. villosa* did not. The results of our trials, in terms of wilt severity and yield, indicate that *T. incarnatum* can be considered a viable alternative to *V. villosa* for management of Fusarium wilt of watermelon. The use of *V. villosa* as a green manure to suppress Fusarium wilt of watermelon has not been widely adopted because growers perceive that the seed may overwinter to become a weed (15). Growers might favor *T. incarnatum* over *V. villosa* because *T. incarnatum* does not reseed as proficiently in the mid-Atlantic region (38). Additionally, *T. incarnatum* does not vine and is less likely than *V. villosa* to outcompete future cash crops. It is possible that some growers might consider reseeding in fall an advantageous cover crop characteristic, because it reduces costs of planting and seed (11).

The magnitude of *V. villosa*'s effects on disease and yield was dependent upon the severity of Fusarium wilt in the field. We did not observe significant cover crop disease-suppressive effects for the field trials conducted in locations with low Fusarium wilt severity. This also occurred in a study conducted in South Carolina, which examined the effects of *V. villosa* on Fusarium wilt of watermelon (16). However, although Fusarium wilt incidence at USDA-BARC was too low to detect significant green manure treatment effects on Fusarium wilt or yield, *V. villosa* and *T. incarnatum* green manures showed a notable, if insignificant, increase in marketable yield for the 2009 field season and a small, nonsignificant reduction in disease for both 2009 and 2010.

Although *V. villosa* and *T. incarnatum* green manures suppressed Fusarium wilt of watermelon on the eastern shore of Maryland and in Delaware, their ability to suppress disease needs to be evaluated under different climates and soil conditions where Fusarium wilt of watermelon is also severe. The variation in Fusarium wilt disease pressure observed in our experiments may have resulted, in part, from differences in the soil environment. Fusarium wilt of watermelon is most prevalent in sandy, slightly acidic soils (pH 5 to 6;

48). The field at USDA-BARC had a higher amount of organic matter and a more neutral pH compared with fields located on the eastern shore of Maryland and Delaware, which are very sandy, low in organic matter (>1%), and have low pH levels. These environmental differences likely contribute to the prevalence of Fusarium wilt of watermelon on the eastern shore of Maryland and Delaware and partially explain why it is less common in central Maryland (44).

In our field trials, *B. juncea*-amended plots increased Fusarium wilt severity in three locations. Although these differences were not always significant, Fusarium wilt severity of watermelon in *B. juncea*-amended plots was never significantly lower than in bare ground plots. The results of our study are similar to the 2011 results of Mazzola et al. (26), who saw an increase in *Fusarium* spp. populations in the soil following brassica seed meal amendments. In vitro assays demonstrated that the isothiocyanate Benzyl ITC present in *Brassica* spp. increased sporulation of *F. oxysporum* isolate 9051C (33). There were observed elevations in *F. oxysporum* spp. populations in watermelon fields following the incorporation of *B. juncea* (28). In the same study, for one field season, Fusarium wilt of watermelon in *B. juncea*-amended plots had 10% more disease than plants in control (bare ground) plots (28). The effects of the secondary compounds of brassica cover crops have been extensively documented for other pathosystems (20,26) but further research on the direct and indirect effects of *Brassica* spp. on *F. oxysporum* f. sp. *niveum* would help elucidate *B. juncea*'s association with increased Fusarium wilt of watermelon.

Actinovate biocontrol resulted in either minimal or inconsistent reductions in Fusarium wilt of watermelon. In addition, we saw no benefit in the combination of the green manures and Actinovate biocontrol treatment on the suppression of Fusarium wilt. This is similar to results in other pathosystems, where Actinovate did not suppress disease. For example, Actinovate treatment did not reduce anthracnose (*Colletotrichum obiculare*) incidence or defoliation in watermelon, corm rot (*F. oxysporum* f. sp. *gladioli*) of gladiolus (*Gladiolus* × *hortulanus*), Fusarium wilt incidence on tomato (*F. oxysporum* f. sp. *lycopersici*), or Fusarium root rot (*Fusarium* spp.) of sweet potato (*Ipomoea batatas*) (6,8,9,13,37). However, Actino-

Table 5. Effects of tilled cover crop and Actinovate AG biocontrol application on marketable watermelon yield (number/hectare) at the University of Maryland's Lower Eastern Shore Research and Education Center (UM-LESREC), the United States Department of Agriculture at the Beltsville Agricultural Research Center (USDA-BARC), and University of Delaware's Carvel Research and Education Center (UD-REC) in 2009, 2010, and 2011^y

Treatment ^z	UM-LESREC 2009	UM-LESREC 2010	UM-LESREC 2011	USDA-BARC 2009	USDA-BARC 2010	UD-REC 2011
Cover crop						
<i>V. villosa</i>	8,353 a	6,261 a	1,416 b	12,333 a	7,400 a	6,159 a
<i>T. incarnatum</i>	—	—	2,883 a	12,223 a	6,355 a	6,745 a
<i>S. cereale</i>	8,073 a	8,120 a	1,089 b	11,417 a	7,736 a	7,486 a
<i>B. juncea</i>	—	7,074 a	815 b	—	6,840 a	6,536 a
Bare ground	8,324 a	7,427 a	1,253 b	11,110 a	8,598 a	6,572 a
<i>P</i> < <i>F</i>	0.2917	0.2247	0.0018	0.3417	0.5514	0.9323
Inoculation						
No treatment	8,259 a	—	—	11,750 ab	7,310 a	—
FON	8,180 a	7,850 a	1,024 b	11,147 b	7,714 a	7,032 a
Actinovate + FON	8,317 a	6,611 a	1,957 a	12,416 a	7,131 a	6,374 a
<i>P</i> < <i>F</i>	0.6834	0.0549	0.0033	0.0080	0.7392	0.4863

^y Marketable watermelon yield is the mean number of watermelon fruit per hectare that weighed more than 3.18 kg. 'Sugar Heart' watermelon was used for all field trials. Means in a column followed by the same letter are not significantly different at $\alpha = 0.05$ according to Fisher's protected least significant difference test. Statistical analysis was conducted using the Statistical Analysis System MIXED procedure; — indicates the absence of the specific cover crop treatment for the given time and location or that there was a loss in sufficient replications.

^z Cover crop seeding rates at UM-LESREC and UD-REC were *Vicia villosa* at 50.44 kg/ha, *Trifolium incarnatum* at 11.21 kg/ha, *Secale cereale* at 125.54–134.50 kg/ha, and *Brassica juncea* at 11.21 kg/ha; and, at USDA-BARC, the seeding rates were *V. villosa* at 44.83 kg/ha, *T. incarnatum* at 28.02 kg/ha, *S. cereale* at 134.50 kg/ha, and *B. juncea* at 6.73 kg/ha. For the inoculation treatment, Actinovate AG's active ingredient is *Streptomyces lydicus* and is produced by Natural Industries. Actinovate was applied as a foliar spray to watermelon plants 2 weeks after seeding at a rate of 0.011 g per 12.2 ml of H₂O per 0.156-m² transplant tray and again a week prior to transplanting to the field as a soil drench around the base of the plant at 0.115 g per 172 ml of H₂O per 1.89 m² per plant. *Fusarium oxysporum* f. sp. *niveum* (FON) was inoculated in 2009 at USDA-BARC and UM-LESREC at 6 ml of 2.45 × 10⁶ CFU/ml by pipetting into a hole (approximately 3 cm in diameter and 8 cm deep) 7 cm away from the crown of each watermelon plant immediately after watermelon transplanting. In 2010 at USDA-BARC, 2 days after cover crop incorporation and a week prior to the laying of black plastic, 60 ml of *F. oxysporum* f. sp. *niveum* inoculum at 2.85 × 10⁸ CFU/ml was mixed into 3.8 liters of H₂O and evenly banded across the center of each 36.92-m² bed (into which watermelon would later be transplanted) with a watering can. In addition, 11 ml of *F. oxysporum* f. sp. *niveum* at 2.33 × 10⁶ CFU/ml was added next to each watermelon plant using the same method employed in 2009. Fields used at UM-LESREC in 2010 and 2011 and UD-REC in 2011 were naturally infested.

vate did reduce *Fusarium* surface rot of sweet potato and dollar spot of Bermuda grass (13,35).

In the trials reported in this article, Actinovate negatively impacted vine length at one location, improved marketable watermelon yield in two of the six field trials, and decreased marketable watermelon yield in one of the six field trials. Other studies that tested Actinovate's growth- and yield-promoting abilities found similar results. Biomass of spinach treated with Actinovate AG + Micro 108 Seed Inoculant was no different than spinach in control plots (5). Tomato treated with Actinovate had yields that were no different than that of control plots (37). Actinovate SP + Latron B1956 treatments did not significantly impact watermelon yield (6).

The mechanism of Actinovate disease suppression has been linked with the ability of its active ingredient, *S. lydicus*, to colonize crop roots (34,40). We were unable to isolate *S. lydicus* from the watermelon plant roots despite repeated attempts. *Streptomyces* spp. are favored by high pH and temperatures near 25°C, and they are able to utilize complex organic materials as an energy source. *S. lydicus* activity is enhanced by high levels of soil organic matter (14,18). The low levels of soil pH and organic matter on the eastern shore of Maryland and in Delaware could have inhibited *S. lydicus* growth, preventing the colonization of watermelon roots. Studies where Actinovate effectively reduced disease examined foliar pathogens or postharvest disease (13,35,41). *S. lydicus* may be a more competitive antagonist in the phyllosphere than in the rhizosphere. Due to the nominal and inconsistent effects of Actinovate on *Fusarium* wilt of watermelon in this study, we do not recommend it for use against *F. oxysporum* f. sp. *niveum*. Actinovate did not have any additive effects on *Fusarium* wilt in combination with any of the cover crop treatments.

Both *V. villosa* and *T. incarnatum* suppressed *Fusarium* wilt of watermelon but the extent of the suppression was dependent on the magnitude of *Fusarium* wilt severity. The yield and soil health benefits of *T. incarnatum* are similar to that of *V. villosa* but *T. incarnatum* is also killed more easily and is less likely to overwinter under mid-Atlantic conditions.

Although the addition of Actinovate did not further reduce *Fusarium* wilt in this study, the combination of *V. villosa* or *T. incarnatum* green manures with other biocontrol products whose ability to colonize the watermelon rhizosphere and suppress *Fusarium* wilt of watermelon are already established could provide improved management of *Fusarium* wilt.

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