Cover Crop Residues Enhance Growth, Improve Yield, and Delay Leaf Senescence in Greenhouse-grown Tomatoes

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Additional index words. Lycopersicon esculentum, Vicia villosa, hairy vetch, Secale cereale, rye, cover crop mulches, nitrogen fertilizer

Abstract. Cover crop management in growing horticultural produce has attracted attention for reducing soil erosion and limiting the input of synthetic fertilizers and pesticides. Hairy vetch (Vicia villosa Roth.), one of the cover crops, exhibits desirable attributes such as high N fixing ability, biomass quality, adaptability to low temperatures, resistance to pests, and fitness in vegetable production, particularly in rotation with tomatoes. The interactions between the cover crop mulch and the tomato plant in the field plots result in delayed leaf senescence and increased disease tolerance. The mechanisms underlying these interactions are largely unknown. Limits in pursuing these studies year-round in the field—growing season and complexity and variability of the field environment—could be circumvented if the observed responses of tomato plants to hairy vetch mulch in the field could be reproduced under greenhouse conditions. We have tested tomato plants for two years in the greenhouse using soil residues brought from field plots where respective cover crops had been previously grown. Treatments were a) bare soil from a fallow, weed-free field plot, b) soil from a field plot that had been planted into a rye cover crop, and c) soil from a field plot that had been planted into a hairy vetch cover crop. Pots with soil from the rye or vetch field plots were further topped with rye or vetch residues, respectively, after transplanting the tomato plants. Additional N was applied to 50% of the plants in each treatment. In the greenhouse, cover crop residue-supplemented tomatoes exhibited higher vigor, higher marketable yield and delayed senescence compared to those grown in bare soil. All treatments responded favorably to additional N from commercial fertilizers. Delayed leaf senescence correlated with the accumulation of rubisco large subunit and chitinase, two proteins central to photosynthesis and pathogenesis, respectively. This study shows that the responses of tomato plants to cover crops seen in the field can be mimicked under greenhouse conditions.

Traditionally, agricultural practice in the old world (Hargrove and Frye, 1987) and early in the America (Betts, 1953; Meisinger et al., 1991) depended on using legumes, such as hairy vetch (Vicia villosa Roth.) and faba bean (Vicia faba), as green manure cover crops. In fact, green manures from leguminous cover crops were the only source of soil fertility, other than animal manures, before synthetic fertilizers came into use (Hargrove and Frye, 1987). The earliest investigations on the use of leguminous cover crops as green manure in the United States focused on their role in soil fertility, particularly with respect to fixing nitrogen (N) and adding organic matter to the soil (Bailey et al., 1930; Coleman, 1939; Evans et al., 1954; Henson and Howell, 1960; Pieters and McKee, 1929; 1938; Ripley, 1941). As agriculture became mechanized in the 1930s involving large cultivation equipment, synthetic fertilizers and pesticides, cover crop use gradually diminished. This new, mechanized, large-scale farming system led to increased crop production and reduced losses due to pests. However, the use of heavy chemical inputs resulted in synthetic fertilizer and pesticide run-offs, therefore negatively impacting agro-ecosystem (National Research Council, 1989; Pryne, 1993). Thus, the need for alternative, environmentally friendly farming systems has grown.

Recent research has focused on cover crop management strategies that would result in reduced soil erosion and reduction in applying synthetic fertilizers and pesticides (Hall et al., 1984; Hoyt, 1985, 1987; Hoyt and Hargrove, 1986; Rice et al., 2001). Tomatoes, sweet corn and other vegetables have been alternated with leguminous cover crops (Abdul-Baki and Teasdale, 1993; Hargrove et al., 1954; Morse, 1995). Hairy vetch, more than any other cover crop, has been shown to exhibit a number of highly desirable attributes—high N fixing ability, biomass quality, adaptability to low temperatures, resistance to pests, and fitness in vegetable production rotations. In several field studies, tomato plant production was more favorable in hairy vetch mulch than bare soil or conventional polyethylene mulch. These responses include a) higher yield and lower production costs (Abdul-Baki et al., 1997; Kelley et al., 1995; Lu et al., 2000); b) longer harvest period, extending by 3 to 4 additional weeks due to delayed senescence (Teasdale and Abdul-Baki, 1997); c) reduced input of N fertilizer (Abdul-Baki et al., 1997); and d) reduced infection by field-borne pathogens (Abdul-Baki et al., 1997; Mills et al., 2002). These positive responses of hairy vetch mulch-grown tomato have economic merit and, consequently, warrant further investigation.

Importantly, the need is to analyze in detail the mechanisms underlying the interactions between cover crop mulch and the tomato plant. One of the limitations in pursuing these studies year-round in the field is imposed by the growing season and the complexity and variability of the field environment. Such limitations could be circumvented if the observed, field responses of tomato plants to hairy vetch mulch could be reproduced under greenhouse conditions. Towards that goal, we carried out studies to test the behavior of tomato plants in the greenhouse using soil and residues from field plots where respective cover crops had been previously grown. We provide evidence that the principles and benefits of alternative agriculture system are upheld in such greenhouse-grown tomatoes. The cover crop residue-supplemented tomatoes exhibited higher vigor, higher marketable yield and delayed senescence compared to those grown in bare soil. This study was initiated to determine if the responses of tomato plants to cover crops seen in the field are mimicked under greenhouse conditions.

Materials and Methods

Source of organic mulches. Two cover crops, hairy vetch and 'Abruzz' rye, were seeded in two adjacent fields in mid-September of 2001 and 2002 at the North Farm, Beltsville Agricultural Research Center in Maryland, at the rate of 45 and 100 kg·ha⁻¹, respectively, using a Brillion seeder (Brillion Iron Works, Brillion, WI). At early flowering of hairy vetch and complete pollination stage of rye, the plants were flail mowed 5 cm above soil surface. The residues were left for several days in the field to dry and then raked and used as mulch residue for the greenhouse-grown tomatoes.

The amount of dry cover crop biomass residue from the mowed hairy vetch and rye plots added as mulch to each pot for growing tomatoes in the greenhouse was calculated using the following information based on many years of field data (Abdul-Baki et al., 1996). Hairy vetch and rye produce an average of 5.5 and 6.0 t·ha⁻¹ of biomass, respectively; field tomatoes are planted in 1.5-m-wide beds and within 45 cm of row spacing, resulting in 14,350 plants/ha; the actual area inhabited by

Received for publication 25 May 2004. Accepted for publication 11 Mar. 2005. We thank Robert Fluhar (PR-1, PR-2 and PR-3), T. Boller (chitinase), and Marvin Edelman (rubisco) for antibodies; Douglas J. Mills for statistical analysis; Peter Ewashkow and Antonio G. Pittarello for help with greenhouse experiments; and Dave Clark for photography.

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the tomato plants at maturity is about 50% of the field area. Based on these assumptions, 380 g of hairy vetch residue or 420 g of rye residue were added to respective pots after tomato seedlings were transplanted.

**Tomato seedlings and soil types.** ‘Sunbeam’ tomato seeds were seeded in April 2002 and 2003, in 128-cell styrofoam trays as described (Abdul-Baki and Teasdale, 1993). Three soil types were used: a) soil from a hairy vetch field plot after mowing and removing the above-ground biomass and leaving the below-ground (roots) biomass in the ground, b) soil from a rye field plot in the same manner as (a), and c) soil from a weed-free, fallow field planted without cover crops. The field plots used had a keyport fine sandy loam, Aquic Hapludult, clayey, mixed, mesic soil, with a 2% slope. Soil was taken from the upper 25 cm of each plot and 28 kg (dry weight) was weighed and placed in plastic pots each having a 35-cm inner diameter. Two 5-week-old tomato seedlings were transplanted into each pot and supported by a stake. Each treatment consisted of eight replicates (one pot with two plants formed a replicate). The experiments were designed using completely randomized block and the plants were grown under natural lighting in the greenhouse, where the minimum and maximum temperatures were, respectively, 25 and 33 °C.

**Supplemental N.** Only one of the two sub-treatments received supplemental N. Recognizing that hairy vetch and rye residues contain about 3.2% and 1.1% N (Teasdale and Abdul-Baki, 1998), respectively, and that the recommended N application for field tomatoes in Maryland is 170 kg ha⁻¹ (Maryland Cooperative Extension, 2003), pots with rye and bare soil received a total of 70 g of N while pots with hairy vetch received 35 g of N per pot. Supplemental N in the form of granular ammonium nitrate was applied weekly, wherever indicated, over a period of 14 weeks starting one week after transplanting.

**Leaf senescence assessment.** Leaf senescence was assessed biweekly using the trapezoidal method as previously described (Mills et al., 2002). Measurements were made visually by estimating the percentage of senescent leaves in the lower, middle, and upper canopy regions of each plant, averaged and analyzed using the trapezoidal method (Campbell and Madden, 1990).

**Biomass and yield.** Dry biomass of tomato plants (roots included) excluding the fruits was measured 14 weeks after transplanting. The entire plants without the fruits were carefully pulled out of pots, roots were rinsed to remove soil from their surfaces, and then dried at 80 °C until constant weight. Fruit yield was determined on duplicate sets of plants in each treatment. Fruits at the breaker or turning stage (Mehta et al., 2002) were harvested every 5 to 6 d. Harvest began 14 weeks after transplanting and continued until the plants stopped producing marketable fruit. Fruit were separated into marketable and nonmarketable categories and only marketable fruit weight was recorded.

**Protein extraction, SDS-PAGE, and immunoblotting.** Total soluble proteins were prepared as described elsewhere (Mattoo et al., 1981) with some modification. Frozen tomato leaf tissue (1 g) was ground to a fine powder in a mortar with a pestle and resuspended in 3 volumes of ice-cold buffer containing 0.4 M sucrose, 0.01 M N-[tris-(hydroxymethyl)methyl] glycine (Tricine)-NaOH, pH 8 at 25 °C, and 0.01 M NaCl. Proteins were quantified by the Bradford method (Bradford, 1976) using BSA as the standard. Total proteins (8 µg) from each leaf sample were electrophoresed on linear 10% to 20% gradient SDS-polyacrylamide gels and electroblotted onto nitrocellulose membrane and then immunodecorated (Callahan et al., 1989). The alkaline phosphatase-based method (BCIP/NBT phosphatase substrate system, NPL) was used as the detection system.

**Statistical analysis.** Analysis of variance was performed on data collected for leaf senescence, biomass, and marketable yield. All analyses were performed using the PROC GLM procedure of SAS version 8.2.

### Results and Discussion

**Total biomass and marketable fruit yield are enhanced in cover crop residue-supplemented greenhouse-grown tomatoes.** In the absence of added N, the total biomass of tomato plants was readily enhanced when rye or hairy vetch residues were used as soil covers compared to bare soil but the effect of hairy vetch was clearly noticeable (Table 1). Supplemental N, as expected, resulted in increasing the total biomass in bare soil-grown tomatoes by an order of magnitude but the maximal values were significantly below those for the cover crop-treated ones. The plant growth responses to the cover crop residues were higher in hairy vetch than in rye, partly because hairy vetch as a legume fixes an appreciable amount of N. In contrast, rye residues do not provide much N and may actually sequester some of the N. In contrast, rye residues do not provide much N and may actually sequester some of the N. Supplemental N in hairy vetch caused a positive, significant response, although the hairy vetch residues contained 12.2 g N per pot. The additional response to supplemental N may be due to either nonavailability or under-utilization of N present in the hairy vetch residue (Abdul-Baki et al., 1997). N release from the hairy vetch residue begins when tomato plants are in the early stages of growth and, therefore, it may not be well synchronized with the demand of the plant (unpublished data).

**Delayed leaf senescence in greenhouse tomato plants grown under cover crop soil cover is associated with sustained presence of Rubisco-large subunit and chitinase.** There was an over-all delay in leaf senescence in cover crop residue-grown tomato plants, which was particularly longer in plants grown under hairy vetch residue compared to either rye or bare soil, with or without supplemental N (Table 1, Fig. 1A and B). Without supplemental N, leaf senescence was observable at 33 d after planting, advanced soon thereafter, and reached about 98% in bare soil and 70% in rye by 108 days after planting with these greenhouse-grown tomato plants. Nonetheless, tomatoes grown with the hairy vetch mulch without supplemental N had higher yields than those grown with the rye residue. Supplemental N in hairy vetch caused a positive, significant response, although the hairy vetch residues contained 12.2 g N per pot. The additional response to supplemental N may be due to either nonavailability or under-utilization of N present in the hairy vetch residue (Abdul-Baki et al., 1997). N release from the hairy vetch residue begins when tomato plants are in the early stages of growth and, therefore, it may not be well synchronized with the demand of the plant (unpublished data).

**Table 1. Effects of mulch treatment with or without nitrogen supplement on tomato plant biomass, marketable fruit yield and leaf senescence.**

<table>
<thead>
<tr>
<th>Soil cover</th>
<th>Supplemental N</th>
<th>Tomato biomass (g)</th>
<th>Marketable yield/ plant (g)</th>
<th>AUDC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare soil</td>
<td>No</td>
<td>19 f</td>
<td>310 e</td>
<td>5089 a</td>
</tr>
<tr>
<td>Bare soil</td>
<td>Yes</td>
<td>101 d</td>
<td>852 f</td>
<td>3558 b</td>
</tr>
<tr>
<td>Rye residue</td>
<td>No</td>
<td>116 d</td>
<td>3208 e</td>
<td>1164 c</td>
</tr>
<tr>
<td>Rye residue</td>
<td>Yes</td>
<td>125 c</td>
<td>2539 b</td>
<td>2166 d</td>
</tr>
<tr>
<td>Hairy vetch residue</td>
<td>No</td>
<td>141 b</td>
<td>1989 c</td>
<td>532 f</td>
</tr>
<tr>
<td>Hairy vetch residue</td>
<td>Yes</td>
<td>188 a</td>
<td>3039 a</td>
<td>1368 e</td>
</tr>
</tbody>
</table>

*aPlants without fruit but roots included.

*bArea under the senescence (defoliation) curve.

*cData from year 2002 test.

*dMeans followed by the same letter are not significantly different at P ≤ 0.05 according to the LSD (least significant difference) test. Marketable yield and AUDC values are the average of two independent experiments carried out in 2002 and 2003.

**Marketable fruit yield per plant was modulated in a similar manner as the total biomass, i.e., it was higher when rye or hairy vetch residues were used as soil covers compared to bare soil but the effect of hairy vetch was more pronounced.** Supplemental N significantly increased marketable yield in all treatments, with the highest response exhibited by the plants grown in bare soil followed by those in the rye residue (Table 1). Irrespective of exogenous N supplementation, the over-all marketable fruit yield was highest under hairy vetch, intermediate under rye residue and lowest in bare soil (Table 1). Analysis of variance for marketable fruit yield, shown in Table 3, indicated significant main and interaction effects as were seen with biomass data.

**Mechanical pollination to maximize fertilization and fruit set was not carried out with these greenhouse-grown tomato plants, nonetheless, tomatoes grown with the hairy vetch mulch without supplemental N had higher yields than those grown with the rye residue.** Supplemental N in hairy vetch caused a positive, significant response, although the hairy vetch residues contained 12.2 g N per pot. The additional response to supplemental N may be due to either nonavailability or under-utilization of N present in the hairy vetch residue (Abdul-Baki et al., 1997). N release from the hairy vetch residue begins when tomato plants are in the early stages of growth and, therefore, it may not be well synchronized with the demand of the plant (unpublished data).
Supplemental N significantly delayed leaf senescence in plants grown in bare soil or under rye residue (Fig. 1B, compare −N with +N panels). Tomato plants grown in rye-mulch with supplemental N exhibited onset of leaf senescence 15 d after those plants that did not receive exogenous N. Senescence progressed at a reduced rate in the N-supplemented bare soil and rye-mulch plants but the effect was pronounced in the latter (Fig. 1B). Surprisingly, supplemental N provided as NH$_4$NO$_3$ actually enhanced leaf senescence in hairy vetch residue-grown plants (Table 1, Fig. 1B). It is known that NH$_4^+$ ions can inhibit NO$_3^-$ uptake by plant roots (Chaillou et al., 1994). Thus, changes in the NO$_3^–$/NH$_4^+$ ratio in the rhizosphere can drastically affect plant growth. Unlike bare soil and rye treatments, vetch also releases relatively more N during its decomposition. This ad-

Table 2. Analysis of variance of biomass of tomatoes grown in pots with or without a cover crop and with or without nitrogen supplement.$^*$

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop (CC)</td>
<td>2</td>
<td>46761</td>
<td>388</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N supplement (N)</td>
<td>1</td>
<td>55148</td>
<td>458</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CC × N</td>
<td>2</td>
<td>1436</td>
<td>11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>120</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$The ANOVA procedure of SAS version 8.2 was used.

Table 3. Analysis of variance of marketable yield of tomato grown in pots with or without a cover crop and with or without nitrogen supplement$^*$.  

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Mean square</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover crop (CC)</td>
<td>2</td>
<td>4434748</td>
<td>239</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>N Supplement (N)</td>
<td>1</td>
<td>4460388</td>
<td>241</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CC × N</td>
<td>2</td>
<td>172780</td>
<td>9.34</td>
<td>0.0004</td>
</tr>
<tr>
<td>Error</td>
<td>42</td>
<td>18506</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^*$The ANOVA procedure of SAS version 8.2 was used.
Field-grown ones—hsp70 level decreased in leaves from 108-d bare soil-grown plants but remained quite high in the similar age plants grown in the field on hairy vetch (Kumar et al., 2004). The reason for this difference remains to be determined but it indicates that hsp70 may be ephemerally related to leaf senescence syndrome.

Conclusions

We show that tomatoes grown in the greenhouse in cover crop residues—from hairy vetch and rye—exhibit enhanced biomass, higher marketable fruit yield and delayed senescence as has been found to be the case with tomatoes grown in cover crops in the field (Abdul-Baki and Teasdale, 1993; Mills et al., 2002; Kumar et al., 2004). These attributes in hairy vetch residue-grown tomatoes were associated with higher content of basic chitinase, an anti-fungal protein (Broglio et al., 1991) and continued presence of the key chloroplast protein, large subunit of Rubisco, which is a determinant in leaf senescence (Mae et al., 1983; Mehta et al., 1992), as compared to the plants grown on bare soil. Thus, the greenhouse system appears to mimic some attributes of tomato plants under field conditions.

The greenhouse system should facilitate the investigation of complex interactions between the plant and its external environment, particularly with regard to the roles of organic matter and soil microbial activity in plant growth. Under greenhouse conditions, the decomposition rate of organic matter and soil microflora population can be analyzed without the interference of climatic changes or changes due to soilborne microorganisms. Temperature, moisture and pests are additional factors that can be better managed in the greenhouse. By controlling some or most of these factors in the greenhouse, experiments can be conducted year-round, and should lead to more consistent results, particularly on the mechanisms involved in alternative agriculture systems, such as the hairy vetch system.

The soil system supplemented with cover crop residues and N under greenhouse conditions should allow plant nutritionists and microbiologists to elucidate the roles of organic mulches and soil microorganisms in plant growth, and to optimize the interactions between the plant, soil nutrients and the microbial system to reduce chemical input, maximize nutrient use efficiency, and minimize nutrient losses to the environment while reducing production costs.

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