Influence of Summer Cover Crops and Mycorrhizal Fungi on Strawberry Production in the Southeastern United States

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Abstract. The effects of eight summer cover crop treatments combined with two arbuscular mycorrhizal (AM) fungal inoculants on strawberry growth and yields were examined in a 2-year field experiment. Cover crop treatments included 1) sudangrass [*Sorghum bicolor* (L.) Moench cv. Piper]; 2) pearl millet [*Pennisetum glaucum* (L.) R.Br. cv. 102 M Hybrid]; 3) soybean [*Glycine max* (L.) Merrill cv. Laredo]; 4) velvetbean [*Mucuna deeringiana* (Bort) Merr. cv. Georgia Bush]; 5) sudangrass/velvetbean combination; 6) pearl millet/soybean combination; 7) a non-mycorrhizal host consisting of rape (*Brassica napus* L. var. *napus* cv. Dwarf Essex) and buckwheat (*Fagopyrum esculentum* Moench) in Year 1 and Year 2, respectively; and 8) no cover crop control. Strawberry tips were inoculated with either a native mixture of several AM fungal species or a single species sold commercially, *Glomus intraradices*. Cover crop treatments were assessed for their aboveground biomass and nutrient uptake as well as their impacts on weed abundance and diversity, soil nutrients, and parasitic nematode populations. Cover crop and AM treatments were assessed for their impact on strawberry growth, yields, AM root colonization, and nutrient uptake. Grass-based cover crop treatments, particularly pearl millet, produced the most aboveground biomass. In both years, all cover crop treatments reduced summer weed biomass compared with the control. Neither cover crop nor AM treatments had an effect on overall strawberry plant growth or yields in either year, although some differences existed at specific growth periods. The results suggest that cover crops are a viable strategy for reducing summertime weeds and that background, native populations of AM fungi in the soil may be just as effective as a commercially available species. It is likely that no overall yield benefit was found among treatments for two reasons: 1) nutrients, especially nitrogen, were not limiting; and 2) the cover crop growth window may have been too short for a significant impact on strawberries over two seasons.

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Strawberries are a high-value crop and strawberry production has strong growth potential, especially in the southeastern United States and in North Carolina where most producers sell directly to consumers through pick-your-own and roadside stands (Sydorovych et al., 2006). In the Southeast, where strawberries are often replanted year after year in the same location, soilborne pathogens, root rot diseases, and weeds can significantly reduce yields (Wing et al., 1995). Conventional producers have traditionally dealt with these pest problems by fumigating with methyl bromide, which is under restricted use for strawberry production (United States Federal Government, 2009). With restrictions on methyl bromide increasing, many producers are considering different synthetic alternatives such as technical-grade 1,3-dichloropropene (Telone II), but the health risks may be significant (National Toxicology Program,

1985). There is a critical need for sustainable alternative approaches to pest and soil management for strawberry production in the southeastern United States and especially for organic production. These alternatives should focus on promoting healthy plants and enhancing beneficial soil organisms while reducing pests and diseases over the long term.

Although not a common practice, summer cover crops can be integrated into strawberry production and may play a critical role in sustainable soil and pest management strategies for strawberry production in the southeastern United States. The incorporation of cover crops with annual crops is an important strategy that can help prevent erosion, increase soil organic matter and fertility (Sarrantonio, 2007), break up hard clay soils, disrupt pest cycles, and reduce weeds (Phatak and Diaz-Perez, 2007). Moreover, legume cover crops can fix atmospheric nitrogen (N), leading to increased N availability and yields of the subsequent crop. Cereal cover crops can produce large amounts of biomass, increasing overall soil organic matter (Snapp et al., 2005). Few studies have documented use of cover crops in strawberry production. LaMondia et al. (2002) found sorghum–sudangrass [*Sorghum bicolor* × *S. sudanense* (Piper) Stapf] and ‘Saia’ oat (*Avena strigosa* Schreb.) cover crops decreased pest populations and increased strawberry yield. Elmer and LaMondia (1999) additionally found oat cover crops combined with (NH₄)₂SO₄ fertilizer reduced the incidence of strawberry black root rot. In general, these studies have demonstrated cover crops to benefit strawberries, but none have been conducted in the southeastern United States, where climatic and growth conditions differ and higher pest pressures exist.

Selective cover crop species may also improve the AM fungal inoculum potential and subsequent crop benefit from AM. Arbuscular mycorrhizas are known to support healthier, higher-yielding crops through increased nutrient acquisition, specifically phosphorus (Smith and Read, 1997) but also potassium, sulfur, copper, zinc, iron (Koide, 1991), and N (Hodge, 2003). Extraradical AM hyphae act as extensions of plant roots, increasing the absorptive surface for nutrient uptake. Plant benefits from AM are diverse and include increased shoot and root biomass, higher tissue nutrient concentrations, greater tolerance for drought conditions (Augé, 2001), and greater resistance to soilborne pathogens (Linderman, 1995). Mycorrhizal fungi have been demonstrated to increase strawberry growth and nutrient acquisition (Taylor and Harrier, 2001) and decrease root damage caused by *Phytophthora* (Norman et al., 1996).

Commercial AM inoculants are available but they generally consist of only one or a few species that may or may not be well adapted to the soil conditions where they are applied. Locally adapted AM fungal isolates can be produced on farm (Douds et al., 2008) and may be more effective than introduced species (Bull et al., 2005). Selection of cover crops that also function as good AM hosts

may increase native AM populations, leading to increased overall belowground functioning (Gosling et al., 2006). Enhancing AM host diversity through crop and cover crop rotations is thought to play a key role in increasing AM inoculum potential and the growth of subsequent AM-dependent crops (Gosling et al., 2006).

There is a variety of summer cover crops species that can be grown in the southeastern United States (Creamer and Baldwin, 2000) and many of these would support AM fungi. Summer cover crops can be integrated into strawberry production in North Carolina where strawberries are harvested by early June and the fields are replanted with new plants in October. During the months of June through August, the fields typically remain fallow. No study that we are aware of has examined the integrated approach of using summer cover crops and beneficial AM inoculants for strawberry production in field conditions. The primary objective of this study was to examine the combined effects of eight summer cover crop treatments (including a no cover crop control) and two AM inoculants on strawberry growth and yields in a 2-year field experiment. Secondary objectives included assessing differences in weed abundance and diversity, soil fertility, and parasitic nematode community among the cover crop treatments.

Materials and Methods

Experimental design. A field experiment was conducted at the Center for Environmental Farming Systems in Goldsboro, NC (lat. 35°36' N, long. 78°04' W) between May 2007 and June 2009. The site was organically managed from 2004 to 2006 as part of a previous strawberry experiment and was planted in perennial rye grass during the intervening months. This field study was designed as a split-plot randomized complete block design with four replications and two factors: cover crop as the main-plot factor (eight treatments) and AM inoculum as the sub-plot, split factor (two treatments). The eight cover crop treatments consisted of two grasses grown alone: 1) sudangrass (SG) [*Sorghum bicolor* (L.) Moench, cv. Piper; Kaufman Seed, Ashdown, AK] and 2) pearl millet (PM) [*Pennisetum glaucum* (L.) R.Br. cv. 102 M Hybrid; Albert Lea Seed House, Albert Lea, MN]; two legumes grown alone: 3) soybean (SB) [*Glycine max* (L.) Merrill cv. Laredo; Kaufman Seed, Ashdown, AK] and 4) velvetbean (VB) [*Mucuna deeringiana* (Bort) Merr. cv. Georgia Bush; Georgia Seed Development Commission, Athens, GA]; two grass/legume combinations: 5) sudangrass/velvetbean (SGVB) and 6) pearl millet/soybean (PMSB); 7) a non-mycorrhizal cover crop host (NONH) consisting of rape (*Brassica napus* L. var. napus cv. Dwarf Essex; Wyatt-Quarles Seed Co., Garner, NC) and common buckwheat (*Fagopyrum esculentum* Moench; Wyatt-Quarles Seed Co.) in 2007 and 2008, respectively; and 8) no cover crop control (NOCC) treatment. Mycorrhizal

pre-inoculation treatments consisted of either a native AM fungal species mix (NAT) or a single AM fungal species, *Glomus intraradices*, from a commercially available inoculant (COM). Cover crop treatments were randomized into four replicated blocks each consisting of eight 6.4 m × 12 m plots for a total of 32 plots. Each cover crop main plot was split into two raised beds 0.91 m × 4.5 m, which were spaced apart (2.44 m on center) to prevent mixing of AM sub-plot treatments. Location of cover crop and AM treatments were conserved both years. Soil samples were taken before cover crop and mycorrhizal treatments were established as a baseline measurement and again after Year 2 strawberry harvest. Soils had high phosphorus (437 mg-dm⁻³) and thus none was recommended for pre-plant fertilization.

Cover crop and field management. Cover crops were planted mid-June each year at the end of strawberry harvest in the piedmont region of North Carolina to correspond to realistic conditions of cover crop adoption. Cover crops were seeded fully across each plot (Table 1) using a Sukup 2055 No-till Grain Drill (Sukup Manufacturing Co., Sheffield, IA), and sudangrass was additionally seeded as a 3.6-m wide buffer between blocks. Legumes were pre-inoculated with either *Bradyrhizobium japonicum* for soybean or *Bradyrhizobium* sp. for velvetbean (INTX Microbials, Kentland, IN). Cover crops were cut with a 6 ft flail mower the first week of September each year and incorporated with a 21 ft disk. Soil fertility was managed based on initial soil analyses and nutrient recommendations for production in the southeastern United States (Poling and Monks, 1994). Organic pre-plant fertilizers, all OMRI-approved (Organic Materials Review Institute, Eugene, OR), were applied each year after a second disk pass before bedding as follows: broadcasted limestone (Crop Production Services, Princeton, NC) at 1800 kg-ha⁻¹; sodium borate spray (Crop Production Services, Princeton, NC) at 5.6 kg-ha⁻¹; broadcasted potassium sulfate 0N-0P-50K (Cooer Farm Supply, Smithfield, NC) at 112 kg-ha⁻¹; and broadcasted soybean meal 7N-1P-1K (J. Milo Pierce Farm Center, Pikeville, NC) at 800 kg-ha⁻¹. Soybean meal additions constituted 63 kg N/ha similar to the recommended 67 kg N/ha.

Mycorrhizal inoculum. The COM inoculum contained a single AM fungal species (*Glomus intraradices*) found in commercially available AM inoculant and was donated by Mycorrhizal Applications, Grants Pass, OR. The NAT mycorrhizal treatment consisted of a mixture of native AM fungal species isolated from the study site before the establishment of the treatments. To develop the NAT mycorrhizal inoculum, 20 random soil samples were taken at a depth of 15 cm from each main plot on 2 Apr. 2007, composited, and established as AM trap cultures in greenhouses at North Carolina State University (NCSSU). Trap cultures consisted of 300 g of each aggregate field plot soil sample mixed with steam-sterilized soil mix in a 1:4

Table 1. Cover crop seeding rates (kg-ha⁻¹) for 2007 and 2008.

Cover crop ²	2007	2008
NONH ³	12.4	73.1
SB	96.8	148.6
VB	124.3	187.7
SG	33.9	33.9
PM	35.0	34.4
SG (VB)	4.8 (86.6)	8.1 (188.3)
PM (SB)	12.9 (66.2)	21.5 (111.9)

²Cover crop treatments include: NONH = non-mycorrhizal host; SB = soybean; VB = velvetbean; SG = sudangrass; PM = pearl millet; SGVB = sudangrass + velvetbean combination; PMSB = pearl millet + soybean combination.

³NONH was dwarf rape in 2007 and buckwheat in 2008.

volume ratio in 6-inch pots. A combination of corn (*Zea mays* L.), sudangrass [*Sorghum bicolor* (L.) Moench], and prickly sida (*Sida spinosa* L.) was used as host plants and grown for 140 d. Each pot was fertilized with 1 tsp Osmocote 19N-6P-12K (Scotts Co., Marysville, OH) and water was limited in the final days to induce AM fungal sporulation.

The following fungal species were identified from the native (NAT) mycorrhizal inoculum: *Acaulospora koskei*, *A. laevis*, *A. mellea*, *A. morrowiae*, *A. scrobiculata*, *Glomus clarum*, *Gl. etunicatum*, *Gl. intraradices*, *Gl. mosseae*, *Gl. tenue*, two undescribed *Glomus* sp. (*Gl.* species 1 and *Gl.* species 2), *Paraglomus occultum*, and *Scutellospora heterogama*. The NAT inoculant was a mix of AM fungal spores, root fragments, and the sand/soil mixture. The COM inoculant was a solution of pure spores combined with a sterilized sand/soil mixture. Only the pure *Gl. intraradices* spores were used rather than the off-the-shelf mycorrhizal inoculant to separate the effects of the AM species from any additional materials commonly present in commercial inoculants such as fertilizers and soil additives.

Strawberry pre-inoculation and management. Each year, new organic Chandler strawberry tips were purchased from a certified nursery. In 2007, Chandler tips were used, but as a result of severe disease problems (*Phytophthora cactorum*) observed in the 2008 Chandler tips, a new set of non-inoculated (without mycorrhizal treatments), non-organic, Camarosa plugs were purchased. As a result of the disease infestation in 2008, the methodology presented in the following paragraph applies to 2007 only. Because the disease took a few weeks to detect, the Camarosa plugs were field-planted ≈1 month late on 13 Nov. 2008.

After the tips were received, they spent 1 week in cold storage (1 °C) and then were transferred to 50-cell trays (cell size: 4.8 cm, 4.8 cm, 5.7 cm) and grown in the NCSSU greenhouses for ≈3 weeks under misting benches. Tips were planted in OMRI-approved SunGro Natural and Organic Planting Mix composed of 75% to 85% Canadian sphagnum peat, perlite, dolomite lime, and an organic wetting agent (SunGro Horticulture, Vancouver, BC, Canada) combined with either the

NAT or COM mycorrhizal inoculant at a 3:1 volume ratio (≈ 25 g inoculant by weight) for each cell. Subsequent analysis revealed at least 40 AM spores per cell. Plants were misted with water three to five times per day for 15 s depending on conditions and fertilized once with Organic Biolink 5N-5P-5K (Westbridge Agricultural Products, Vista, CA) foliar spray (rate: 1.5 oz per gallon of water) during the third week of greenhouse growth. Plants were placed outside under shade to acclimate 1 week before field planting.

Plasticulture beds were formed 2 weeks before planting using a Kennco bed shaper and mulch layer (Kennco Manufacturing, Ruskin, FL). Twenty-six strawberry plugs were planted in each AM treatment bed in two rows at 12-inch (30.48 cm) spacing within each row for a total of 52 plants per cover crop main plot. Only the center 12 plants in each bed were used for yield data collection. Five of the remaining plants were used for biomass collection, leaving nine additional buffer plants. From 7 Mar. to 7 Apr. 2008, potash 0N-0P-60K (Crop Production Services, Princeton, NC) and OMRI-approved Phytamin 800 7N-0P-0K (California Organic Fertilizers, Hanford, CA) were each applied separately through drip irrigation at the recommended rate of 67.25 kg-ha⁻¹ each over 5 weeks. This resulted in a weekly application rate of Phytamin 800 at 240 kg-ha⁻¹ in Weeks 1 and 2 and 160 kg-ha⁻¹ in Weeks 3 through 5. In 2009, fertilizers were applied during the same time and the potash rate remained the same, whereas sodium nitrate 16N-0P-0K (Wood Creek Farm Supply, Cana, VA) comprised 20% of the total N applied. In 2009, Phytamin 800 was applied at the weekly rate of 192 kg-ha⁻¹ in Weeks 1 and 2 and 128 kg-ha⁻¹ in Weeks 3 through 5 and sodium nitrate was applied at the weekly rate of 21 kg-ha⁻¹ in Weeks 1 and 2 and 14 kg-ha⁻¹ in Weeks 3 through 5.

Data collection. Soil fertility and nematode community structure were analyzed from composited soil samples collected in Spring 2007 and Summer 2008. Cover crop plots were evaluated in Summer 2007 and 2008 for total weed aboveground biomass and weed diversity at 4 and 8 weeks after planting using three randomized 0.25-m² quadrates per plot. Shannon Index (H') was used for diversity calculations (Shannon and Weaver, 1949) according to the equation:

$$H' = - \sum_{i=1}^S p_i \ln p_i - \left[\frac{(S-1)}{2N} \right]$$

where p_i is the proportion of the total number of individuals of species i and can be found by $[p_i = n_i/N]$, where n_i is the number of individuals in species i , N is the total number of individuals collected in the entire sample, and S is the number of samples.

Eight weeks after planting the cover crops, a 0.5 m² quadrat was randomly placed in each cover crop plot and all cover crop aboveground biomass within the quadrat was cut at ground level, collected, dried at 60 °C for 96 h, and weighed. Subsamples of this

material were sent to the North Carolina Department of Agriculture (NCDA) Agronomic Services Laboratory for shoot nutrient analyses. In mixed cover crop treatments, the individual species were separated before analysis.

One representative strawberry plant per mycorrhizal treatment bed was collected at five time periods each year in 1) early January; 2) early March; 3) early April; 4) early May; and 5) late May (2008) or early June (2009). These dates were chosen based on key phenological stages of strawberry growth (Fernandez et al., 2001). Whole strawberry plants were removed from the field, washed thoroughly, and leaves, petioles, crowns, roots, and fruits/flowers (if any) were separated, dried at 60 °C for 96 h, and weighed. Before drying, fresh leaf area (cm²) was measured using a LI-COR LI-3100C Area Meter (LI-COR Biosciences, Lincoln, NE). Dried roots were later randomly subsampled for mycorrhizal colonization assessment, rehydrated, cleared, and stained (modified from Philips and Hayman, 1970). Percent mycorrhizal colonization of strawberry roots was assessed using a grid-line intersect method (Giovannetti and Mosse, 1980). Mycorrhizal fungal diversity was assessed through identification of fungal spores from trap cultures developed from five composited soil samples collected from each plot in May 2006 before the treatments were established and again in June 2009. Leaf nutrient analyses for the third and fifth biomass harvests were performed by the NCDA Agronomic Services Laboratory.

During harvest, marketable yield, cull yield, and average berry weight data were collected on the same 12 plants within each COM or NAT treatment bed. Average berry weight was calculated based on the number of berries harvested from the same 12 plants; if the number of berries exceeded 25, the calculation was based on a random sampling of 25 marketable berries. Culls were any berries deemed to be unmarketable as a result of size (less than 12 g), disease, or deformity.

Statistical analysis. All data were analyzed using the PROC GLM and PROC MIXED procedures of SAS Version 9.1.3 (SAS Institute, Cary, NC). Statistical significance was expressed at the $P < 0.05$ level. Log or square root transformations were applied to data before analysis if necessary to fulfill normality and homogeneity of variance assumptions. Pairwise mean comparisons were performed using Tukey's honestly significant difference. Treatments of cover crop, mycorrhiza, and their interactions were considered fixed effects, whereas rep and rep*cover crop were considered random effects. Data collected over time considered time as a crossed fixed-effect factor. Soil variables and cover crop biomass were analyzed following a randomized complete block design with four repetitions and eight cover crop treatments. Strawberry biomass, yields, and plant nutrient status were analyzed following a split plot for each time point and as a split-split plot with time as a sub-subplot factor for the combined analysis of all time

points. A three-way multivariate analysis of variance (MANOVA) was used to analyze the overall treatment effects for the biomass responses (root, crown, leaves, flowers, fruit) recognizing the dependency structure of these responses. Subsequent separate analyses of variance for each variable were also conducted.

Results

Cover crops. Cover crop biomass differed significantly in both 2007 ($P < 0.01$) and 2008 ($P < 0.01$) with treatments that included grasses generally being higher (Fig. 1). In both years, the fast-growing PM and SG grasses produced significantly more biomass than other cover crops in Week 4 (data not shown), but this difference among treatments was reduced by Week 8. Nutrient analysis of the cover crop shoots in 2007 revealed significant differences for all macro- and micro-nutrients (data not shown), whereas in 2008, all nutrients differed except iron ($P = 0.09$), manganese ($P = 0.57$), and copper ($P = 0.05$). Across both years, the PMSB and SGVB mixed treatments contained significantly higher concentrations of all nutrients analyzed. The average legume biomass component of the mixes was 9% for PMSB and 16% for SGVB.

After 8 weeks of growth, all cover crops had significantly less weed biomass than the controls in 2007 ($P < 0.01$) and 2008 ($P < 0.01$) with the PM and SG treatments showing the most consistent weed reduction (Fig. 1). Treatments containing grasses significantly reduced weed biomass compared with legume species alone ($P < 0.01$) and all cover crop treatments significantly reduced weed biomass compared with the no cover crop treatment ($P < 0.01$). Weed diversity (H') was significantly affected by cover crop treatments in 2008 ($P = 0.02$), showing reduced diversity in the PM and PMSB treatments compared with all other treatments. No difference in weed diversity was found in 2007 ($P = 0.83$) (Fig. 2).

Soil and nematode analyses. In 2007 and 2008, no significant differences were found in the soil fertility analyses performed on each cover crop plot when compared with the baseline measurement and among treatments (data not shown, $P = 0.51$). Analyses of the nematode community structure in 2007 and 2008 found no elevated levels of pathogenic nematode populations in baseline and among treatments (data not shown).

Strawberry yields, growth, nutrients, and mycorrhizas. Cover crop and mycorrhizal treatments did not significantly affect average berry weight, cull yield as a percent of total harvest, marketable yield, or total yield in 2008 or 2009 (Table 2) nor were there any interactions among treatments. A MANOVA of the root, shoot, crown, fruit, and flower weights and leaf area per plant across all sampling dates showed no significant effects from the cover crop or mycorrhizal treatments in either 2008 or 2009 (data not shown), although some of these biomass variables varied significantly between mycorrhizal treatments on individual dates.

Fig. 1. Mean aboveground cover crop and weed biomass ($\text{kg} \cdot \text{ha}^{-1}$) sampled 8 weeks after planting in 2007 and 2008. Cover crop treatments include NONH = non-host, dwarf rape in 2007 and buckwheat in 2008; SB = soybean; VB = velvetbean; SG = sudangrass; PM = pearl millet; SGVB = sudangrass + velvetbean combination; PMSB = pearl millet + soybean combination. Gray areas in the combination treatments represent the legume fraction. Error bars represent SE, and bars with the same letter are not significantly different according to Tukey's honestly significant difference ($P = 0.05$). Each year was analyzed separately as a one-way analysis of variance.

Native (NAT) mycorrhizal plants had significantly greater root, crown, and leaf dry weights (g) and leaf area cm^2 per plant on the 11 Jan. 2009 sample date and greater crown and leaf dry weights on the 11 May 2009 sample date compared with COM mycorrhizal plants (data not shown).

A MANOVA of strawberry leaf nutrients showed no significant effects from cover crop treatments in either the April (Wilks' lambda $P = 0.51$) or May 2008 sampling periods (Wilks' lambda $P = 0.87$; data not shown). In 2009, cover crop treatment did affect magnesium (Mg) concentration during the April sampling period (the PMSB treatment had significantly lower Mg than

all others, $P = 0.02$), but no differences were found in the June 2009 sample for any nutrient (Wilks' lambda $P = 0.176$; data not shown). Mycorrhizal treatments did significantly differ in their effect on overall nutrient analysis in April (Wilks' lambda $P = 0.02$) and late May 2008 (Wilks' lambda $P < 0.01$) as well as the next year in Apr. 2009 (Wilks' lambda $P < 0.01$; data not shown). Mycorrhizal treatments did not significantly differ in their effect on the overall nutrient status of plants harvested in June 2009 (Wilks' lambda $P = 0.25$). When analyzed individually, in Apr. 2008, the NAT mycorrhizal treatment had significantly higher levels of calcium, sulfur, copper, and boron

compared with the COM treatment. Additionally, the NAT mycorrhizal treatment had significantly higher levels of N, iron, and copper but lower levels of phosphorus, potassium, Mg, sulfur, and sodium when compared with the COM treatment in late May 2008 (data not shown). In 2009, many of these mycorrhizal treatment effects did not persist and only copper levels were greater in the NAT mycorrhizal treatments in April 2009, whereas manganese and zinc levels were higher in COM treatments in June 2009.

In 2008 and 2009, cover crop treatments did not significantly affect the percent mycorrhizal colonization in strawberry roots

Fig. 2. Mean Shannon Index (H') of weed diversity in 2007 and 2008. Cover crop treatments include: NOCC = no cover crop control; NONH = non-host, dwarf rape in 2007 and buckwheat in 2008; SB = soybean; VB = velvetbean; SG = sudangrass; PM = pearl millet; SGVB = sudangrass + velvetbean combination; PMSB = pearl millet + soybean combination. Error bars represent SE, and bars with the same letter are not significantly different according to Tukey's honestly significant difference ($P = 0.05$). Each year was analyzed separately as a one-way analysis of variance.

Table 2. Marketable and total yield, percent cull, and average berry weight for cover crop and mycorrhiza treatments in 2008 and 2009.

	Marketable yield (g/plant)		Total yield (g/plant)		Percent cull (unmarketable)		Avg fruit wt (g/berry)	
	2008	2009	2008	2009	2008	2009	2008	2009
Cover crop treatment²								
NOCC	425 ^a	113	488	173	9.5	37.6	17.7	16.0
NONH	486	92	537	143	11.4	35.2	18.8	14.8
SB	499	115	546	175	10.2	31.9	17.4	16.9
VB	521	99	576	189	8.1	34.1	18.7	17.2
SG	484	120	529	184	9.6	29.1	18.5	17.5
PM	431	133	481	163	8.5	32.7	17.5	16.9
SGVB	452	106	511	158	9.2	32.8	17.8	15.9
PMSB	489	128	540	146	12.6	32.1	18.0	16.1
<i>P</i> value	0.41	0.45	0.64	0.47	0.09	0.56	0.51	0.66
Mycorrhiza treatment³								
COM	482	108	533	161.0	9.4	34.1	18.2	16
NAT	464	118	519	171.0	10.4	32.3	17.9	16.8
<i>P</i> value	0.33	0.29	0.50	0.31	0.28	0.37	0.54	0.10
Interactions								
<i>P</i> value	0.64	0.21	0.64	0.18	0.91	0.46	0.48	0.47

²Cover crop treatments include: NOCC = no cover crop control; NONH = non-mycorrhizal host; SB = soybean; VB = velvetbean; SG = sudangrass; PM = pearl millet; SGVB = sudangrass + velvetbean combination; PMSB = pearl millet + soybean combination. NONH was dwarf rape in 2008 and buckwheat in 2009 harvest season.

³COM = commercial mycorrhiza; NAT = native mycorrhiza.

⁴Tukey's honestly significant difference showed no significant differences ($P < 0.05$) between treatments for the yield data presented here.

(2008 $P = 0.47$; 2009 $P = 0.08$; Fig. 3). The COM treatment had a significantly higher ($P < 0.01$) mean percent root colonization per strawberry plant in 2008 (37.8% versus 26.3%), but this treatment effect did not carry over into 2009 when the plants were not pre-inoculated ($P = 0.99$). The collection date was significant ($P < 0.001$) across both years

with a general trend of the percent AM colonization increasing over time (Fig. 3).

In baseline measurements, 16 AM fungal species were identified and *Gl. etunicatum* was the dominant species found in 88% overall the plots (Table 3). A native *Gl. intraradices* was found in 32% of the plots in baseline measurements. After strawberry

harvest in 2009, a total of 23 AM fungal species were identified and *Gl. etunicatum* again dominated and was found in 100% of NAT subplots and 97% of COM subplots. *Gl. intraradices* was not recovered in any of the COM subplots and was found in only 22% of NAT subplots.

Discussion

The ability of cover crops to reduce weeds was one of the main findings in this study. Results from our study showed a 67% reduction of weed biomass averaged overall cover crop treatments ($144 \text{ kg} \cdot \text{ha}^{-1}$) when compared with the no cover crop (bare ground) control ($440 \text{ kg} \cdot \text{ha}^{-1}$) over both years. The high biomass producers—pearl millet and sudangrass grown alone and the combination of pearl millet with soybean—reduced weed biomass by more than 98% compared with the no cover crop control. Cover crops are well known as an effective weed management strategy in organic farming systems and similar weed reductions have been found with other cover crop species (Mennan et al., 2009).

Although cover crops greatly reduced weed biomass, this did not result in cover crops enhancing strawberry total or marketable yields in either year. The positive benefit of cover crops to soil fertility, and any resulting strawberry yields enhancement, may accumulate over a longer period beyond the timeframe of this experiment. Although many other studies have found summer cover crops to enhance crop yields, including lettuce (Wang et al., 2008), okra (Wang et al., 2006), and organic kale (Mennan et al., 2009), these are likely the result of a longer time for cover crop growth. In a review of the effects of cover crops on soil health and subsequent cash crops, Fageria et al. (2005) found that when there was a crop benefit from cover crops, it came primarily from the effects of improved nutrient availability through added N from leguminous covers and/or increased soil organic matter from cover crop residues.

We believe we did not observe a strawberry yield benefit from cover crops in our study for three main reasons, which are not mutually exclusive: 1) the weather conditions and later planting date in the second year may have superseded any treatment effects; 2) the 8-week window for summer cover crops may not have been of sufficient duration to maximize cover crop biomass and organic matter and nutrient deposition; and 3) nutrients, especially N, were not limiting to strawberries and thus there was not an opportunity for improved nutrient availability from the cover crops. The major differences in strawberry yields were found between 2008 (526 g/plant) to 2009 (166 g/plant). This 68% yield reduction between years is a result of the combination of the later planting in 2008 and 2009 weather conditions. The cold and rainy weather in early Spring 2009 had a considerable effect on reducing yields and strawberry producers in this region experienced a 50% yield reduction this year (Poling, 2009).

Fig. 3. Percent arbuscular mycorrhizal (AM) colonization of strawberry roots from whole plants sampled at different days after planting for commercial (COM) and native (NAT) AM inoculants in 2008 and 2009. Vertical dashed lines represent peak stages of strawberry growth (as labeled). No significant difference was found among cover crop treatments in either year and therefore the data are not presented here. Values are mean percentages ($\pm$ SE; $n = 4$).

A general challenge for maximizing summer cover crop benefits in strawberry production in the southeastern United States is the limited growth period. In most of the region, cover crops cannot be planted until the second half of June (after strawberry harvest) and then must be cut by the end of August to leave time to prepare the strawberry beds for an early October planting. Increases in soil organic matter from the use of cover crops is a process that takes several years (Sarrantonio, 2007) and thus full benefits are not realized immediately. An important approach to enhancing benefits from cover crops and rotation in strawberry production will be to rotate fields such that strawberries would not follow each other in

the same field each year. This is a challenge for many strawberry growers in the southeastern United States who run roadside pick-your-own operations and simply do not have the space available or economic resources to rotate out of strawberry production.

It may be possible to observe benefits of enhanced nutrient availability from summer cover crops in strawberry production, even without rotation of fields, if either pre-plant fertilizers or fertilizer additions through drip irrigation are reduced. In our experiment, we followed the recommended fertility rates for strawberry production in the southeastern United States and thus, nutrients, especially N, were sufficient and any nutrient enhancement from cover crops was potentially

Table 3. Arbuscular mycorrhizal fungal species identified from trap cultures sampled from baseline soils in Spring 2007 and from COM and NAT and mycorrhizal treatments in June 2009. *Glomus intraradices* was the only species present in the COM inoculum.

AM species	Baseline	COM treatment	NAT treatment
<i>Acaulospora koskei</i>	X		X
<i>A. laevis</i>	X	X	X
<i>A. mellea</i>	X	X	
<i>A. morrowiae</i>	X	X	X
<i>A. rehmsii</i>		X	
<i>A. scrobiculata</i>	X	X	X
<i>A. spinosa</i>	X		
<i>A. species 1</i>			X
<i>Entrophospora species 1</i>			X
<i>Gigaspora margarita</i>		X	X
<i>Gi. rosea</i>			X
<i>Glomus clarum</i>	X	X	X
<i>Gl. etunicatum</i>	X	X	X
<i>Gl. intraradices</i>	X	X	X
<i>Gl. mosseae</i>	X	X	X
<i>Gl. tenue</i>	X		
<i>Gl. species 1</i>	X		X
<i>Gl. species 2</i>	X	X	
<i>Paraglomus occultum</i>	X		X
<i>Scutellospora heterogama</i>	X	X	X
<i>Sc. nigra</i>		X	X
<i>Sc. pellucida</i>	X	X	X
<i>Sc. persica</i>		X	X
Total AM species	23	16	14

COM = commercial mycorrhiza; NAT = native mycorrhiza; AM = arbuscular mycorrhizal.

obscured. Reducing N in drip irrigation, especially in organic strawberry production, could also help save producers considerable money (Sarrantonio, 2007). Cover crop residues may be releasing nutrients slowly over the strawberry season, yet it is difficult to assess when and how much nutrients are available and how this coincides with specific strawberry nutrient requirements and uptake physiology. Additionally, strategies that enhance cover crop biomass production (e.g., enhanced seeding rates or an addition of compost), reduce fertilizers, especially N, and enhance soil biological activity are important priorities for both organic and conventional strawberry producers.

No significant differences were found for strawberry yields between the AM fungal inoculants; thus, enhancement or pre-inoculation with the native, on-farm AM mixed species may be just as effective as purchasing a commercial AM fungal species. AM commercial inoculants may be necessary in instances when the native AM fungal populations are very low or missing, especially from the results of extensive methyl bromide applications. Although the COM treatment had higher strawberry root mycorrhizal colonization in 2008, no correlation has been shown between AM root colonization and host growth (Williams et al., 1992). No difference was found in the 2009 AM colonization

percentages, likely because we could not pre-inoculate the strawberry plugs and the native AM fungal species likely outcompeted any *Gl. intraradices* remaining from the COM inoculum. The native, on-farm AM inoculant had similar effects on strawberry yields and in several cases, the NAT treatment improved growth over the COM treatment. In the eight instances that the mycorrhizal treatments significantly differed, the NAT inoculant increased strawberry growth characteristics (root, crown, and leaf biomass and leaf area) over the COM inoculant 75% of the time. Furthermore, nutrient status of the strawberry plants between the two AM treatments showed significant variation during the third and fifth biomass harvests for specific nutrients (data not shown), favoring the NAT treatment early in the harvest season and the COM treatment later, yet neither the AM treatment differences in biomass growth nor the differences in nutrient uptake led to any significant difference in final strawberry yields. From a producer's perspective, it may be better to promote practices that enhance native AM diversity than to purchase an off-the-shelf inoculant based on one (or a few) AM species. Schreiner and Bethlenfalvay (1997) found that a mixed AM inoculum outperformed a single species inoculum on pea (*Pisum sativum*) plants. Klironomos (2003) also found that the greatest host benefit occurs between native mycorrhizas paired with native plants. It is likely that single species AM inoculants are being outcompeted by the native mixed species inoculants or that mixed inoculants have a better chance of initiating a more favorable host-endophyte relationship and offering a variety of host benefits.

Associations with AM can enhance crops in various ways, through increased nutrient uptake (especially phosphorus), disease suppression, tolerance to water stress, and improvement of the soil structure (Gosling et al., 2006). Dodd et al. (1990) found that using pre-crops (similar to cover crops) of cassava (*Manihot esculenta* Crantz), kudzu [*Pueraria phaseoloides* (Roxb.) Benth.], and sorghum (*Sorghum* sp.) increased AM colonization and subsequent yield of cowpea [*Vigna unguiculata* (L.) Walp.]. Although we found no link between the interaction of cover crops and AM treatments in the strawberry yields, the possibility remains that, over time, a rotation of cover crops could influence the underlying AM fungal community to benefit strawberry yields.

The yield benefits to strawberries from association with AM fungi are wide-ranging. Douds et al. (2008) found that AM inoculation increased strawberry yields by 17% compared with non-inoculated controls, translating to an average 3.6 additional fruit per plant. Mycorrhizal inoculation has been observed to increase strawberry yields with certain combinations of fungi and cultivars (Vestberg, 1992). Severity of *Phytophthora fragariae* was reduced by inoculation with AM (Norman et al., 1996), although other studies looking at different fungal species found no reduction (Baath and Hayman,

1984), highlighting the importance of pairing hosts, cultivars, and AM species appropriately. Moreover, organic production practices may also help promote AM populations. Bull et al. (2005) found no benefit to applying commercial AM inoculants in organic and non-fumigated strawberry systems when native AM were present.

Mycorrhizal functioning and benefits to crops can differ greatly between greenhouse and real-world field conditions. Our study was the first to evaluate the integrated effects of summer cover crops and inoculation with AM fungi on strawberry production in field conditions. The importance of investigating the effects of cover crops and AM together in field conditions cannot be overemphasized, because soil types, climate, fertility, weed pressures, and other variables can affect the outcome compared with greenhouse conditions where these factors are regulated. The complex functions of AM fungi in agroecosystems and the best management practices thereof are just beginning to be understood. The task remains to determine which cover crops and AM associations most benefit strawberries grown in the southeastern United States and how to package this information into systems-level practices that growers can adopt.

Literature Cited

- Augé, R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11:3–42.
- Baath, E. and D.S. Hayman. 1984. No effect of VA mycorrhiza on red core disease of strawberry. *Trans. Br. Mycol. Soc.* 82:534–536.
- Bull, C.T., J. Muramoto, S.T. Koike, J. Leap, and C. Shennan. 2005. Strawberry cultivars and mycorrhizal inoculants evaluated in California organic production fields. *Crop Management*. doi: 10.1094/CM-2005-0527-02-RS.
- Creamer, N.G. and K.R. Baldwin. 2000. An evaluation of summer cover crops for use in vegetable production systems in North Carolina. *HortScience* 35:600–603.
- Dodd, J.C., I. Arias, I. Koomen, and D.S. Hayman. 1990. The management of populations of vesicular-arbuscular mycorrhizal fungi in acid-infertile soils of a savanna ecosystem: I. The effect of pre-cropping and inoculation with VAM-fungi on plant growth and nutrition in the field. *Plant Soil* 122:229–240.
- Douds, D.D., G. Nagahashi, J. Shenk, and K. Demchak. 2008. Inoculation of strawberries with AM fungi produced on-farm increased yield. *Biol. Agr. Hortic.* 26:209–219.
- Elmer, W.H. and J.A. LaMondia. 1999. Influence of ammonium sulfate and rotation crops on strawberry black root rot. *Plant Dis.* 83:119–123.
- Fageria, N.K., V.C. Baligar, and B.A. Bailey. 2005. Role of cover crops in improving soil and row crop productivity. *Commun. Soil Sci. Plant Anal.* 36:2733–2757.
- Fernandez, G.E., L.M. Butler, and F.J. Louws. 2001. Strawberry growth and development in an annual plasticulture system. *HortScience* 36:1219–1223.
- Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection in roots. *New Phytol.* 84:489–500.

- Gosling, P., A. Hodge, G. Goodlass, and G.D. Bending. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agr. Ecosyst. Environ.* 113:17–35.
- Hodge, A. 2003. Plant nitrogen capture from organic matter as affected by spatial dispersion, interspecific competition and mycorrhizal colonization. *New Phytol.* 157:303–314.
- Klironomos, J.N. 2003. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology* 84:2292–2301.
- Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* 117:365–386.
- LaMondia, J.A., W.H. Elmer, T.L. Mervoch, and R.S. Cowles. 2002. Integrated management of strawberry pests by rotation and intercropping. *Crop Prot.* 21:837–846.
- Linderman, R.G. 1995. Roles of mycorrhizae in biocontrol, p. 1–26. In: Pflieger, F.L. and R.G. Linderman, (eds.). *Mycorrhizae and plant health*. APS Press, St. Paul, MN.
- Mennan, H., M. Ngouajio, E. Kaya, and D. Isik. 2009. Weed management in organically grown kale using alternative cropping systems. *Weed Technol.* 23:81–88.
- National Toxicology Program. 1985. Toxicology and carcinogenesis studies of Telone II [technical-grade 1,3-dichloropropene (CAS No. 542-75-6) containing 1.0% epichlorohydrin as a stabilizer] in F344/N rats and B6C3F1 mice (gavage studies), NTP TR 269. No. 85-2525. National Toxicology Program, Research Triangle Park, NC.
- Norman, J.R., D. Atkinson, and J.E. Hooker. 1996. Arbuscular mycorrhizal fungal-induced alteration to root architecture in strawberry and induced resistance to the root pathogen *Phytophthora fragariae*. *Plant Soil* 185:191–198.
- Phatak, S.C. and J.C. Diaz-Perez. 2007. Managing pests with cover crops, p. 25–33. In: Clark, A. (ed.). *Managing cover crops profitably*. 3rd Ed. Sustainable Agriculture Network, Beltsville, MD.
- Philips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55:158–161.
- Poling, B. 2009. Brief advisory—May 12. Berry mg email newsletter. 10(47).
- Poling, B. and D.W. Monks. 1994. Strawberry plasticulture guide for North Carolina, AG-505. NC State Univ. Coop. Ext. Service, Raleigh, NC.
- Sarrantonio, M. 2007. Building soil fertility and tilth with cover crops, p. 16–24. In: Clark, A. (ed.). *Managing cover crops profitably*. 3rd Ed. Sustainable Agriculture Network, Beltsville, MD.
- Schreiner, R.P. and G.J. Bethlenfalvay. 1997. Mycorrhizae, biocides, and biocontrol. 3. Effects of three different fungicides on developmental stages of three AM fungi. *Biol. Fertil. Soils* 24:18–26.
- Shannon, C.E. and W. Weaver. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, IL.
- Smith, S.E. and D.J. Read. 1997. *Mycorrhizal symbiosis*. 2nd Ed. Academic Press, San Diego, CA.
- Snapp, S.S., S.M. Swinton, R. Labarta, D. Mutch, J.R. Black, R. Leep, J. Nyiraneza, and K. O'Neil. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agron. J.* 97:322–332.

- Sydorovych, O., C.D. Safley, L.M. Ferguson, E.B. Poling, G.E. Fernandez, P.M. Brannen, D.M. Monks, and F.J. Louws. 2006. Economic evaluation of methyl bromide alternatives for the production of strawberries in the southeastern United States. *HortTechnology* 16:118–128.
- Taylor, J. and L. Harrier. 2001. A comparison of development and mineral nutrition of micropropagated *Fragaria* × *ananassa* cv. Elvira (Strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. *Appl. Soil Ecol.* 18:205–215.
- United States Federal Government. 2009. Protection of stratospheric ozone: The 2009 critical use exemption from the phaseout of methyl bromide. 40 CFR, Part 82. Fed. Regist. 74:19878–19900.
- Vestberg, M. 1992. The effect of vesicular–arbuscular mycorrhizal inoculation on the growth and root colonization of ten strawberry cultivars. *Agr. Sci. Fin.* 1:527–534.
- Wang, G., M. Ngouajio, M.E. McGiffen, Jr., and C.M. Hutchinson. 2008. Summer cover crop and in-season management system affect growth and yield of lettuce and cantaloupe. *HortScience* 43:1398–1403.
- Wang, Q., Y. Li, and W. Klassen. 2006. Summer cover crops and soil amendments to improve growth and nutrient uptake of okra. *HortTechnology* 16:328–338.
- Williams, S.C.K., M. Vestberg, M. Uosukainen, J.C. Dodd, and P. Jeffries. 1992. Effects of fertilizers and arbuscular mycorrhizal fungi on the post-vitro growth of micropropagated strawberry. *Agronomie* 12:851–857.
- Wing, K.B., M. Pritts, and W. Wilcox. 1995. Biotic, edaphic and cultural factors associated with black root rot in New York. *HortScience* 30:86–90.