



Single season effects of mixed-species cover crops on tomato health (cultivar Celebrity) in multi-state field trials



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ABSTRACT

Cover crop use can help mitigate the deleterious effects of common cropping practices (e.g., tillage) and is, therefore, an important component of soil health maintenance. While known to be beneficial in the long-term, the short-term effects of cover crops, specifically mixed-species cover crops in organic systems are less clear. Cover crop effects on tomato productivity and disease severity were recorded over three field seasons (2010, 2011 and 2012) at sixteen field sites in three states, Maryland, New York and Ohio (MD, NY and OH), each with distinct soilborne disease pressure. Plots of five state-specific cover crop treatments were established the season prior to tomato production; the resulting plant residue was incorporated the following spring approximately four weeks before tomato planting. Total fruit yields along with early-season shoot height and fresh weight were used to compare treatment effects on productivity. Treatment disease severity ratings relied on natural inoculum. Interestingly, the effect of a single season of cover cropping on total yield was significant in no more than 25% of all site years. Similarly, cover crop effects on tomato disease levels were significant in 0–44% of the sixteen field sites. However, significant field-specific patterns were observed in every state across multiple years for some treatments. For example, in New York in 2010, tomato yields following all mixed cover crops were greater than the single rye cover crop in one field, but this pattern was reversed in the adjacent field. Thus, no general recommendation of a specific cover crop mixture can be made for near-term enhancement of tomato productivity or for reduction of disease. Therefore, growers should focus on location and operation-specific variables when choosing cover crops.

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1. Introduction

Organic agriculture relies on ecologically based methods of crop production, employing a variety of techniques for integrated pest management and retention of soil fertility. Planting cover crops is one strategy, used for centuries, that has been shown to

increase organic matter, reduce erosion, improve physical characteristics of the soil, prevent leaching of soil nitrogen, suppress weeds and reduce disease incidence (Snapp et al., 2005; Thurston, 1990). Recommendations for specific cover crops have been provided based on the unique advantages each is supposed to confer. For example, Gramineous species of cover crop (e.g., annual rye (*Lolium multiflorum* Lam.) and winter rye (*Secale cereale* M. Bieb)) improve soil physical structure, produce ample biomass adding to organic matter and sequester excess nitrogen in the soil, which prevents leaching (Snapp et al., 2005; Treadwell et al., 2010). Leguminous species (e.g., hairy vetch (*Vicia villosa* Roth), crimson clover (*Trifolium incarnatum* L.) and alfalfa (*Medicago sativa* L.)) can provide additional nitrogen through symbioses with nitrogen-fixing rhizobacteria (Snapp et al., 2005). Some members of the family Brassicaceae (e.g., forage radish (*Raphanus sativus* var.

Abbreviations: GLM, general linear model; CCT, cover crop treatment; AUDPC, area under disease progress curve; ANOVA, analysis of variance; HSD, Honestly Significant Differences; PPN/PAN, plant-pathogenic nematodes/plant-associated nematodes; RKN, root-knot nematodes.

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longipinnatus L.) and forage turnip (*Brassica rapa* var. *rapa* L.) have a long taproot, which facilitates water infiltration and breaks up compaction (Chen and Weil, 2009). This family also produces glucosinolates that hydrolyze to release various volatile compounds, the most biologically active of which is isothiocyanate. These compounds have been found to serve as effective biofumigants in some systems, acting similarly to the synthetic pesticide metam sodium, which generates methyl isothiocyanate when in contact with damp soil (Matthiessen and Kirkegaard, 2006). Mixing species of cover crops in one planting is a strategy to take advantage of the benefits promoted by each. For example, the carbon to nitrogen ratio can be balanced, facilitating a slower release of nitrogen, which can be better utilized by crops during the growing season. By mixing species with complementary growth patterns, a grower can also maximize weed control, nitrogen sequestration, biomass production and diversity of beneficial insect populations (Treadwell et al., 2010). For example, by pairing rye and vetch, the quick-growing rye will prevent weed establishment and utilize nitrogen added by the vetch, which grows more slowly (Snapp et al., 2005).

Numerous experiments have shown that cover crops can increase yield and protect crop plants from soilborne disease (Abawi and Widmer, 2000; Abdul-Baki et al., 1996; Bulluck and Ristaino, 2002; Larkin and Griffin, 2007; Mazzola and Mullinix, 2005; Zhou and Everts, 2004). However, not all studies have observed a benefit from specific cover crops or green manures on yield, disease severity or suppression of plant-pathogenic nematode (PPN) populations (Chellemi, 2006; Hartz et al., 2005). The complex interaction of various factors such as cover crop species or cultivar, soil characteristics, crop-pathogen system and environment determines the extent to which cover crops can beneficially impact vegetable crop health. Interpreting the impacts on crop health can be complicated. For instance, one study found that cover crops improved crop health, thereby leading to decreased seedling mortality despite increased disease severity caused by *Fusarium* spp. and *Pythium* spp. (Medvecky et al., 2007).

Fewer studies have investigated mixed-species green manures. A rye-vetch green manure reduced incidence of Southern blight on tomato and increased populations of beneficial Pseudomonads (Bulluck and Ristaino, 2002). A rye-legume mixture also increased yield of tomatoes and suppressed weeds more effectively than a rye monocrop (Teasdale and Abdul-Baki, 1998). A rye and a rye-field pea mixture of cover crops both had positive effects on tomato growth and yield as compared to bare ground (Akemo et al., 2000). Mixed species of hay used on land in transition to organic management reduced damping-off of tomato by *Pythium* spp. and *Rhizoctonia solani* by 3–30% (Baysal et al., 2008).

The goal of this study was to investigate the near-term effects of mixed-species green manures on subsequent plantings of tomato (*Solanum lycopersicum* L.) in organic production systems. The experiment was conducted in three states at multiple locations, each with different types of soilborne disease pressure, including Phytophthora blight caused by *Phytophthora capsici* Leonian (NY), Southern blight caused by *Sclerotium rofsii* Sacc. (MD), plant-pathogenic nematodes (MD) and *Rhizoctonia* root rot caused by *R. solani* J.G. Kühn (OH). Crop and soil variables were chosen to examine the effects of a single season application of cover crops on tomato productivity and disease severity. The hypothesis tested was that a one-season application of mixed-species cover crops would provide enhanced productivity and reduce disease severity regardless of location or year, as compared to single-species cover crop applications or a bare ground control. Statistical testing was performed for each field and year separately in order to detect site-specific effects.

Table 1
Seeding rates for CCTs in MD, NY and OH.

	Treatments and seeding rate (kg/ha)
<i>MD</i>	
1	Hairy vetch (79) + winter rye (79)
2	Hairy vetch (25)
3	Hairy vetch (42) + forage radish (42)
4	Mixed-species hay (125) ^a
5	No cover
<i>NY</i>	
1	Hairy vetch (34) + winter rye (79)
2	Crimson clover (10) + annual rye (18)
3	Forage turnip (15) + winter rye (45)
4	Winter rye (135)
5	No cover
<i>OH</i>	
1	Mixed-species hay (56/112) ^a
2	Winter rye (150)
3	Hairy vetch (50)
4	Hairy vetch (25) + winter rye (75)
5	Forage radish (10)

^a Mixed-species hay included red fescue (*Festuca rubra* L.), orchard grass (*Dactylis glomerata* L.), timothy (*Phleum pratense* L.), red clover and alfalfa. Composition was determined by equal seed number in MD and OH. Seeding rates in OH were 56 kg/ha in 2010 and 112 kg/ha in 2011 and 2012.

2. Materials and methods

2.1. Experimental design and management

Research was conducted in 2010, 2011 and 2012 at the University of Maryland Lower Eastern Shore Research and Education Center, Salisbury, the New York State Agricultural Experimental Station, Phytophthora blight research farm in Geneva and the Ohio Agricultural Research and Development Center, Wooster. The three states and years included in the study sum to sixteen different field-site years and a total of 370 separate plots.

The experiment was conducted as a randomized complete-block design with four blocks (NY and OH) or six blocks (MD) and five treatments per block. Each year different fields were used in each location in order to test the single season impacts of the cover crop treatments.

In MD, the trial was conducted in one field per year (5 treatments × 6 blocks) for a total of 30 plots per year. Plots were 64 m × 122 m and had two rows of black plastic on 21 m centers. A single row of tomatoes were transplanted 0.6 m apart within each row. Soil at this location was Fort Mott loamy sand and Rosedale loamy sand.

In NY, the trial was conducted in two fields per year (5 treatments × 4 blocks) for a total of 40 plots per year. Plots were 24 m × 76 m with one row of plastic on 31 m centers. A single row of tomatoes were transplanted 0.6 m apart within the row. Soil at this location was Odessa silt loam.

In OH, the trial was conducted in three fields (5 treatments × 4 blocks) for a total of 60 plots in 2010 and 2011, but only one field (5 treatments × 8 replicates) for a total of 40 plots in 2012. Plots were 31 m × 61 m with four rows of black plastic on 153 m centers. A single row of tomatoes were transplanted 0.6 m apart within each row. In this location, the soil was a Wooster Riddles silt loam.

The five treatments of mixed-species green manure combinations were different in each state based on local growing conditions and practices. The treatments and seeding rates are listed in Table 1. Cover crop seed was sown in the fall and the cover crop was mowed and tilled in as a green manure the following spring three to five weeks before transplanting the tomatoes (Supplementary Table A). Before tilling in the cover crop biomass, the fresh above-ground plant residue was weighed from a 12 m × 12 m portion of each plot

and recorded. Fields in all states had raised beds with black plastic and drip irrigation. Tomatoes were grown using standard organic practices including trellising appropriate to each location. Plants were irrigated throughout the season as needed.

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Tomato seeds were sown into a locally produced organic potting mix in 50-cell flats (TO Plastics, Clearwater, MN) and maintained in a greenhouse with 16 h of both natural and supplemental light per day. Seedlings were moved into a cold frame for at least 24 h before transplant. Cultivar Celebrity (Johnny's Select Seed, Winslow, ME) was used. This cultivar has disease resistance to Verticillium wilt, Fusarium wilt Races 1 and 2, root-knot nematodes, Alternaria stem canker and tobacco mosaic virus (Rutgers Cooperative Extension, 2013). This variety was chosen because it is widely grown in the test region and has fairly standard disease resistance. Because host resistance is part of an effective integrative disease management program, cultivar Celebrity was deemed to be part of a grower-relevant model for assessing cover crop effects.

2.2. Soil and plant productivity analysis

At the time of transplant (Supplementary Table A), 473 mL of soil was collected at a depth of 15–18 cm from each plot in 2010 and 2011 and analyzed at soil testing labs in each state, specifically Dairy One, Ithaca, NY, the University of Delaware Soil Testing Laboratory, Newark, DE and the Service Testing and Research lab, Wooster, OH. The analysis included organic matter (%), pH, phosphorus, potassium and calcium measurements.

Effects on early plant vigor were measured by comparing the height and fresh shoot weight of the above-ground portion of two tomato plants per plot at four weeks post-transplant (Supplementary Table A). Plant height was measured in the field from the crown at soil-level to the apical meristem and is reported as shoot height. The total above-ground weight of plants cut off at soil-level was measured in the field using a portable scale in NY (Fisher Scientific, Pittsburg, PA) or transported to the lab and weighed in MD (Sartorius Universal, Goettingen, Germany) and OH (Mettler Toledo, Toledo, OH) and is reported as shoot weight.

Tomatoes were harvested and weighed from each plot to determine the total yield (Supplementary Table A). Harvest began when at least 10% of the plots had ripe (>stage 5) fruit ready to harvest and each harvest was separated by 7–10 days. Only ripe fruit was harvested during the initial harvests, while all fruit larger than 4 cm in diameter were picked during the last harvest. For MD, in 2010, two plants per plot were harvested three times. In 2011, four plants per plot were harvested eight times. In 2012, four plants per plot were harvested five times. In NY, six plants per plot were harvested three times for 2010, 2011 and 2012. In OH, two plants per plot were harvested three times in 2010 and 2012 and two times in 2011.

2.3. Disease severity analysis

In Maryland, plots were rated for disease at approximately 10-day intervals during the growing season. Early blight (*Alternaria solani* Sorauer and *A. tomatophila* Simmons), Septoria leaf spot (*Septoria lycopersici* Speg.), and Southern blight (*Sclerotium rolfsii* Sacc.) were present each year. Early blight and Septoria leaf spot were rated as the average percentage of infected leaf area in three 1 m sections of each plot. Southern blight incidence was recorded as the percent of plants within a plot that were symptomatic. In Maryland only, each plot was also tested for the presence of nematodes. For counts of stylet-bearing nematodes, soil samples were collected four times each year: (1) pre-incorporation and (2) post-incorporation of green manures, (3) mid-season and (4) harvest

(Supplementary Table A). On each sampling date, six soil samples (25 cm diam. × 20 cm deep) were randomly collected from each plot with a soil core probe and combined (with the exception that 2 soil samples were collected per plot on 4/16/2010). Nematodes were extracted from 100 cm³ soil by centrifugal flotation (Jenkins, 1964). At mid-season and harvest (Supplementary Table A), one tomato root system was harvested from each plot (two root systems per plot on 8.26.10). These root systems were rated for root galling, and nematode eggs were then collected from the roots (method similar to Hussey and Barker, 1973) and enumerated.

In New York, plots were rated for disease weekly over the duration of the growing season. Three diseases were present each year: Phytophthora blight (*P. capsici* Leonian), Septoria leaf spot, and early blight. In NY fields 3 and 4 (2011), leaf mold (*Fulvia fulva* Cooke) and late blight (*Phytophthora infestans* (Mont.) de Bary) were present. The research farm where the six NY fields (2 per year) were located is known to be infested with *P. capsici* (Dunn and Smart, 2012). Phytophthora blight disease severity (%) was determined for each plot by assessing wilt, yellowing and stunting for all plants in the plot. Disease severity (%) of early blight, Septoria leaf spot, late blight and leaf mold was rated by percentage of leaf tissue affected for all plants in each plot.

In Ohio, plots were rated for disease at approximately 10-day intervals during the growing season. Early blight and Septoria leaf spot were present each year. Early blight and Septoria leaf spot were rated as the average percentage of infected leaf area in three 1 m sections of each plot.

2.4. Statistical analysis of productivity and disease severity data

Every crop productivity and disease-related factor from each field, state and year was analyzed separately using a general linear model (GLM) with cover crop treatment (CCT) and cover crop biomass run as fixed effects and block as a random effect. Cover crop biomass was included in the model because the biomass generated by each cover crop likely plays a large role in the effects measured. Therefore, the relationship between the cover crop species and the response variables after controlling for biomass could be evaluated. This avoided a possible erroneous correlation of effect to specific cover crop species, which may instead have been tied to the biomass generated by the species. Because cover crop biomass and treatment are inherently correlated, multicollinearity can inflate the standard errors of the model parameters. This would result in finding fewer significant pairwise differences and is therefore a more stringent test of CCT effects. However, since the biomass effects were deemed important as well, a separate ANOVA was run for the biomass of each treatment and results were added to the state-specific tables (Supplementary Tables B–G) for comparison between the results of the measured effect with the biomass generated by each treatment. In addition to the *P*-values generated by the statistical model, bar graphs were evaluated in order to mine for trends that may have been undetected by the statistical model. If the main effect of CCT was significant at *P* < 0.1, then a Tukey's Honestly Significant Differences (HSD) test was used to test for significant differences among CCTs. Analyses were performed using the statistical program R (R Development Core Team, 2011) with packages lme4 (Douglas et al., 2011), multcomp (Hothorn et al., 2008) and RLRsim (Scheipl et al., 2008). For disease severity data, the area under disease progress curve (AUDPC) was calculated from the rating measurements, data was confirmed to be normal, then used in the GLM analysis (Madden et al., 2007).

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Nematode data were transformed ($\log(x+1)$) and subjected to analysis of variance (ANOVA) with the GLM procedure of

SAS JMP (SAS, 2009). Nematode population density means were compared using Tukey's HSD test following a significant *F* test. Non-transformed data showing the actual number of nematodes per unit soil are presented for convenience in comparison to other papers and to numbers reported from grower's fields. Significant differences ($P < 0.10$) are discussed unless stated otherwise. Means were calculated excluding soil samples from which nematode counts were zero; the latter were excluded from analyses on the assumption that nematodes were present but populations were so low that individual nematodes were not detected in the subsamples.

3. Results

3.1. Soil and plant productivity analysis

In order to visualize the significance of the overall effects of CCT on all measured variables in the experiment, results for all fields are presented in Table 2. Overall, just 25% of the sixteen sites tested responded significantly to CCT in total yield or shoot weight early in the growing season. Similarly, soil variables measured within one week of tomato transplanting (three to five weeks following cover crop incorporation) differed significantly by CCT at no more than 31% of the tested sites (Table 2). More detailed results for each variable are presented in state-specific tables (Supplementary Tables B–D), which present the mean values and Tukey assignments for each variable when the effect of CCT was significant based on the GLM analysis. These tables are presented to allow for overall comparison of CCT effects. In order to visualize trends indicating an effect of treatment and cover crop biomass, bar graphs with productivity measurements and disease severity data were examined for topographical patterns, both among factors and between factors (Supplementary Data Graphs).

Soil analysis was conducted on a total of 290 plots from 13 fields in the three states over two years. Specifically, CCT significantly affected the pH of soil samples in four of thirteen fields tested. In MD 2012, bare ground and mixed-species hay CCT had a higher pH than other CCT (Supplementary Table B). In OH field 1 (2010), mixed-species hay, rye and vetch + rye had a significantly higher pH than radish. In OH field 5 (2011), rye had a significantly higher pH than vetch. In OH field 6 (2011), radish had a higher pH than vetch or rye (Supplementary Table D). Overall, the effect of CCT on pH was inconsistent. Likewise, soil organic matter (%) was significantly affected by CCT in just three out of the thirteen fields tested (Table 2). In MD 2012, the vetch + rye CCT had significantly more organic matter than bare ground plots or the vetch CCT (Supplementary Table B). In NY field 2 (2010), bare ground, vetch + rye and clover + rye had significantly higher organic matter than rye and turnip + rye CCT (Supplementary Table C). In OH field 2 (2010) all treatments provided significantly more organic matter than the mixed-species hay (Supplementary Table D). The effects of CCT on soil mineral availability were also examined. In three of the thirteen fields, potassium content was affected by CCT (Table 2). In MD 2012, the vetch + rye CCT contained significantly more potassium than all other treatments (Supplementary Table B). However, in OH field 5, the mixed-species hay treatment contained significantly more potassium than only the vetch + rye-treated plots. In OH field 6 (2011), mixed-species hay and rye CCT had significantly more potassium than vetch. In only one field of thirteen, OH field 4 (2011), CCT affected phosphorus in soil, where radish had significantly higher phosphorus content than vetch + rye and vetch (Supplementary Table D).

Rare and inconsistent effects of CCT on tomato plant vigor were noted in this study. In just two fields out of sixteen, OH field 2 and OH field 6, tomato shoot height was significantly affected by CCT (Table 2). Vetch-treated plants were significantly taller than the

Table 2
Significance of *F*-tests assessing effects of cover crop treatments (CCT) on productivity related variables by field in 2010, 2011 and 2012.^a

	Maryland						New York						Ohio							Total S/total ^c		
	2010		2011		2012		2010		2011		2012		2010			2011			2012			
	1 ^b	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	7			
Total yield	<0.01						0.7	<0.01	0.81	<0.01	0.73	0.17	0.25	0.41	0.81	0.61	0.7	0.7	0.01	4/16		
Shoot height	0.56	0.14	0.27	0.35	0.14	0.27	0.89	0.09	0.45	0.47	0.47	0.15	0.03	0.71	0.51	0.54	<0.01	0.51	0.51	2/16		
Shoot weight	0.01	0.32	0.32	0.84	0.32	0.32	<0.01	<0.01	0.17	0.44	0.43	0.36	0.13	0.82	0.63	0.14	0.7	0.01	0.01	4/16		
pH	0.46	0.19	0.02	0.19	0.02	0.02	0.89	0.18	0.64	0.33	ND	0.01	0.91	0.67	0.02	0.03	0.06	0.06	ND	4/13		
Organic matter	0.3	0.13	<0.01	0.13	<0.01	<0.01	0.32	0.16	0.15	0.15	ND	<0.01	<0.01	0.21	0.1	0.1	0.05	0.05	ND	3/13		
Phosphorus	0.73	1	0.49	0.58	0.58	0.49	0.58	0.49	0.49	0.49	ND	0.11	0.33	0.97	0.02	0.16	0.73	0.73	ND	1/13		
Potassium	0.11	0.96	<0.01	0.96	<0.01	<0.01	0.24	0.24	0.36	0.36	ND	0.68	0.59	0.51	0.47	<0.01	0.04	0.04	ND	3/13		

^a Numbers are *P*-values from the GLM test for cover crop treatment effects on the outcome of the measured variable. Values in bold are significant at $P < 0.1$ by GLM analysis as well as by Tukey analysis. Values of $P < 0.1$ but not highlighted in bold were not significant according to Tukey analysis. Abbreviation 'ND' signifies that no data was acquired for the measured variable in a given field.

^b Field number, with two fields per year in NY, three fields in OH for 2010 and 2011 and one field in OH for 2012 MD had one field per year. This totals to sixteen distinct fields.

^c Total significant (S)/total, where total (n) equals the number of fields where a variable was measured. If the measured variable was available in all three states, in each field in each state and for three years then $n = 16$. Total significant (S) is the total number of fields where one or more CCT significantly affected the productivity measurement at $P < 0.1$ as well as Tukey analysis.

mixed-species hay-treated plants in field 2, and in field 6 tomatoes grown following a radish CCT were significantly shorter than tomatoes in all other plots (Supplementary Table D). However, fresh shoot weight was affected by CCT in four out of sixteen fields (Table 2). In MD 2010, tomatoes planted in radish + vetch-treated plots weighed significantly more than tomatoes from all other treatments (Supplementary Table B). In NY field 1, the tomatoes planted in the bare ground plots weighed significantly more than those of all other treatments except those in vetch + rye-treated plots, while in NY field 2, plants in the bare ground plots weighed significantly more than those of all other treatments (Supplementary Table C). Finally, in OH field 7 (2012), tomatoes from radish CCT weighed more than those from mixed-species hay or vetch + rye CCT (Supplementary Table D).

The total yield was affected significantly by cover crop in just four out of sixteen test fields. These instances occurred in 2010 and 2011 for MD and NY. For these cases, the CCT conferring the greatest yield was not consistent (Supplementary Tables B and C, Supplementary Data (Productivity Graphs)). In MD 2010, bare ground, mixed-species hay and vetch-treated plots had significantly greater yields than radish + vetch or vetch + rye-treated plots. However, in MD 2011, vetch-treated plots yielded significantly more than bare ground or mixed-species hay-treated plots (Supplementary Table B). Interestingly, though not showing statistically significant differences among treatments, tomato yields following all mixed cover crops planted in NY field 1 (2010) were greater than the single rye cover crop, but this pattern was reversed in the field adjacent to it, NY field 2 (2010), where bare ground and rye-treated plots yielded higher than other treatments. The following year, the vetch + rye-treated plots in NY field 4 (2011) yielded the highest along with bare ground and rye-treated plots (Supplementary Table C).

The cover crop biomass also had an effect on total yield in all three states, but this also was not consistent (Supplementary Data (Productivity Graphs)). For instance, in NY field 2 (2010), the two highest yields were from the rye cover crop, which had the largest biomass, and the bare ground control, with no biomass (Supplementary Table C).

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2014.01.010>.

3.2. Disease severity analysis

In order to visualize the overall effect of CCT on disease severity, all results for all fields are presented in a table summarizing all statistical outcomes (Table 3). Overall, 0–44% of the tested sites with a noted disease were found to differ significantly across one or more CCT treatments. More detailed results for each disease that was found to be significant are presented in state-specific Supplementary Tables E–G as well as comparative graphs in Supplementary Data (Disease Graphs).

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3.2.1. Early blight

Early blight was consistently found in all three states in every year of the field trials. As seen in Table 3, in six out of fifteen fields, CCT affected early blight severity. Looking more closely at the significant results using Tukey analysis (Supplementary Tables E–G) as well as all field-year instances using comparative graphs (Supplementary Data (Disease Graphs)), it becomes clear that no one treatment had a significant beneficial or detrimental effect on the level of disease. In MD 2010, vetch had significantly more disease than radish + vetch. In MD 2011, mixed-species hay had significantly more disease than all other treatments except bare ground

Table 3
Significance of F-tests assessing effects of CCT on disease^a in 2010, 2011 and 2012.^b

	Maryland			New York			Ohio							Total S/total ^d
	2010		2012	2010		2011	2010		2011		2012			
	1 ^c	2		3	4		1	2	3	4	5	6	7	
Early blight	<0.01	<0.01	0.04	0.09	0.30	0.70	<0.01	0.01	0.18	0.84	0.2	ND	ND	6/15
Septoria blight	<0.01	<0.01	<0.01	0.45	0.05	ND	ND	ND	ND	0.71	0.57	ND	ND	4/9
Phytophthora blight	ND	<0.01	ND	0.67	0.01	0.67	ND	ND	ND	ND	ND	ND	ND	2/6
Leaf mold	ND	ND	ND	0.20	0.27	NA	ND	ND	ND	ND	ND	ND	ND	0/2
Late blight	ND	ND	ND	0.19	0.62	NA	ND	ND	ND	ND	ND	ND	ND	0/2
Southern blight	<0.01	ND	0.11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1/3

^a Disease measurements compared were AUDPC values calculated from approximately weekly ratings of disease severity over the season.

^b Numbers are P-values from the GLM test for cover crop treatment effects on the outcome of the measured variable. Values in bold are significant at $P < 0.1$ by GLM analysis as well as by Tukey analysis. Values of $P < 0.1$ but not highlighted in bold were not significant according to Tukey analysis. Abbreviation 'ND' signifies that no data was acquired for the measured variable in a given field.

^c Field number, with two fields per year in NY, three fields in OH for 2010 and 2011 and one field in OH for 2012 MD had one field per year. This totals to sixteen distinct fields.

^d Total significant (S)/total, where total (n) equals the number of fields where a disease was observed. If the disease was observed in all three states, in each field in each state and for three years then $n = 16$. Total significant (S) is the total of instances significant at $P < 0.1$ by both GLM analysis as well as Tukey analysis.

Table 4
Soil population densities of stylet-bearing nematodes in field plots in Salisbury, MD. Counts are per 100 cm³ soil. Plant-parasitic/plant-associated nematode genera detected included *Criconeoides*, *Ditylenchus*, *Helicotylenchus*, *Heterodera*, *Meloidogyne*, *Mesocriconema*, *Paratylenchus*, *Pratylenchus*, *Trichodorus*, *Tylenchorhynchus* and *Tylenchus*. Fungivores were species of *Aphelenchus* and *Aphelenchoides*.

	2010				2011				2012			
	16-April	29-April	25-June	26-August	14-April	16-May	6-July	13-September	25-April	24-May	24-July	20-September
<i>Plant-parasitic/plant-associated nematodes</i>												
Vetch + rye	299a ^a	300a	163a	148a	250ab	550ab	154a	133a	183a	105a	33a	37a
Mixed-species hay	371a	275a	84a	116a	300a	410ab	110a	137a	139ab	32b	58a	55a
Vetch	245a	148ab	90a	74a	123ab	315bc	59a	71a	141ab	150a	91a	33a
Radish + vetch	402a	298a	87a	80a	216ab	737a	129a	125a	139ab	80ab	60a	50a
Bare ground	143a	106b	105a	64a	40b	105c	40a	100a	68b	30b	25a	39a
<i>Fungivores</i>												
Vetch + rye	33a	75a	41a	66a	60a	205b	69a	35a	16b	108a	50a	30a
Mixed-species hay	78a	68a	50a	74a	68a	303ab	88a	38a	18ab	143a	38a	20a
Vetch	63a	65a	56a	53a	77a	408ab	115a	52a	54a	155a	56a	36a
Radish + vetch	78a	75a	137a	47a	40a	503a	131a	92a	44ab	108a	56a	30a
Bare ground	29a	31a	74a	41a	80a	150b	58a	52a	19ab	60a	75a	30a

^a Means within a column followed by the same letter in a specific nematode classification are not significantly different ($P < 0.10$) on a particular date according to Tukey's HSD test.

(Supplementary Table E). In NY field 2 (2010), the bare ground treatment had significantly more early blight than the turnip + rye or clover + rye treatments (Supplementary Table F). CCT significantly affected early blight in OH for 2010 and 2012. However, early blight severity was lowest in the mixed hay CCT in all three years (Supplementary Table G; data for 2011 not shown). Additionally, in OH field 1 (2010), the vetch + rye treatment had more early blight than mixed-species hay and rye. In OH field 2 (also 2010), rye-treated plots only had significantly more early blight than mixed-species hay-treated plots. In OH field 7 (2012) the vetch + rye treated fields had more early blight than vetch, mixed-species hay and rye-treated plots (Supplementary Table G).

3.2.2. *Septoria blight*

The only other disease found across all three states and years was *Septoria blight*, which affected nine out of sixteen fields. Of the nine fields affected, four fields indicated a significant effect of CCT on *Septoria blight* severity (Table 3). In MD 2010, vetch-treated plots had more severe disease than bare ground, mixed-species hay or radish + vetch-treated plots. However, in MD 2012, bare ground-treated plots had significantly more disease than all other treatments except mixed-species hay (Supplementary Table E). In NY field 2 (2010), bare ground plots had more disease than clover + rye and turnip + rye-treated plots, while in the nearby field 1 (also NY 2010), clover + rye and vetch + rye-treated plots had significantly more disease than rye-treated plots (Supplementary Table F). Overall, CCT effects on *Septoria blight* severity were infrequent and variable.

3.2.3. *State-specific diseases*

Maryland fields in 2010, 2011 and 2012 were rated for naturally occurring Southern blight. Out of the three years, only 2010 saw a significant effect of CCT, where vetch + rye-treated plots had significantly more disease than mixed-species hay-treated plots (Supplementary Table E). However, PPN/PAN levels tended to be higher in mixed CCT plots than single CCT or bare ground treatments, although the differences were not always significant (Table 4).

Of the plant-pathogenic nematodes/plant-associated nematodes (PPN/PAN) collected from the MD fields, the genus occurring most often and in the highest numbers within most plots was *Tylenchus* spp. This genus has been isolated from numerous plant hosts, but is considered to be root-associated rather than a true plant parasite (Yeates et al., 1993; Simard et al., 2008). *Tylenchus* spp. were identified from 313 of the 360 soil samples (87%), and accounted for 90.5% of all individual nematodes counted over

the three-year period in the PPN/PAN group. *Tylenchorhynchus* spp. were the most abundant PPN, counted from 90 of the 360 soil samples (25%) over the three years and comprising 4.9% of all individual PPN/PAN counted. Other PPN detected in plots were [genus (number of plots, year(s) collected; percent of all PPN/PAN counted)]: *Criconeoides* (3, 2012; 0.1%), *Ditylenchus* (20, 2010–2012; 0.7%), *Helicotylenchus* (8, 2010, 2011; 0.3%), *Heterodera* (6, 2010–2012; 0.2%), *Meloidogyne* (3, 2010; 0.1%), *Mesocriconema* (2, 2010; <0.03%), *Paratylenchus* (2, 2010; 0.2%), *Pratylenchus* (36, 2010–2012; 2.0%), and *Trichodorus* (23, 2010–2012; 1.0%). The other stylet-bearing nematodes collected from these samples were *Aphelenchoides* and *Aphelenchus*, which are primarily fungivores (Yeates et al., 1993). *Aphelenchoides* was collected from 11% of the soil samples (2010–2012; 4.1% of the individual fungivores counted over the three years), and *Aphelenchus* from 76% of the soil samples (274 soil samples, 2010–2012; 95.9% of fungivores).

Root systems were sampled at mid-season and harvest each year; nematode eggs were counted from ca. 13%, 37% and 3% of the collected root systems (mid-season) and 65%, 23%, and 17% (harvest) in 2010, 2011 and 2012, respectively. However, no galling or egg masses were observed on these root-knot nematode (RKN)-resistant tomato root systems, and it could not be determined by morphology which non-RKN taxa were the source(s) of the eggs.

Prior to green manure incorporation in the spring of 2010, population densities of PPN/PAN were similar among all cover crop treatments (Table 4; 16 April, $P < 0.10$). However, shortly after incorporation (29 April), the population densities were lowest in bare ground plots, with ca. 2.5–3 times more PPN/PAN in all other treatments except vetch. No significant differences were recorded among treatments at mid-season or harvest in 2010.

In 2011, PPN/PAN populations were 7.5 times higher in mixed-species hay plots than in bare ground plots after a winter of cover crop growth (Table 4, 14 April). Similar to 2010, after incorporation of green manures, densities were lower in bare ground plots on 16 May than in all treatments except vetch, with ca. 4–7 times more PPN/PAN in vetch + rye, mixed-species hay, and radish + vetch treatments than in bare ground plots. PPN/PAN counts from radish + vetch plots were also more than 2 times greater than in vetch plots. As in 2010, there were no significant differences among treatments at mid-season or harvest.

In 2012, spring (25 April) PPN/PAN densities in the soil with the winter cover crop plantings were lowest in bare ground plots, as in 2011. However, the only treatment with significantly higher numbers was vetch + rye, with ca. 2.5 times more PPN/PAN than bare ground plots (Table 4). Similar to the two previous years, population numbers were low in bare ground plots post-incorporation

(24 May), but in 2012 were also low in mixed-species hay plots. As in 2010, vetch + rye plots had more PPN/PAN after incorporation of green manures than bare ground plots (3.5 times greater in the former). Unlike the previous years, the vetch green manure also resulted in high PPN/PAN counts, with 5 times more than bare ground plots. Also, both vetch + rye and vetch green manures resulted in higher counts than the mixed-species hay green manure (24 May). No significant differences were found among treatments later in 2012.

Population densities of fungivores did not differ among treatments at any time in 2010 (Table 4). In 2011, population numbers were ca. 2.5–3 times higher in radish + vetch after incorporation of green manures (16 May) than in bare ground or vetch + rye treatments, but no differences were recorded at mid-season or harvest. In 2012, the only differences in fungivore populations were observed in the spring prior to green manure incorporation (25 April), with more than 3 times the number of fungivores counted in vetch plots than in vetch + rye plots.

Data from Table 4 and Supplementary Table B indicated that highest and lowest nematode populations did not follow the same trends as biomass quantity, except that the bare ground treatments, which had the lowest biomass, also tended to have the lowest numbers of PPN/PAN each year in the post-incorporation sampling time.

In NY, *Phytophthora* blight occurred in all fields and years (total of 6), where two fields saw a significant effect of CCT (Table 3). In NY field 2 (2010), turnip + rye-treated plots had significantly more disease than all other treatments except clover + rye. In NY field 4 (2011), turnip + rye had more disease than clover + rye, but not significantly more than other treatments (Supplementary Table F).

In NY fields 3 and 4 (2011), leaf mold and late blight were also rated. There was no significant effect from CCT on these diseases (Table 3).

4. Discussion

Mixed-species cover crops appeal to organic growers for a variety of reasons. However, in this study, we found no near-term effects of mixed-species green manures on total yields (Table 2) and disease levels (Table 3) of subsequent plantings of tomato (*S. lycopersicum* L.) in organic production systems across the Northeast region. The lack of consistent response of plant diseases to CCTs seems to be due to variations in both cultural practices and environmental factors. For example, cover crops were found to reduce severity of *Phytophthora* blight in North Carolina, but the mechanism was through reduced pathogen dispersal by splash, where the CCT was not tilled under (Ristaino et al., 1997). In this study, where we tilled in the cover crops, we found a contrasting pattern, with *Phytophthora* blight typically higher in mixed (and single) CCTs as compared to the bare ground control in NY. It may be that the act of incorporation provided material to support pathogen growth. But cultural factors are not likely the only culprit. For example, in MD, a tilled hairy vetch green manure significantly reduced disease by *Fusarium* wilt on watermelon both in the greenhouse and the field (Zhou and Everts, 2004). However, in the Maryland sites used in this study, there were no consistent reductions in any of the noted tomato diseases by tilled vetch, alone or in combination with other species (Supplementary Table E).

We think that the variable effects of CCT detailed in this large multi-site study are likely due to site-specific variations in environmental variables. Soil types varied from state to state, with Odessa silt loam in NY, Wooster Riddles silt loam in OH and loamy sand in MD. Cover crop establishment varied from year to year in each location, with limited growth in some cases due to environmental factors such as heavy rain or cold temperatures. Interactions between cover crops and crop plants appear to be

highly dependent on the environment and timing factors such as cover crop incorporation and time of transplant. This may be due to allelopathy as well as changes in microbial populations due to plant matter decomposition (Welbaum et al., 2004). Approximately four weeks were allowed between tilling of cover crops and transplant in order to prevent allelopathy, but cooler and drier conditions can slow decomposition. The measurements of plant height and shoot weight at four weeks post-transplant were useful in detecting possible effects of allelopathy on early crop growth. In two cases out of sixteen, both in 2010 NY fields when only three weeks were allowed between tilling and transplant, the bare ground control treatment conferred a significantly larger shoot weight than other treatments, except vetch + rye in field 1 (Supplementary Table C). This verifies the importance of waiting a minimum of four weeks between tilling and transplant.

Beneficial cover crop effects may be closely linked to the accumulation of organic matter and the resulting improvement of soil structure over time. Cover crops have been observed to increase soil aggregate stability, an important property of healthy soils (Magdoff et al., 2000), even after one season (Liu et al., 2005). While no correlation between cover crop biomass and tomato productivity were observed in this study (Supplementary Graphs and Supplementary Tables B–G), increases in total soil organic matter can improve soil and crop health under some soil conditions (Abawi and Widmer, 2000). Interestingly, each cover crop mixture produced a significantly different weight of biomass in most fields (thirteen out of sixteen). However, soil analyses showed few differences in soil organic matter (four out of sixteen) between treatments at around the time of transplanting, less than six weeks after incorporation (Table 2). Thus, while additions of organic matter may improve soil health and productivity, we found no consistent evidence for such an effect over a single cropping season.

In the MD fields, which combined a resistant tomato cultivar with green manure treatments in a field with low PPN pressure, there were two consistent results over the three-year period. These were: (1) shortly after incorporation of green manures, bare ground plots had lower PPN/PAN populations (comprised primarily of *Tylenchus* spp. and *Tylenchorhynchus* spp.) than plots with green manures, particularly plots with vetch + rye; and (2) the effect did not last throughout the growing season, as no differences were found among treatments at mid-season and harvest. Variable results have been reported from use of green manures for managing PPN populations, with some studies demonstrating suppression and others showing population increases (Thoden et al., 2011). Many parameters are involved, including status of the cover crop as a host plant, plant chemistry, biomass, and effects on other soil organisms (Oka, 2010; Thoden et al., 2011). Studies with rye and nematotoxic compounds from rye have resulted in suppression of PPN populations in some cases but not others (Meyer et al., 2009; Timper et al., 2011; Zasada et al., 2000). Combined with hairy vetch, rye did not decrease population numbers in this study. While incorporated rye cover crops increased populations of PAN (such as Tylenchidae) and fungivores in earlier MD studies (Gruber et al., 2010), no effect was observed on fungivores in the current study. Gruber et al. (2010) found no effect of radish on Tylenchidae or fungivores, which was generally the case with the radish + vetch treatment in our study. *R. sativus* green manure also was not nematotoxic to *Globodera rostochiensis* (Valdes et al., 2011). Results likely vary with nematode taxon, amount of plant biomass, cultivar and growing conditions.

While vetch can be a nematode host (Clark, 2007), this green manure showed some activity against nematodes in our study, but the results were not consistent. There was a trend the first two years (2010 and 2011) in which the hairy vetch green manure resulted in low PPN/PAN numbers after incorporation. This trend did not occur in 2012, when nematode populations in vetch plots were

high shortly after green manure incorporation. The lack of trends may be due to fact that the variety of tomato used in this study is resistant to RKN. Interestingly, in a 2012 greenhouse pot trial in which soil was taken from the MD fields immediately post green manure incorporation, inoculated with *Meloidogyne incognita*, and planted to a susceptible tomato variety (BHN 444), the vetch green manure resulted in the lowest number of *M. incognita* eggs on plant roots of all of the treatments (Meyer, unpubl.), indicating possible efficacy as a soil amendment against *M. incognita*.

Depending on the nutrient and organic matter content, weed pressure, erosion propensity or amount of compaction in the field, different cover crops or mixtures can serve the specific needs of a grower (Snapp et al., 2005). Our study clearly suggests that mixed-species and single-species cover crops are not able to consistently affect tomato crop productivity or suppress disease after a single season of incorporation across locations. However, repeated patterns in relative productivity and disease levels were observed for some CCT-site combinations, as discussed above. This indicates that it may be necessary for growers to closely evaluate the responses that occur on their land to any given CCT to ensure a positive return on investment. Such knowledge is important for soil management practices, where the recommendation has been to apply cover crops over various seasons in order to ensure benefits.

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