

Article

# Allelopathic Impacts of Cover Crop Species and Termination Timing on Cotton Germination and Seedling Growth

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**Abstract:** The integration of cover crops into cotton (*Gossypium hirsutum*, L.) production remains challenging. One potential negative impact of cover crops on cotton is allelopathy. Proper selection of cover crop species and termination timing could potentially reduce the impacts of allelopathy on cotton seedlings. Two studies were conducted to determine cotton germination and growth sensitivity to cover crop leachate, which were measured using (I) five cover crops species, including: oats (*Avena sativa* L.), hairy vetch (*Vicia villosa*), winter pea (*Lathyrus hirsutus*), winter wheat (*Triticum aestivum*), and annual rye (*Lolium multiflorum*), and (II) a blend of cover crops at four termination timings, including: at planting, three weeks prior to planting, six weeks prior to planting, and a split termination, where a 25 cm band in the top of the bed was terminated six weeks prior to planting, and the remaining cover crop was terminated at planting (referred to as strip 6-wk). Samples for Experiment I were collected on May 24th and for Experiment II on March 22nd (Strip/6-wk and 6-wk), April 30th (3-wk), and May 11th (at planting) in 2018. The effect of 0 (deionized water), 25, and 50 (v/v) cover crop leachate extract on cotton seed germination was evaluated in a series of controlled environmental studies. All cover crop species' leachates negatively impacted cotton germination and seedling growth ( $p < 0.05$ ). Germination inhibition rates declined numerically by species, with winter pea  $\geq$  hairy vetch  $\geq$  oats  $\geq$  annual rye  $\geq$  winter wheat at the 50 v/v concentrations. Winter pea germination inhibition on cotton equaled 47.0% and cotton radicle length was decreased by 62.8%. Termination at planting suppressed cotton germination more than the other termination timings, with the 50 v/v treatment resulting in a germination inhibition of 60.0%. Proper selection of cover crop species and termination timing prior to planting cotton will be critical in maximizing the benefits and minimizing the risks of a cover crop.

**Keywords:** cover crops; termination timings; allelopathy; cotton

## 1. Introduction

Interest in cover crops in no-till farming is increasing [1]. Conservation agriculture systems that utilize high residue cover crops offer many benefits, including enhanced water infiltration, lower soil water evaporation, increased soil organic matter, and increased soil biodiversity. Additionally, high biomass yielding cover crops can suppress early-season weeds [2]. Moreover, cover crops can suppress weeds through the production of allelopathic secondary metabolites. Allelopathy has been defined as any direct or indirect harmful or beneficial effect by one plant on another through production of chemical compounds that escape into the environment [3,4].

These compounds have been used as natural herbicides [5]. Unfortunately, these chemicals can also impact the growth and development of the following row crop [6,7]. Subsequently, it is important

to determine the allelopathic compatibility of cover crops with row crops before incorporating them into agricultural systems, as phytotoxins released by cover crops could affect the establishment of row crops [8].

In their study, Allen et al. [7] showed that the use of rye and wheat in the rotation reduced the growth of cotton, and that rye allelopathy was the cause of this suppression. Regardless of specific chemical involvement, the suppression of cotton following small grain cover crops was repeatable [7]. Wheat and rye have been recognized for their suppression of germination and radicle elongation of several species via the release of benzoxazinoid allelochemicals [9]. Bauer and Reeves [10] reported that black oat (*Avena strigosa* Schreb), rye and crimson clover (*Trifolium incarnatum* L.) residues inhibited tap root elongation in greenhouse grown radish (*Raphanus sativus* L.) and cotton (*Gossypium hirsutum* L.) plants. Price et al. [6] indicated that cotton radicle elongation was inhibited by extracts of forage rape ( $\leq 19\%$ ), wheat ( $\leq 23\%$ ), rye ( $\leq 26\%$ ), triticale ( $\leq 28\%$ ), black oat ( $\leq 34\%$ ), crimson clover ( $\leq 30\%$ ), white lupin ( $\leq 40\%$ ), sunn hemp ( $\leq 35\%$ ), hairy vetch ( $\leq 45\%$ ), and black medic ( $\leq 33\%$ ). Cover crop species differ in their allelopathic potential [11,12]. Cereal rye and soft red winter wheat (*Triticum aestivum* L.) are the two most commonly recommended winter cover crops for row crop production in the southeastern U.S. [6]. Both of these cover crops contain allelopathic compounds that inhibit weed growth [6–14].

Based on germination and radicle elongation of the response species tested in a study by Geddes et al. [9], hairy vetch did not chemically inhibit germination of any response species, and was in fact found to stimulate the germination of lamb's quarters under the study conditions. Therefore, hairy vetch might not be a good candidate species for weed-suppressive allelopathic mulch [9]. Baldwin and Creamer [15] reported that winter pea (*Pisum sativum*) grows fast and vigorously, and while growing as a cover crop, can suppress weeds due to its allelopathic impact.

A winter cover crop's allelopathic potential is an important attribute which is typically overlooked (Price et al.) [6]. The information that ranks the relative allelopathic potential of these cover crops, or of other traditional and non-traditional cover crops available to producers, is limited [6,7]. The complexity of allelopathic interactions complicates conclusive field research [16]. In row crops production, it is extraordinarily difficult to distinguish mechanisms of interference between chemically allelopathic and physical mulch-residue effects in the no-till system [12–16]. Therefore, laboratory screening allows for the selection of promising species or genotypes for field evaluation [6–16].

To minimize the risk of integrating cover crops into the cotton production system, cotton producers need more information on how allelopathic properties change with different cover crop species and cover crop termination dates. Therefore, the current study was designed to fill the gap in the allelopathic potential ranking of available cover crop species. The objectives of this study were to assess the relative effects of (1) different cover crop species and (2) different termination timings of a blend cover crop species' extracts on cotton germination and seedling development in controlled environments.

## 2. Materials and Methods

### 2.1. Experiment I

#### 2.1.1. Cover Crop Species

In order to examine the allelopathic effect of oats (*Avena sativa* L.), hairy vetch (*Vicia villosa*), winter pea (*Lathyrus hirsutus*), winter wheat (*Triticum aestivum*) and annual rye (*Lolium multiflorum*) on germination and seedling growth of cotton (*Gossypium hirsutum* L.), a field trial was initiated on 20 November, 2017, at the West TN Research and Education Center (WTREC). Single cover crop treatments including oats (112 kg/ha), hairy vetch (33.6 kg/ha), winter pea (56 kg/ha), winter wheat (100 kg/ha), and annual rye (100 kg/ha) [17] were established in a randomized, complete block design with four replicates. The topgrowth of cover crop species was harvested two weeks prior to planting cotton on 4 June 2018. The cover crop species allelopathy trial was executed in complete block design with six replications. Plot size was 4 rows (4.0 m) wide and 9.1 m long. The average temperature in

the field between November 2017 and the middle of May 2018 was 9–10 °C. Plots were kept under rainfed condition.

### 2.1.2. Sampling and Extract Preparation

Afterwards, samples were oven dried at 60 °C for five days, ground to pass a 1 mm screen and then stored in a refrigerator at 2–4 °C until used [18]. Ten grams of each sample was soaked in 100 mL deionized water at 25 °C for 24 h on a shaker. The extract was then filtered through four layers of cheesecloth and centrifuged at 4000 rpm for one hour. The supernatant was then vacuum filtered through Whatman no. 42 paper. Stock extracts were made fresh for each time the cotton seeds received cover crop leachates.

### 2.1.3. General Culture and Seed Bioassay

Stock extract was diluted appropriately with sterile, distilled water to give the final concentrations of 0, 25, and 50 *v/v* rates. Four milliliters for each treatment was then pipetted to Whatman no. 2 filter paper and then placed in a 9 cm diameter plastic petri dish. Ten non-treated seeds of 'PHY 490 W3FE' (Dow AgroSciences, Indianapolis, IN, USA) cotton cultivar were surface sterilized with 2% (*v/v*) commercial bleach solution for three minutes, rinsed with deionized water and then placed on Whatman paper in the petri dish. Seeds were then placed on filter paper containing extract in each petri dish. The petri dishes were placed in a lighted room at 25 °C. Four milliliters of each cover crop extract was added to the cotton seeds replicated in petri dishes every other day for eight days.

### 2.1.4. Data Collection

Germination rate was calculated as the number of germinated seeds (radicles >1 mm long). Measurements were repeated at 24 hour intervals. Hypocotyl and root lengths were measured for all seedlings in each petri dish at 168 h after placing the seeds on the medium. After 168 h, fresh and dry weights of the root and seedling lengths and germination percentage in each petri dish were measured.

## 2.2. Experiment II

### 2.2.1. Termination Timing

In the fall of 2017, a mixed cover crop was planted consisting of cereal rye (33.6 kg/ha), annual ryegrass (2.25 kg/ha), crimson clover (9 kg/ha), winter pea (9 kg/ha), hairy vetch (2.25 kg/ha), daikon radish (4.5 kg/ha) and buckwheat (4.5 kg/ha) in the field [17]. In 2018, the cover crops blend was terminated (1) immediately prior to planting, (2) three weeks prior to planting, (3) six weeks prior to planting, and 4) in a split termination, where a 25 cm band over the furrow was terminated six weeks prior to planting, and the remaining cover crop was terminated at planting (strip 6-wk). Plot size was designed based on County Standard trial (CST) in West Tennessee during 2018. Therefore, plot size was 12 rows wide (11.5 m) and approximately 25 m in length. The average temperature in the field between November 2017 and the middle of May 2018 was 9–10 °C. Plots were kept under rainfed conditions. Samples from each termination timing were taken at planting. Above ground biomass of the cover crop was clipped and processed in the laboratory, in the same way as *Experiment I*.

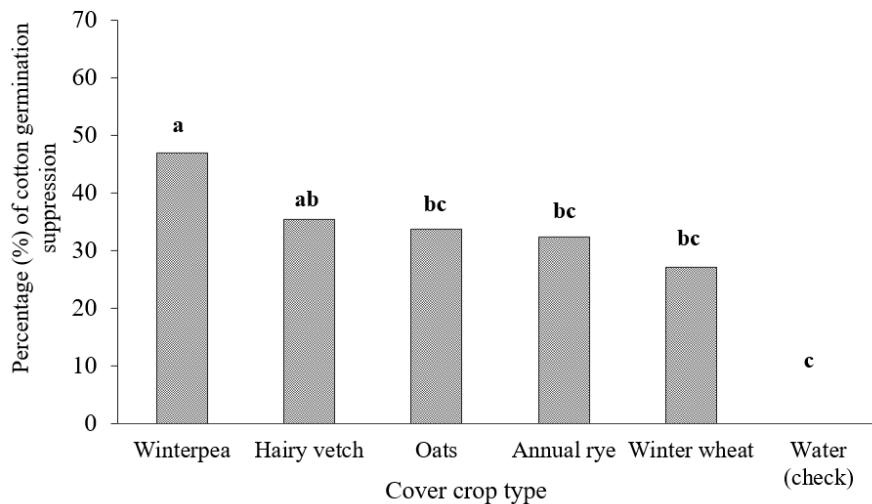
### 2.2.2. Data Collection and Statistical Analysis

Treatments were arranged in a factorial, randomized complete block design with six replications. Statistical analysis was completed in JMP Pro v.15 (SAS Institute, Cary, NC, USA). The six data points, considering all replications for each parameter in each measurement, were averaged to give a value per extract treatment. Then, values for cotton parameters for each experiment (i.e., I and II) 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).

### 3. Results

#### 3.1. Experiment I

While all cover crop extracts negatively impacted cotton germination and seedling growth, the germination percentage of cotton seeds was most inhibited by winter pea. The highest concentrate of winter pea extract (i.e., 50 v/v) inhibited cotton germination by over 47% and drastically reduced the root length of cotton seedlings (Figure 1 and Table 1).



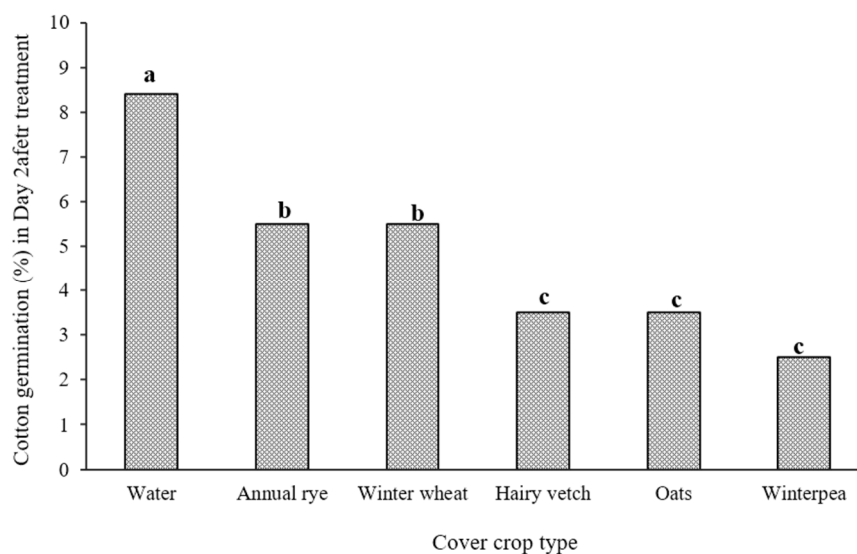
**Figure 1.** Cotton seeds were treated with extracts of cover crops (i.e., 50 v/v) including ‘winter wheat’, ‘annual rye’, ‘oats’, ‘hairy vetch’, and ‘winter pea’. The extract resulted from ‘winter pea’ suppressed cotton germination percentage the most (Experiment I). Means with different letters are significantly different from one another ( $p < 0.05$ ).

**Table 1.** Cotton seeds were treated with cover crops extract including ‘annual rye’, ‘winter wheat’, ‘oats’, ‘hairy vetch’, ‘winter pea’ and water (check), Experiment I.

Extract (v/v)	Cover Crop Type	Germination Percentage (%)	Germination Rate (Ng/d) <sup>‡</sup>	Root Length (cm)	Shoot Length (cm)	Seedling Length (cm)	Dry Weight (g)
25	Water (check)	60.4 a	2.63 a	1.4 a	2.7 a	4.12 a	0.12 a
	Annual rye	44.4 b	1.95 b	1.0 a	2.6 ab	3.62 ab	0.07 b
	Winter wheat	48.4 ab	1.90 b	1.0 a	1.9 b	2.87 cd	0.08 b
	Oats	44.0 b	1.70 b	1.0 a	1.9 b	3.06 bcd	0.07 b
	Hairy vetch	52.2 ab	2.00 b	1.1 a	2.3 ab	3.42 abc	0.06 b
50	Winter pea	45.0 b	1.70 b	0.6 b	2.0 b	2.70 d	0.06 b
	Water (check)	60.4 a	2.63 a	1.4 a	2.7 a	4.12 a	0.12 a
	Annual rye	40.9 bc	1.67 b	0.89 b	2.2 ab	3.09 b	0.05 bc
	Winter wheat	44.0 ab	1.62 b	0.74 bc	1.5 cd	2.31 cd	0.05 bc
	Oats	40.0 bc	1.30 bc	0.63 cd	1.7 bcd	2.41 cd	0.05 bc
	Hairy vetch	39.0 bc	1.61 bc	0.82 bc	2.0 abc	2.83 bc	0.05 bc
	Winter pea	32.1 c	1.24 c	0.52 d	1.4 d	1.98 d	0.04 c

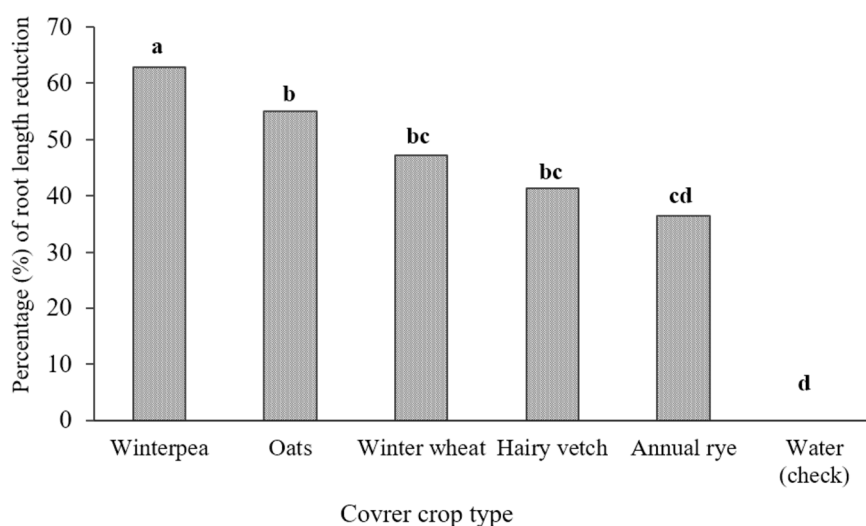
<sup>‡</sup> Germination rate (Ng/d): number of germinated seeds per day. Means with different letters are significantly different from one another  $p < 0.05$ .

Increasing extract concentration of cover crops (i.e., 50 v/v) resulted in delayed germination by species (winter pea  $\geq$  hairy vetch  $\geq$  oats  $\geq$  winter wheat  $>$  annual rye) compared to deionized water (check) in Day 2 (Figure 2). The initial rate of germination was reduced by cover crops extract compared to deionized water (check) (Figure 2). Overall, increasing extract concentration of cover crops to 50 v/v resulted in a considerable decrease in total cotton germination to less than 27% for ‘winter wheat’, 32% for ‘annual rye’, 33% for ‘oats’, 35% for ‘hairy vetch’, and 47% for ‘winter pea’ over deionized water (check), respectively (Figure 1).



**Figure 2.** Effect of cover crop type extracts (i.e., 50 v/v) in a controlled environment on delaying cotton germination percentage two-days after treatment (Experiment I). Means with different letters are significantly different from one another ( $p < 0.05$ ).

Cotton germination percentage by leachates of three cover crops, including ‘annual rye’, ‘winter wheat’, and ‘oats’ were suppressed less than ‘winter pea’ and ‘hairy vetch’ extracts (Table 1). Declines in cotton germination rate, root and shoot lengths, dry weight, and overall seedling length with increasing cover crops residue rate were significant (Table 1). The cover crop, ‘winter pea’ leachate (i.e., 50 v/v) significantly reduced cotton root (62%) and seedling (52%) lengths compared to water (check) and deionized water (Figure 3 and Table 1). In contrast, ‘annual rye’ leachate (i.e., 50 v/v) had the lowest impact on root (36%) and seedling (25%) lengths reduction, compared to all other cover crops and water (check) (Figure 3 and Table 1).



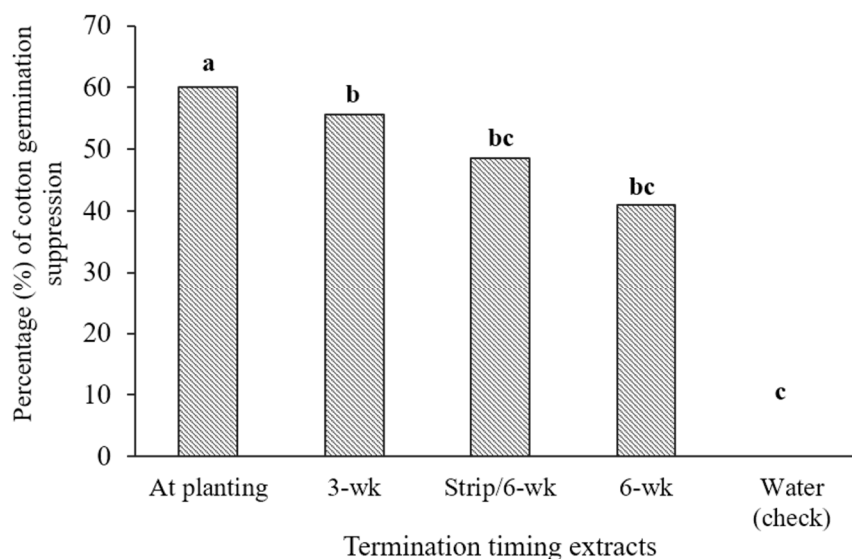
**Figure 3.** Cotton root lengths reduction percentage (%) when cotton seeds were treated with cover crop type extracts at 50 v/v rate (Experiment I). Means with different letters are significantly different from one another ( $p < 0.05$ ).

### 3.2. Experiment II

Among the termination timing treatments, the allelopathic impacts on cotton seed germination were most detrimental from the ‘at planting’ termination timing. Termination timing reduced cotton

seed germination percentage by 60% for 'at planting' termination, 55% for '3-wk' termination, 48% for 'Strip/6-wk' termination, and 40% for '6-wk' before planting compared to deionized water (check) (Figure 4 and Table 2).

Negative allelopathic effects were reduced with earlier termination timings (Table 2). Shoot lengths of cotton seedlings were affected by termination timing extracts, and root growth was significantly reduced as the extract concentration increased (Figure 5 and Table 2). It was also observed from this study that the application of termination timing concentration extracts on cotton seeds, compared to deionized water (check), reduced the final dry weight (Table 2).

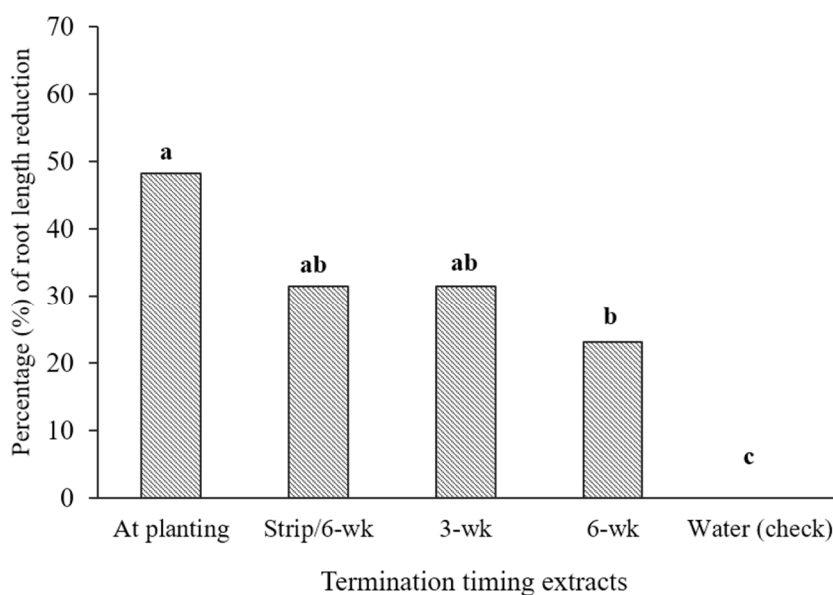


**Figure 4.** Effects of a mixed cover crop with termination timings on cotton germination suppression percentage (%) at 50 v/v extracts rate (Experiment II). Means with different letters are significantly different from one another ( $p < 0.05$ ).

**Table 2.** Cotton seeds were treated with extracts of a mixed cover crop [cereal rye (33.6 kg/ha), annual ryegrass (2.25 kg/ha), crimson clover (9 kg/ha), winter pea (9 kg/ha), hairy vetch (2.25 kg/ha), daikon radish (4.5 kg/ha), and buckwheat (4.5 kg/ha)] which were sampled at different termination timings (6-wk, strip/6-wk, 3-wk, and at planting), Experiment II.

Extract (v/v)	Cover Crop Termination Timing	Germination Percentage (%)	Germination Rate (Ng/d) <sup>£</sup>	Root Length (cm)	Shoot Length (cm)	Dry Weight (g)
25	Water (check)	52.5 a	1.62 a	1.43 a	2.58 a	0.07 a
	6-wk	35.0 b	1.60 a	1.13 ab	1.51 b	0.04 b
	Strip/6-wk	32.0 b	1.27 b	1.11 ab	1.68 b	0.04 b
	3-wk	25.4 b	1.17 bc	0.78 b	1.41 b	0.03 b
	At planting	23.4 b	0.98 c	1.10 ab	1.67 b	0.04 b
50	Water (check)	52.5 a	1.62 a	1.43 a	2.58 a	0.07 a
	6-wk	31.0 b	1.30 ab	1.10 ab	1.65 b	0.04 b
	Strip/6-wk	27.0 bc	1.06 bc	0.98 b	1.52 b	0.04 b
	3-wk	23.3 bc	1.02 bc	0.98 b	1.26 c	0.03 b
	At planting	21.0 c	0.85 c	0.74 c	1.50 b	0.04 b

<sup>£</sup> Germination rate (Ng/d): number of germinated seeds per day. Means with different letters are significantly different from one another  $p < 0.05$ .



**Figure 5.** Effects of a mixed cover crop with termination timings on root length of cotton. The root length was determined at seven days after seeding on the filter paper wetted with the 50 v/v extracts rate (Experiment II). Means with different letters are significantly different from one another ( $p < 0.05$ ).

#### 4. Discussion

Cotton seeds placed within cover crops extracts (i.e., ‘annual rye’, ‘winter wheat’, ‘oats’, ‘hairy vetch’, and ‘winter pea’) were characterized by delayed and inhibited germination relative to deionized water (check) (Figure 2). The allelopathic effect on germination varied and depended on extracted species (Table 1). This suggests that the allelopathic potential of the cover crops is variable. This may be very important to a producer in selecting a cover crop cultivar and understanding the negative impacts allelopathy might have on cotton seedlings.

In the current study, the decline in cotton germination rate, root and shoot lengths, dry weight, and overall seedling length with increased cover crops residue rate were significant (Table 1). Sahoo et al. [19] indicated in a study that the aqueous leaf extracts of *Leucaena leucocephala* and *Tectona grandis* reduced the fresh and dry weights of maize (*Zea mays* L.). Bruce et al. [20,21] reported negative impacts of wheat residue on emergence and growth of canola (*Brassica napus* L.). In a study by Allen et al. [7], the observed suppression effect of cover crops on cotton lint and seed yield was consistent with results of the long-term system study when both rye and wheat were present in the rotation, providing further evidence of a relationship between suppressed cotton growth, development, and yield with allelopathic compounds.

In our study, the ‘winter pea’ extracts drastically reduced the length of cotton seedlings compared to other cover crop types (Table 1). Bauer and Reeves [10] reported that the tap root elongation in greenhouse grown radish (*Raphanus sativus* L.) and cotton (*Gossypium hirsutum* L.) plants were inhibited by the residues of black oat (*Avena trigose* Schreb), rye and crimson clover (*Trifolium incarnatum* L.) [10]. Hulugalle et al. [22] showed that in general, reductions in germination, emergence, dry matter production and root length density of cotton seedlings were affected by winter legumes, summer legumes, cereal extracts and a control group, in that order. They indicated that compared with cereals, leguminous rotation crops, particularly winter legumes, reduced emergence, growth and lint yield, and resulted in poor fiber quality in cotton [22]. Hill et al. [23] concluded that effects of aqueous extracts of ‘hairy vetch’ and ‘cowpea’ vary with species and extract concentration. The allelopathic effects are generally attributable to the production and exudation of particular toxic chemicals, which differ in concentrations among cultivars of the same species and are perceived with varying levels of susceptibility among cultivars of other species [24,25].

Price et al. [6] reported that cover crop extracts decreased cotton radical elongation  $\leq 49\%$  depending on species. The cotton radicle elongation was inhibited by forage ‘rape’ ( $\leq 19\%$ ), ‘wheat’ ( $\leq 23\%$ ), ‘rye’ ( $\leq 26\%$ ), ‘triticale’ ( $\leq 28\%$ ), ‘black oat’ ( $\leq 34\%$ ), ‘crimson clover’ ( $\leq 30\%$ ), ‘white lupin’ ( $\leq 40\%$ ), ‘sunn hemp’ ( $\leq 35\%$ ), ‘hairy vetch’ ( $\leq 45\%$ ), and ‘black medic’ ( $\leq 33\%$ ). In greenhouse studies, in which the leachates of ground dried residues of the three cover crops were evaluated based on germination, plant height, and dry weight of goosegrass, smooth amaranth (*A. hybridus* L.), bell pepper, and tomato, Adler and Chase [26] found that goosegrass (*Eleusine indica* L.) germination was inhibited in a similar manner by residues of the three cover crops by up to 80% or less compared to the control [26]. Selection of crop species based on their allelopathic potential is perhaps one of the best strategies for reducing their negative impact on row crops and taking advantage of allelopathy for suppressing weeds.

In the current study, all extracts of a blend cover crop at different termination timings inhibited root elongation and germination rate, with the most negative impacts occurring under termination at planting (Table 2 and Figure 5). The current results show that cover crop termination timing can minimize the potential allelopathic impacts on an emerging cotton crop, and early termination timing will minimize the allelopathic impacts on emerging weeds. Furthermore, applying split termination of cover crops within the row ‘strip /6-wk’ has shown the potential to minimize the negative allelopathic impacts of the cover crop on the row crop germination by 22% compared with ‘at planting’, while achieving cultural control benefits for weed management. Finally, the longer the interval between cover crop termination and row crop planting, the less likely allelopathic compounds will affect crop emergence and growth. Differences in the sensitivity of crop cultivars to allelopathic impacts of cover crops may also occur [26].

## 5. Conclusions

Cover crop species selection and termination timing are important considerations in managing the allelopathic impact of cover crops. Producers must understand the influence of cover crop species and termination timing on allelopathy and must consider the positive benefits that allelopathy would have on weed suppression along with the negative impacts that allelopathy might have on cotton seedlings. Overall, the current experiment indicates that caution should be exercised when delaying cover crop termination until planting, since the negative impacts of allelopathy on cotton seedlings are reduced with early termination timings, especially in relation to winter pea or hairy vetch. Use of split termination of the blend cover crop within the row ‘strip /6-wk’, has shown the potential to minimize the negative allelopathic impact of cover crops in comparison with treatment ‘at planting’. Further research is needed to evaluate the impact of cover crops extract to minimize the negative allelopathic impacts of the cover crop on the row crop, while achieving cultural control benefits for weed management.

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