Allelopathic Impact of Cover Crop Species on Soybean and Goosegrass Seedling Germination and Early Growth

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Abstract: Cover crops can provide a variety of benefits to an agricultural system: weed suppression, soil quality improvement, and soil water infiltration. Although there is ample research documenting weed suppression from cover crops, the mechanics of the suppression are not implicitly understood. Along with the aforementioned positive attributes, negative allelopathic effects on row crops planted into cover crop systems have been documented. The objective of this study was to evaluate the allelopathic potential of certain cover crop species on soybean (Glycine max L.) and goosegrass (Eleusine indica L.) germination and early seedling growth under controlled environments in petri dish and pot experiments. Leachates from above-ground biomass of five cover crop species, wheat (Triticum aestivum L.), cereal rye (Secale cereale), hairy vetch (Vicia villosa), crimson clover (Trifolium incarnatum L.), and canola (Brassica napus L.), from two locations (East and Middle Tennessee) were extracted and applied at 0 (water) and 50 v/v. In experiment I, both soybean and goosegrass seeds were examined, and, in experiment II, only soybean seeds were examined under the application of cover crop leachates. Most cover crop leachates from both locations significantly reduced the soybean seedling root length ($p < 0.01$). Overall, the application of canola extract (East Tennessee) suppressed soybean seed germination the most (28%) compared to deionized water. For goosegrass, the wheat cover crop leachate significantly reduced seeding root length ($p < 0.01$). In experiment II, the soybean root nodulation was significantly increased with the wheat extract treatment compared to deionized water. While the results indicate that the location and environment may change cover crop species allelopathic potential, the wheat cover crop leachate had the most potent allelopathic impact on goosegrass germination and growth; however, had the lowest observed adverse effect on our tested row crop, soybean.

Keywords: allelopathy; cover crop species; goosegrass (Eleusine indica L.); soybean

1. Introduction

The adoption of conservative farming practices, such as no-tilling systems, has increased the utilization of cover crops in agronomic systems [1]. Cover crops offer many potential benefits to a production field, including weed suppression, soil quality improvement, and soil water infiltration [2,3]. These known benefits are only part of the equation; naturally produced allelochemicals from cover crops could suppress herbicide-resistant weeds and support a sustainable agricultural production system [4]. However, research has also documented negative allelopathic effects on row crops planted into cover crop systems [5,6], specifically, the suppression of seedling growth on succeeding cash crops [1,7,8]. Allelopathy involves the positive or negative effects of a plant (donor), including microorganisms, on neighboring plants (targets) through the release of chemical compounds into the environment, mostly in the soil [4]. Xuan et al. [9] reported that the most common pathways for allelochemicals to be introduced in the soil are through leaching, volatilization, root exudation, and the death and decay of the fallen plant parts via biotic or abiotic means.

Understanding the link between cover crop management and allelopathic dynamics can help avoid negative impacts on the growth and productivity of the subsequent row
crop [1,6]. It is important to determine the allelopathic compatibility of cover crop species with row crops before incorporating them into agronomic systems, as phytotoxins released by cover crops could affect the establishment of row crops [10].

Furthermore, allelopathy has offered a new alternative for the development of ecofriendly agricultural practices, with the dual purpose of enhancing crop productivity and maintaining ecosystem stability [4]. Therefore, finding a biological solution to minimize the perceived hazardous impacts from herbicides and insecticides in agriculture production is critical [9]. Strategies for weed control vary largely among cropping systems, but mainly rely on the application of synthetic herbicides [11]. Continuous use of the few available active ingredients in herbicides has enhanced the selection of resistant weed populations [12], which have increased immensely in the last decades, thus demanding that weed management strategies evolve accordingly [11].

The objectives of this study were to assess the allelopathic impacts of (i) five cover crop species on germination of soybean (Glycine max L.) and goosegrass (Eleusine indica) in a lab bioassay, and (ii) three cover crop species on the growth and development of soybean seedlings in a greenhouse environment.

2. Materials and Methods

2.1. Cover Crop Species and Harvesting Samples

Five cover crop species, namely, wheat (Triticum aestivum L.), cereal rye (Secale cereale), hairy vetch (Vicia villosa), crimson clover (Trifolium incarnatum L.), and canola (Brassica napus L.), were considered in this study. Cover crop species were planted at two locations, Tennessee AgResearch and Education Center in Spring Hill (35.7187–86.9657) (Middle Tennessee (MT)) and Tennessee AgResearch and Education Center in Knoxville (35.9020–83.9575) (East Tennessee (ET)) in October 2019. Cover crop species seeding rates were based on recommendations in the University of Tennessee extension publication “Forage and Field Crop Seeding Guide for Tennessee” [13]. Soil information for both locations is presented in Table 1. The experimental design was a randomized complete block design with three replications in each location. Plot size was four rows (4.0 m) wide and 9.1 m long. The average temperature in the field between October 2019 and the middle of May 2020 was 11–12 °C. Plots were kept under rainfed conditions.

Table 1. Soil properties for the Tennessee AgResearch and Education Center in Knoxville (ET) and the Tennessee AgResearch and Education Center in Spring Hill (MT).

| Location | pH  | Phosphorus (kg ha⁻¹) | Potassium (kg ha⁻¹) | Calcium (kg ha⁻¹) | Magnesium (kg ha⁻¹) | Soil Type                        |
|----------|-----|----------------------|---------------------|-------------------|---------------------|----------------------------------|
| ET       | 6.3 | 82                   | 111                 | 1388              | 173                 | Sequatchie loam (fine-loamy, siliceous, semiactive, thermic Hapludult) |
| MT       | 5.6 | 101                  | 102                 | 1960              | 214                 | Maury silt loam (fine, mixed, active, mesic Typic Paleudalf)             |

In May 2020, the above-ground biomass of cover crop species was hand-harvested from two replicated plots of each cover crop species and transported back to a lab at the West Tennessee AgResearch and Education Center in the University of Tennessee (35.6301–88.8577), where leachates were extracted from the fresh cover crops and used as a supplement for soybean and goosegrass plants from seedlings.

2.2. Extract Preparation

The five aforementioned cover crop samples were prepared in a multistep process as described by Shekoofa et al. [1]. The samples were oven-dried at 60 °C for 5 days, ground to pass a 1 mm screen, and then kept in a refrigerator at 2–4 °C until used. Ten grams of each cover crop sample was soaked overnight in 100 mL of deionized water at 25 °C for 24 h. The following day, a beaker containing the mixture solution with a magnet inside was
placed on a shaker and run for 15 min at 900 rpm. The extract was then filtered through four layers of cheesecloth and centrifuged at 4000 rpm for 30 min. The liquid was then filtered through Whatman no. 42 paper. Stock extracts were made fresh, the day prior to initiating the experiment, and were kept refrigerated for the duration of the experiment.

2.3. Experiment I

2.3.1. Culture and Seed Bioassay

Stock extract was diluted with sterile, deionized water to give the final concentration of 50% (v/v) rate for each cover crop, while the deionized water treatment served as a check. Four milliliters of each cover crop extract treatment were then pipetted onto Whatman no. 2 filter paper in a 9 cm diameter plastic petri dish. The soybean genotype ‘Ellis’ [14] seeds, along with goosegrass seeds received from The University of Tennessee Weed Diagnostics Center, were surface-sterilized with 2% (v/v) commercial bleach solution for 3 min, rinsed with deionized water, and allowed to air dry. Ten soybean and 30 goosegrass seeds were then placed in respective petri dishes. Each cover crop treatment was replicated four times, resulting in 44 petri dishes each of soybean and goosegrass (i.e., two locations, five cover crop species extract plus a check with deionized water, and four replications), totaling 88 petri dishes. The petri dishes were placed on a lab bench in a lit room at 25 °C. Four milliliters of each treatment extract was added to the petri dishes every other day for 8 days.

2.3.2. Data Collection

Germination rate (Ng/day) was calculated as the number of germinated seeds (radicles >1 mm long). Measurements were repeated at 24 h intervals. Hypocotyl and root lengths were measured for all seedlings in each petri dish 8 days after placing the soybean and goosegrass seeds on the medium. Fresh and dry weights of the root and seedling lengths and germination percentage (%) in each petri dish were then measured.

2.4. Experiment II

Extending this study into the greenhouse increased the scope of cover crop materials needed to produce the amount of extracts. Therefore, only three of the five cover crops were selected for the greenhouse study, including ‘hairy vetch’, ‘canola’, and ‘wheat’ from both locations (ET and MT) and deionized water (check/no treatment), to evaluate the physiological effects of cover crop on soybean plants further growth and development.

The three cover crop leachates were extracted using the same method described above for experiment I with one change; instead of 10 g, 100 g of each sample was soaked overnight in 1000 mL of deionized water at 25 °C for 24 h.

2.4.1. Plant Material and Cover Crop Extract Application in the Greenhouse

The soybean cultivar ‘Ellis’ [14] was grown in a greenhouse at the West TN AgResearch and Education Center. Four seeds per pot were sown at a depth of 2 cm into a moistened soil composed of 50% sand and 50% Lexington silt loam (fine-silty, mixed, active, thermic Ultic Hapludalf) in 3.8 L pots (18 cm × 19 cm) and inoculated with N-Dure™ soybean inoculant (Verdesian Life Sciences, Cary, NC, USA). There were three pots in each treatment group. Pots were covered with aluminum foil immediately after planting to aid in retaining moisture and then removed 2 days after planting (DAP) as the application of extracts treatments commenced. Soybean plants were thinned to two plants per pot at 12 DAP, and aluminum foil was reapplied so that the soil surface was covered and water loss via transpiration was reduced. Natural light was supplemented with 600 W high-pressure sodium lamps (P. L. Light Systems (Lincoln, ON, Canada) to maintain 500–550 µmol·m⁻²·s⁻¹ at the plant level, in a 15 h day and 9 h night schedule. Temperature and relative humidity in the greenhouse were recorded every 5 min with EL-USB-2-LCD data loggers (Lascar Electronics Ltd., Erie, PA, USA). Daily temperature was maintained at 32 °C, and relative humidity was maintained at 60%.
Treatments were applied every 4 days (150 mL) at 2, 6, 10, 14, 18, and 22 DAP with supplemental applications (150 mL) of tap water at 4, 8, and 12 DAP. Treatment applications at 14, 18, and 22 DAP increased to 200 mL to account for increased water loss from the pots due to plant growth. Hairy vetch ET and canola MT treatments were terminated at 22 DAP due to limited cover crop biomass, and final sampling was conducted; the remaining treatments continued until 26 DAP.

2.4.2. Data Collection

Emergence was monitored daily after planting to calculate soybean germination percentage. Plant height was measured as the height of each plant from the top of the soil level to tip of the plant, conducted approximately every 4 days, beginning at 8 DAP, on 12, 16, 20, 22, and 26 DAP. Leaf number per plant was observed after the first trifoliate leaf was fully developed; it was measured at 16, 20, 22, and 26 DAP. Chlorophyll content was measured at 20 DAP using a Chlorophyll Meter SPAD 502 (Konica Minolta, Tokyo, Japan) on the two youngest, fully expanded leaves on each plant. Stem diameter was measured at 22 and 26 DAP with UltraTech digital calipers (General Tools & Instruments, New York, NY, USA). At the conclusion of the study, plants were cut at soil level. Leaf area for each plant was measured with a Licor LI-300C leaf area meter (Licor Biosciences, Lincoln, NE, USA) before the above-ground biomass was dried in an oven at 60 °C for 12 h and then weighed. The root mass was gently removed from the pot and the soil was washed away. Tap root length was noted and a total root length was also noted if lateral roots extended beyond the tap root when fully extended. Root nodules were counted before all below-ground tissue was dried in an oven at 60 °C for 12 h and weighed.

2.5. Statistical Analysis

2.5.1. Experiment I

Treatments were arranged in a randomized complete block design with four replications. Statistical analysis was completed using JMP Pro v.15 (SAS Institute, Cary, NC, USA). Replications for each parameter in each measurement were averaged to give a value per extract treatment. Then, values for soybean and goosegrass parameters were compared using the one-way ANOVA model. Statistical significance was set at $p \leq 0.05$. The treatment means were separated using Tukey’s HSD test (SAS Institute, Cary, NC, USA).

2.5.2. Experiment II

The experimental design for experiment II was a completely randomized design with three replicate pots in each treatment and two plants in each pot. The germination percentage measurement was analyzed per pot, while measurements on plants were recorded individually. Group means for each parameter on each day and cumulative total for germination percentage were analyzed using JMP Pro 14 (SAS Institute, Cary, NC, USA) with a mixed model ANOVA. The 95% confidence intervals for each group mean were calculated and are presented in Table 2.
Table 2. Germination percentage, leaf number per plant, and plant height of soybean seedlings throughout experiment II. Time of measurement is represented by days after planting (DAP). Group means are presented ± the 95% confidence interval.

| Time Period | Cover Crop Extract | 4 DAP | 6 DAP | 8 DAP | 10 DAP | Mean |
|-------------|--------------------|-------|-------|-------|--------|------|
|             | Hairy Vetch ET     | ±16.7 | ±16.7 | ±16.7 | ±16.7  | ±16.7|
|             | Wheat ET           | ±25.0 | ±28.9 | ±28.9 | ±28.9  | ±28.9|
|             | Canola ET          | ±50.0 | ±50.0 | ±50.0 | ±50.0  | ±50.0|
|             | Hairy Vetch MT     | ±33.3 | ±33.3 | ±33.3 | ±33.3  | ±33.3|
|             | Wheat MT           | ±41.7 | ±41.7 | ±41.7 | ±41.7  | ±41.7|
|             | Canola MT          | ±16.7 | ±16.7 | ±16.7 | ±16.7  | ±16.7|

| Leaf number per plant | Cover Crop Extract | 4 DAP | 6 DAP | 8 DAP | 10 DAP | Mean |
|------------------------|--------------------|-------|-------|-------|--------|------|
| Hairy Vetch ET *       | ±2.0 a             | ±0.0  | ±0.4  | ±0.4  | ±0.4   | ±0.4 |
| Wheat ET               | ±1.8 a             | ±0.4  | ±0.3  | ±0.3  | ±0.3   | ±0.3 |
| Canola ET              | ±1.8 a             | ±0.3  | ±3.3  | ±3.3  | ±3.3   | ±3.3 |
| Hairy Vetch MT         | ±1.6 ab            | ±0.5  | ±5.6  | ±5.6  | ±5.6   | ±5.6 |
| Wheat MT               | ±1.7 a             | ±0.4  | ±2.7  | ±2.7  | ±2.7   | ±2.7 |
| Canola MT *            | ±1.2 b             | ±0.3  | ±2.7  | ±2.7  | ±2.7   | ±2.7 |
| Check (Water)          | ±2.0 a             | ±0.0  | ±2.8  | ±2.8  | ±2.8   | ±2.8 |

| Soybean plant height (mm) | Cover Crop Extract | 4 DAP | 6 DAP | 8 DAP | 10 DAP | Mean |
|---------------------------|--------------------|-------|-------|-------|--------|------|
| Hairy Vetch ET *          | ±77.5 a            | ±11.8 | ±128.2ab | ±9.5 | ±171.7ab | ±11.8 | ±195.7ab | ±14.4 | ±240.2a | ±24.4 |
| Wheat ET                  | ±66.0 a            | ±20.3 | ±115.0ab | ±24.8 | ±146.8b | ±32.2 | ±181.5ab | ±37.0 | ±230.7a | ±45.2 |
| Canola ET                 | ±80.9 a            | ±19.0 | ±135.5a | ±14.4 | ±187.3a | ±13.5 | ±214.5a | ±27.5 | ±256.0a | ±31.4 |
| Hairy Vetch MT            | ±69.7 a            | ±21.1 | ±120.2ab | ±27.4 | ±142.6b | ±27.4 | ±166.0b | ±28.5 | ±211.0ab | ±27.7 | ±245.8b | ±25.5 |
| Wheat MT                  | ±66.7 a            | ±12.8 | ±119.3ab | ±14.5 | ±148.3b | ±14.4 | ±181.2ab | ±22.2 | ±227.0a | ±19.9 | ±276.2ab | ±15.6 |
| Canola MT *               | ±44.2 b            | ±15.3 | ±77.5c | ±22.6 | ±98.5c | ±29.6 | ±118.0c | ±35.3 | ±164.3b | ±43.5 |
| Check (Water)             | ±57.8 ab           | ±11.5 | ±107.5b | ±14.3 | ±140.7b | ±17.5 | ±169.3b | ±21.2 | ±214.8a | ±32.8 | ±256.2b | ±33.5 |

* Results for 26 DAP are not shown due to early termination of treatment. Means with different letters are significantly different from one another p < 0.05.
3. Results
3.1. Experiment I
3.1.1. Soybean

There was significant variation in soybean germination percentage among cover crop treatments for the Middle Tennessee (MT) location. Germination percentages for MT canola and cereal rye were significantly ($p < 0.05$) higher compared to MT wheat and crimson clover. For East Tennessee (ET), germination percentage was numerically lower for cover crop treatments compared to the check (water), but not statistically significant (Figure 1a). All cover crop treatments resulted in a lower germination rate (Ng/day) than the check (water). Reductions in germination rate (Ng/day) among cover crop treatments were statistically significant ($p < 0.05$) compared to the check (water) for ET canola and cereal rye, and from both locations for wheat, crimson clover, and hairy vetch (Figure 1b). Similarly, soybean seedling root length was reduced in all cover crop treatments compared to the check (water). Statistically significant ($p < 0.05$) reductions in root length occurred in treatments that received cover crop extracts from ET canola and wheat from both locations (MT and ET) compared to the check (water) (Figure 1c).

Figure 1. Effects of cover crop extracts on germination percentage (a), germination rate (b), and root length (c) for soybean seeds. Error bars represent the mean ± SE. Means with different letters are significantly different from one another $p < 0.05$. ‘ET’: East Tennessee location; ‘MT’: Middle Tennessee location.
3.1.2. Goosegrass

There was no significant interaction with location among cover crop extract treatments for goosegrass germination and growth parameters; therefore, the data are combined within this section. Hairy vetch extract was found to stimulate the germination percentage of goosegrass (Figure 2a) compared to the check (water), cereal rye, and wheat. Crimson clover, cereal rye, and wheat extracts all reduced the germination percentage of goosegrass seeds compared to the check (water). The germination rate (Ng/d) followed a similar pattern, with the hairy vetch treatment slightly enhancing germination rate compared to the check (water). The cereal rye and wheat extract treatments had a significantly lower germination rate (Ng/d) compared to the check (water) (Figure 2b). Canola extract increased root length by about 40% and 148% respectively, and shoot length by 135% and 57%, respectively, compared to the check (water) and wheat (Figure 2c,d). Canola and hairy vetch extracts promoted root and stem elongation of germinated goosegrass seeds compared to the check (water). In contrast, the wheat extract significantly reduced goosegrass germination percentage (29%), germination rate (48%), and root length (43%) compared to water (Figure 2a–c). The shoot length of germinated goosegrass seeds was significantly reduced in the check (water) compared to all other cover crop extracts.

![Figure 2](image_url)

**Figure 2.** Effects of cover crop extracts on germination percentage (a), germination rate (b), root length (c), and shoot length (d) for goosegrass seeds. Error bars represent the mean ± SE. Means with different letters are significantly different from one another *p* < 0.05. Data averaged over the two locations (i.e., ET and MT) were first analyzed with a mixed model ANOVA to rule out any effect of location.

3.2. Experiment II

All cover crop extracts negatively impacted soybean germination percentage compared to the check (water), with the exception of hairy vetch ET. These differences in germination percentage of soybean plants among cover crop extract treatments were significant (*p* = 0.05) at 4 DAP only (Table 2), with a decrease in magnitude of differences among the extract treatments and check (water) as soybean growth progressed. Soybean germination percentages in pots with extract treatments of hairy vetch MT, canola MT, and wheat ET were significantly lower (*p* < 0.05) than the check (water). At 10 DAP, the...
differences in soybean germination percentage among extract treatments were reduced (Table 2). While the soybean germination percentage was suppressed under application of most of the cover crop extracts compared to the check (water), the largest differences were observed when wheat ET and canola MT extract treatments were applied (Table 2).

The soybean plants treated with the hairy vetch MT extract had a significantly higher number of leaves per plant (6.6) at 22 DAP compared to soybean plants treated with all other extract treatments and the check (water), which ranged from 3–3.67 leaves per plant (Table 2). However, there were no significant differences in total leaf area at termination (data not shown). At 22 DAP, soybean plant height ranged from 164.3 mm to 256 mm. Soybean plants treated with canola MT extract had a significantly lower height than those treated with the check (water) or the remaining cover crop extracts, with the exception of hairy vetch MT (Table 2).

Soybean root nodule count was significantly higher at termination (26 DAP) for soybean plants treated with both wheat extracts (i.e., MT and ET) than the check (water), canola ET, and hairy vetch MT extract treatments (Figure 3). No significant differences were found in SPAD, stem diameter, biomass, or root length results among soybean plants treated with cover crop extracts compared to the check (water) (Table A1).

Figure 3. Root nodules per soybean plant at termination in each treatment. Error bars represent the mean ± SE. Means with different letters are significantly different from one another p < 0.05. ‘ET’; East Tennessee location; ‘MT’; Middle Tennessee location.

4. Discussion

The results of this study indicate that allelopathy can affect both weed and row crop germination and initial growth. Multiple factors such as the source of cover crop material, medium of application (in vitro vs. soil), and target species contributed to how the cover crop treatments resulted in changes during early plant growth and development.

The location had a significant impact on the results of experiment I, specifically for soybean. This can likely be explained by field and environmental variabilities where the cover crop species were growing (Table 1). In their study, Qu et al. [15] reported that soil pH, soil organic carbon, and total nitrogen content lead to some plants and soil organisms releasing allelochemicals. The bioactivities of the allelochemicals are influenced by the chemical properties of the soil (pH, solute concentration, and cation exchange capacity), as well as by cultural practices. Field history, slope, soil type, water-holding capacity, temperature, precipitation, and relative humidity all affect growing conditions and allelochemicals [4,16,17]. An additional factor which could have also affected the concentrations of allelochemicals in the cover crops is any abiotic stress that was present during the period when cover crops were growing. According to Hussain et al. [18], the concentration of allelochemicals in sorghum (Sorghum bicolor L.), a highly allelopathic species, can be affected by nutrient, temperature, and water stress, as well as herbicide applications.
Previous studies have shown that different cover crop species (i.e., cereal rye, crimson clover, wheat, and hairy vetch) can have diverse allelopathic impacts on cotton seedling growth [1,19–21]. Allen et al. [7] observed a suppression of cotton lint and seed yield when rye and wheat were utilized as cover crops in the cropping rotation. This result provides further evidence of a relationship among suppressed crop growth, development, and yield with allelopathic compounds. Similarly, Abou Chehade et al. [22] found that application of aqueous extracts of cereal rye significantly reduced shoot growth in two different weed species. The current study results suggest that these effects are also applicable to soybean and goosegrass seedling growth.

In terms of applied cover crop management with respect to allelopathy, questions still remain. For example, the wheat extract treatment in experiment I significantly reduced goosegrass seed germination percentage, rate, and root length compared to the check (Figure 2). In their study, Price et al. [21] identified cereal rye and winter wheat as cover crops that inhibit weed growth as a result of their allelopathic properties. This characteristic could explain the popularity of cereal rye and winter wheat as the most prevalently endorsed winter cover crops for row crop production in the southeastern US [21]. However, in soybean, while wheat extract application significantly increased root nodulation, germination percentage was inhibited. Clearly, a successful allelochemical for weed management should inhibit the germination of several weed species and not inhibit the germination of the crop [23].

In experiment II, one of the most significant results was the increase in soybean root nodulation in pots treated with wheat extract from both locations (Figure 3). While research is limited in the effects of cover crops on soybean nodulation, one study by Cordeiro et al. [24] found that soybean plants grown in plots that were cover-cropped with black oats (*Avena strigosa*) had higher levels of nodulation than those that were grown in a fallow field or preceded by a white lupine (*Lupinus albus*) cover crop. While that study attributed the level of nodulation to differing levels of soil N, these findings do imply that different cover crop residues may influence the prevalence of nodulation in soybean plants.

Differing results between experiment I and experiment II were likely due to the added complexity of the soil component in experiment II. The intricate relationship between soil and allelochemicals has been widely described through many studies; once infiltrated into the soil by the donor plant, allelochemicals are introduced into a complex plant–soil system in which different factors affect their availability and, consequently, their effective influence on target plants [4,16,17,25,26]. Phytotoxicity can be affected through a complex of soil characteristics, climatic conditions, and plant factors between both the donor and the target plants [25]. The relationship between the donor and the target plant exerts multiple effects on retention, transport and transformation processes of allelochemicals in the soil [4]. In addition, the microbial degradation/transformation of allelochemicals in the soil affects the dose of allelochemicals that can cause plant inhibition [26–28]. One well-researched example of this is the bacterial degradation of juglone, an allelochemical produced by black walnut trees [29].

Allelopathic potential is highly variable among cultivars within a cover crop species, as well as within a cultivar [21]. According to the current study results, the wheat cover crop leachate had the most potent allelopathic impact on goosegrass germination and growth, in addition to the lowest observed adverse effect on the row crop, soybean. To be able to accurately make recommendations to producers, varieties of cover crop species under variable conditions and locations should continue to be thoroughly evaluated. Further research into cover crops with allelopathic potential for weed management should focus on identifying cultivars and growing conditions which maximize allelochemical production. Research into management strategies which reduce the negative effects to row crops such as termination timings and techniques would also be useful in developing allelopathy tools as a viable option for producers.
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Abbreviations

Days after planting, DAP; East Tennessee, ET; Middle Tennessee, MT.

Appendix A

Table A1. SPAD, stem diameter, biomass, and root length of soybean seedlings throughout Experiment II. Time of measurement is represented by days after planting (DAP).

| Cover Crop Extract | Time Period |
|---------------------|-------------|
|                     | 20 DAP      | 26 DAP      |
| SPAD                |             |             |
| Hairy Vetch ET      | 43.5 ± 1.0  |             |
| Wheat ET            | 36.7 ± 1.5  |             |
| Canola ET           | 40.6 ± 1.9  |             |
| Hairy Vetch MT      | 40.0 ± 2.5  |             |
| Wheat MT            | 33.9 ± 2.1  |             |
| Canola MT           | 41.0 ± 1.0  |             |
| Check (Water)       | 37.8 ± 1.7  |             |

Stem diameter

| Cover Crop Extract | 22 DAP | 26 DAP |
|--------------------|--------|--------|
| Hairy Vetch ET *   | 2.7 ± 0.1 |        |
| Wheat ET           | 2.7 ± 0.1 | 2.7 ± 0.5 |
| Canola ET          | 2.3 ± 0.1 | 2.5 ± 0.8 |
| Hairy Vetch MT     | 2.6 ± 0.1 | 2.7 ± 0.19 |
| Wheat MT           | 2.4 ± 0.1 | 2.6 ± 0.07 |
| Canola MT *        | 2.2 ± 0.1 |        |
| Check (Water*)     | 2.2 ± 0.1 | 2.4 ± 0.10 |

Biomass

| Cover Crop Extract | 22 DAP | 26 DAP |
|--------------------|--------|--------|
| Hairy Vetch ET *   | 1.2 ± 0.2 |        |
| Wheat ET           | 1.1 ± 0.1 |        |
| Canola ET          | 1.4 ± 0.1 |        |
| Hairy Vetch MT     | 1.5 ± 0.2 |        |
| Wheat MT           | 1.0 ± 0.1 |        |
| Canola MT *        | 0.7 ± 0.1 |        |
| Check (Water)      | 1.0 ± 0.1 |        |

Root length

| Cover Crop Extract | 22 DAP | 26 DAP |
|--------------------|--------|--------|
| Hairy Vetch ET *   | 25.8 ± 3.5 |        |
| Wheat ET           | - ± - | 38.9 ± 5.8 |
| Canola ET          | - ± - | 37.1 ± 4.3 |
| Hairy Vetch MT     | - ± - | 28.7 ± 1.3 |
| Wheat MT           | - ± - | 33.9 ± 4.1 |
| Canola MT *        | 21.6 ± 6.2 |        |
| Check (Water)      | - ± - | 28.9 ± 0.7 |

* Terminated at 22 DAP.
