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## Relative Contribution of Physical Interference and Allelopathy to Weed Suppression by Winter Annual Cover Crop Mixtures


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**RELATIVE CONTRIBUTION OF PHYSICAL INTERFERENCE AND  
ALLELOPATHY TO WEED SUPPRESSION BY WINTER ANNUAL COVER  
CROP MIXTURES**

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

By

Alyssa D. Travlos

December 2018

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ALLELOPATHY TO WEED SUPPRESSION BY WINTER ANNUAL COVER CROP  
MIXTURES**

Agriculture

Missouri State University, December 2018

Master of Science

Alyssa D Travlos

**ABSTRACT**

Cover cropping systems are widely used in crop production systems to prevent erosion, improve soil health, and suppress weeds. Common cover cropping systems include combinations of cereal rye (*Secale cereale* L.), Brassica species, legumes, and other winter annual species. Three cover crop mixtures (cereal rye alone, cereal rye plus winter pea, and cereal rye plus winter pea plus radish) were applied using three methods (fresh residue, dried leached residue, and leachate) to common waterhemp (*Amaranthus tuberculatus* var. *rudis*) and large crabgrass [*Digitaria sanguinalis* (L.) Scop.]. The experiment was conducted once in a greenhouse and once in a growth chamber. Significant interactions ( $\alpha=0.05$ ) were observed in the greenhouse study between treatment and days after planting (DAP) for emergence, height, and leaf count for common waterhemp. In addition, fresh cover crop residues suppressed common waterhemp emergence relative to dried leached residue. The interaction of treatment and DAP was also significant for large crabgrass emergence. In the growth chamber study, common waterhemp data were inclusive due to poor emergence. No significance was observed with the large crabgrass, but trends suggest that fresh residue was more effective than other applications. In conclusion, the results of this study suggest that cover crop mixtures did not influence weed response, but data suggest that allelopathy had an important contribution in both environments.

**KEYWORDS:** cover crop, cover crop mixtures, herbicide resistance, allelopathy, common waterhemp, large crabgrass

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December 2018

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In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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## INTRODUCTION

Populations of herbicide resistant weeds are increasing, making row crop production more difficult for producers. There are a total of 495 unique cases of herbicide resistant weeds globally. There are currently 26 sites of action with evolved resistance to 23 of them (HRAC 2018). This increase in resistance and lack of new chemical technology is making it imperative for producers to have other methods to help manage weed populations that are present in the production system. Cover crops are planted for the protection and enrichment of the soil and not for a harvestable product. Cover crops have many benefits upon implementation to ranging from soil health to weed control. Cover crops are able to physically modify seed germination by altering the growing environment. This could be by reducing light availability or by changing the soil temperature and moisture (Creamer et al. 1996). Some cover crops also have the ability to suppress weeds with allelopathy (Zimdahl 2013).

## LITERATURE REVIEW

### Importance of Soybean Production

Soybeans [*Glycine max* (L.) Merr] are a crucial product in multiple industries outside of and including agriculture. An individual soybean plant generally produces between sixty and eighty pods with each pod containing two to four pea-sized beans. In the United States, soybeans were originally grown as a forage crop. They were first planted as a row crop during the 1940s, but it wasn't till the 1980s that an increase in planted acreage was observed (Missouri Crop Resource Guide 2018). In 2016, the United States produced 4.31 billion bushels of soybeans from 83 million acres. Nearly half of the 2016 crop, 2.03 billion bushels, were exported, representing 47% of world export production for the year. Soybean is the top-ranked agricultural commodity in Missouri, followed by corn (USDA NASS 2017). Missouri currently ranks sixth in United States soybean production with a five-year average of 5.3 million acres harvested yielding 40.4 bushels per acre (Missouri Crop Resource Guide 2018). According to the 2012 agriculture census for the state of Missouri, 148,826,538 bushels were harvested off of 5,250,275 acres (USDA Ag Census 2012).

### Weeds

Weeds are something that agricultural producers have struggled against since the beginning of farming. Weed management is an important aspect of crop production because they compete with crops for nutrients, light, and water. This competition can result in decreased crop yield and quality (Zimdahl 2013). Some weed species are harder to manage than others. Some of the most troublesome weed species in Missouri soybean fields include common waterhemp

[*Amaranthus tuberculatus* (Moq.) J. D. Sauer] and large crabgrass [*Digitaria sanguinalis* (L.) Scop.].

**Common Waterhemp.** Common waterhemp is a summer annual commonly found throughout the central and eastern parts of the United States that can reduce soybean yield by as much as 44% (Steckel and Sprague 2004). Common waterhemp is a dioecious species with complex terminal inflorescences (Crespo 2012). Common waterhemp seeds measure between 0.8 mm to 1 mm in diameter. Seed production can be as much as 1.5 times more than that of other pigweeds. Each plant generally produces 250,000 seeds but can produce as much as 1 million or more in optimal conditions. Research shows that 12% of seeds remain viable in the soil seedbank after 4 years (Buhler and Hartzler 2001). Common waterhemp emerges throughout the growing season, with a greater percentage of plants emerging later than most summer annuals, which is related to the presence of a C4 photosynthetic pathway (Crespo 2012). Hartzler (1999) found that in the upper Midwest, most plants emerged in late June to early July. Later emergence allows plants to avoid PRE applications of herbicides and POST applications of nonresidual herbicides (Crespo 2012).

**Large Crabgrass.** Large crabgrass is a summer annual that can be found across the United States. This species is more commonly found in agronomic crops, horticultural crops, and in turf/landscapes in nearly all soil types (Uva et al 1997). Large crabgrass reproduces by seed and spreads via tillering. A single plant can produce 150-700 tillers and up to 150,000 seeds (University of Massachusetts Extension 2011). Once the seeds shatter, they remain dormant for a period of time before germinating. When the soil temperature has been 11°C for four consecutive days they will begin to germinate (University of Massachusetts Extension 2011). Once established, large crabgrass can tolerate high temperatures and dry conditions as a result of being

a C4 plant. Large crabgrass continues vegetative growth through midsummer and then begins reproductive growth. Seed heads will continue to form until the first killing frost (University of Massachusetts Extension 2011).

**Herbicide Resistance.** Populations of herbicide resistant weed biotypes are a growing problem in crop management. Herbicide resistant weeds were first discovered in the United States in the late 1960s in a nursery that repeatedly sprayed simazine, a triazine herbicide, to control common groundsel (*Senecio vulgaris* L.; Holt 1992). However, the first confirmed case of an herbicide resistant weed in Missouri wasn't until 1992 when common cocklebur (*Xanthium strumarium* L.) resistant to ALS-inhibiting (Group 2) herbicides was reported (Heap 2018). Two years later, in 1994, Missouri had three more confirmed cases of herbicide resistant weeds. Those weeds were waterhemp resistant to ALS inhibitors and photosystem II inhibitors (Group 5) and barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv] resistant to ALS inhibitors (Heap 2018).

Weed populations typically become resistant to herbicides after repeated use of an herbicide for many years in a row. When weeds become resistant to an herbicide, the population changes in favor of members with differences at the biochemical site of action. Herbicide site of action is the site in the plant that the herbicide disrupts to interfere with plant growth and development (Peterson et al. 2015). According to the International Survey of Herbicide Resistant Weeds, there are a total of 26 sites of action and weed biotypes resistant to 23 sites of action have been described. Currently, the site of action with the most resistant weed species is ALS inhibitors with 160 resistant species. Following ALS inhibitors, photosystem II inhibitors, ACCase inhibitors, EPSPS inhibitors, and T1R1 auxin receptors have the most resistant species (Heap 2018). Common waterhemp currently has biotypes that are resistant to ALS inhibitors,

photosystem II inhibitors, PPO inhibitors, EPSPS inhibitors, T1R1 auxin receptors, and HPPD inhibitors. Large crabgrass has reported resistance to ACCase inhibitors, ALS inhibitors, and photosystem II inhibitors (Heap 2018). Some herbicide resistant weed biotypes have multiple pathways of resistance while some possess just a single pathway.

### **Integrated Pest Management**

Development of an integrated pest management program to manage herbicide resistant weed populations is crucial. An integrated pest management program is an ecological approach for the management of multiple pests with a variety of tactics (Flint 2012). In addition to chemical control, producers also use mechanical and cultural management. Mechanical management methods, such as tillage, mowing, hand weeding, or mulching, have the longest history and are commonly used developing countries. Cultural management techniques are based on the plant growth environment. Cultural management methods include planting date, planting population, and cover cropping. While these methods are unlikely to control weeds on their own, when used in conjunction with other methods, they can be very effective (University of California IPM 2016).

### **Cover Crops**

Cover crops are planted for the protection and enrichment of the soil, not for production of a harvestable product. Cover crops have many benefits to offer, from erosion control to improved soil health. These benefits not only increase cash crop production but protect the environment as well. According to Hartwig et al. (2002) the primary benefit of cover cropping is the reduction of water runoff and soil erosion, which increases soil productivity. Hall et al.

(1984) found that when corn was planted in birdsfoot trefoil or crown vetch living mulch on a 14% slope, water runoff, soil loss, and pesticide loss were reduced 95% to 99% when compared with conventional tillage. Cover crops can also increase soil organic matter, which also improves soil structure and tilth. Danso et al. (1991) reported that cover crops improve the soil structure, tilth, and water-holding capacity, which results in reduced risk of the environmental pollution by nitrogen fertilizers.

**Cover crops and Weed Management.** Cover crops also serve as a barrier to weed emergence or as competitors with weeds that might occur in a field. They serve as a physical barrier by smothering the weeds, suppressing seed germination and growth, or by lowering soil temperatures. Recent increased interest in cover crops gives rise to the question as to what benefits might be obtained from planting mixtures instead of monocultures. Research conducted in 2012 by the University of Nebraska-Lincoln showed that on an equivalent land basis area, cover crop mixtures were more productive than monocultures, but they didn't result in an increase in cash crop productivity or establishment of the cover crop (Wortman et al. 2012).

A second method by which cover crops contribute to weed control is allelopathy. Allelopathy is a form of plant interference that occurs when one plant inhibits with growth of another through a chemical inhibitor (Zimdahl 2013). The production of allelochemicals varies with the environment and the accompanying stresses of the environment. Einhellig (1996) found that environmental conditions can modify the rate of allelochemical production, meaning environmental stress could enhance the relative biological activity of an allelochemical so that a lower concentration may inhibit growth of other plants. Allelochemicals enter the environment in many ways, primarily through root exudation, movement through the soil via leaching, or



volatilization (Zimdahl 2013). The mode and time at which the chemical enters can alter the effects that take place.

**Commonly Used Species and Their Allelochemicals.** While there is an array of plant species that can be used for cover cropping, some species are more commonly used than others. In Missouri, some commonly used species include cereal rye (*Secale cereal L.*), brassica species such as radish (*Raphanus sativus L.*), and legume species such as winter pea (*Pisum sativum L.*). All of these species are known to produce allelochemicals and each has unique advantages and disadvantages for use.

Cereal rye can be seeded later in the fall than most cover crops and still produce sufficient biomass. Rye outperforms most other cover crops when planted on land that is infertile, sandy, acidic, or poorly prepared. Rye overseeds readily into many agronomic crops and resumes growth quickly in the spring. This allows for a timely killing by either rolling, mowing, or herbicide application (SARE 2012). Rye is easy to establish with the ability to become established in many different soil types and environments. For use as a winter annual cover crop, seeding in late summer to midfall is recommended for optimal growth. In the spring, rye matures and grows quickly. Rye can immobilize nitrogen, depending on its maturity at termination. An early kill of rye has the possibility of reducing nitrogen immobilization and conserving soil moisture. However, a late kill of rye can deplete soil moisture and produce more residue (Clark et al. 1997). This increase in residue could be difficult for a tillage system to handle due to an increase in above ground biomass (SARE 2012).

The common allelochemicals found in cereal rye are DIBOA (2,4-Dihydroxy-1,4-benzoxazin-3-one), and its metabolite, BOA (2-benzoxazonlinone; Kelton 2012). DIBOA exists as a nontoxic, stable glucoside that is released through enzymatic hydrolysis. This process

is activated upon wounding or tissue death. At elevated temperatures, DIBOA spontaneously degrades to BOA which is a less toxic and more stable metabolite (Barnes 1987).

Brassica species are commonly known for rapid growth in the fall, high biomass production and nutrient foraging ability. Brassicas are normally used as a cover crop in vegetable and specialty crop production but are gaining popularity in row crop production. This is primarily due to their ability for capturing nutrients, trapping nematodes, and for their biofumigation activity. Brassicas act as biofumigants with the release of chemical compounds that can be toxic to soil borne pathogens and pests. For establishment of brassicas, it is best to plant them 4 weeks before the average of the first freeze with the maximum soil temperature of 85 degrees Fahrenheit and the minimum 45 (SARE 2012). The allelochemical found in brassica species are gluconsinolates (Kelton et al. 2012). Gluconsinolates are secondary metabolites that contain sulfur and nitrogen. They are enzymatically hydrolyzed by myrosinase in the presence of water to form isothiocyanates (Haramoto et al. 2005).

Winter peas exhibit rapid growth in cool, moist weather. They can withstand temperatures as low as -12°C. However, they don't consistently grow well in areas colder than moderate hardiness zone 6. For optimal performance, pea should be established in soils that are well-drained with a neutral pH and moderate fertility. Winter pea excels at fixing nitrogen and produces abundant amounts of vining forage. This helps contribute to short-term soil conditioning. Termination can be accomplished easily with herbicides or by disking and mowing after full bloom (SARE 2012). The allelochemicals found in winter pea are coumarins (Kelton, 2016). These are compounds that can also be found in the Apiaceae and Asteraceae families (Razavi 2011).

A producer can realize many benefits by implementing cover crops into their integrated pest management program. Managing populations of herbicide resistant weeds requires additional, nonchemical control options such as cover crops. The implementation of cover crops will not only improve weed management, but it will also improve the overall land management. The main goal of producers is to help feed the world, but it is also to help preserve the land and leave it better off than when they found it. Cover cropping systems have the capability to aid producers in all of these aspects of their operation.

### **Objectives**

The overall objective of this study was to evaluate control of common and troublesome weeds found in Missouri cropping systems using cover crops. Specifically, this study sought to identify effective cover crop mixtures and evaluate the relative contribution of allelopathy and physical suppression of selected weed species. The weed species for the present study were chosen because they are commonly found in Missouri fields, grow rapidly, are prolific seed producers, and have the potential to be difficult to control. Cover crop species were chosen due to being popular for use with producers and for their allelopathic capabilities.

## **MATERIALS AND METHODS**

### **Methods Common to Both Experiments**

Greenhouse and growth chamber studies were conducted in Greene County, Missouri during the summer of 2018 to evaluate the relative contribution of physical interference and allelopathy to weed suppression by winter annual cover crop mixtures. This study evaluated the effects on two weed species, large crabgrass and common waterhemp. The experiment had a factorial treatment arrangement plus a non-treated control. There were three cover crop mixtures, a cereal rye monoculture, a cereal rye plus winter pea mixture, and a cereal rye plus winter pea plus radish mixture. There were also three methods of application, a fresh biomass, dried leached biomass, and leachate (Table 1). Treatment applications were assigned using a random number generator resulting in a randomized complete block design. There were four replications for each species, with ten pots per replication.

### **Preparation Common to Both Studies**

Cover crops were grown in a greenhouse located on the Missouri State University campus located at 37.19905°N, -93.27618°E. Cereal rye (45 g area<sup>-1</sup>, Beck's. Atlanta, IN), Austrian winter pea (20 g area<sup>-1</sup>, Green Seed Inc. Springfield, MO), and radish (25 g area<sup>-1</sup>, Beck's. Atlanta, IN) were seeded into greenhouse flats on April 22, 2018. Seed was planted into Promix BX with mycorrhizae (Pro Mix®, Quakertown, PA). These planting rates were used to simulate those similar to what producers would plant in the field (Nathan and Reinbott, 2011). Three-week old shoots were harvested on May 13, 2018 and combined into mixtures. Mixtures consisted of cereal rye monoculture (14.4 g), cereal rye plus Austrian winter pea (7.2 g of each),

and cereal rye, Austrian winter pea, and radish (4.8 g of each; Table 2). Mixtures were dried for 48 hours at 50°C in a forced-air dryer (Cascade Tek®, Cornelius, OR). Oven-dried material was cut into 1-2 cm pieces and placed in 200 mL of distilled water at a pH of 6. Cover crop mixtures were then shaken for 24 hours at room temperature at a speed of 350 rotations per minute (VWR® Incubating Mini Shaker, Radnor, PA). After 24 hours, mixtures were vacuum-extracted through 4 layers of cheesecloth until no additional leachate was removed. Original residue was resuspended in distilled water (pH 6) and re-extracted after an additional 48 hours on shaker. The same vacuum-extraction procedure was performed. Extracts (250 mL) were centrifuged (Beckman® J2-HS, Indianapolis, IN) at 4,000xg for 10 minutes. Leachate was stored in the dark at 7°C for four days until applied. The leached residues were re-dried at 50°C for 48 hours and stored at room temperature until treatments were applied (Burgos et al. 1999). Fresh residues were maintained in a greenhouse until study initiation and applied immediately after harvest. All cover crop treatments were applied immediately after planting.

### **Greenhouse Studies**

Common waterhemp and large crabgrass were planted on May 26, 2018 in 15-cm plastic azalea pots containing 49.5cm<sup>3</sup> of growing medium (Promix BX with mycorrhizae, Pro Mix®, Quakertown, PA). Each pot contained 20 seeds of each species.

Fresh cover crop residue was applied at a rate of 1.2 g of residue per pot to designated pots. The leached residue application was 0.07 g per pot for the cereal rye monoculture, 0.09 g per pot of the cereal rye and winter pea mixture, and 0.06 g per pot of the cereal rye, winter pea, and radish mixture. There is a difference between biomass weights for dried leached residue because application weight was based on remaining biomass after extraction and drying process.

All mixtures started with the same fresh biomass weight. For all applications of leachate, 50 mL were applied. Plants grew for 42 days (harvested July 7, 2018) and pots were evaluated daily. During the study, the average maximum temperature in the greenhouse was 37.1°C and the average minimum temperature was 19.2°C. The average photoperiod was 14 hours and 44 minutes.

### **Growth Chamber Studies**

Common waterhemp and large crabgrass were planted in 0.9-mL Styrofoam cups. Seed was planted into a 100% sand mixture. Each pot contained 10 seeds of the designated plant. Treatments were applied similarly to the greenhouse study, except for the mass of fresh and dried residue. Fresh residue was applied at a rate of 2.1g, 0.13g, and 0.1g of the one-, two-, and three-way mixtures, respectively. Variation in rates were seen to help make it comparable to the dried leached residue applications. Leached residue was applied at 0.13g per pot for the cereal rye monoculture, 0.1g per pot for the cereal rye and winter pea mixture, and 0.09g per pot for the cereal rye, winter pea, and radish mixture. There is a difference between biomass weights for dried leached residue because application weight was based on remaining biomass after extraction and drying down process. All mixtures started with the same fresh biomass weight. For leachate applications, 50mL was applied. Plants grew for 28 days and cups were evaluated daily and watered as needed. The growth chamber settings were programmed to simulate day and night time with different light settings and temperatures. Day time was set from 10am to 6pm with the temperature at 27°C. Night time was set from 7pm to 9am with temperature at 15°C. The growth chamber used was a Low Temperature Diurnal Illumination Incubator (2015-2, Sheldon Manufacturing Inc®, Cornelius, OR).

## **Harvest Measurements**

Plants were harvested 42 days after planting (DAP) in the greenhouse and 29 DAP in the growth chamber. Throughout the duration of the greenhouse study, emergence counts, height measurements, and leaf counts were recorded. Height and leaf counts were measured on the tallest plant in each pot. Upon harvest, final emergence counts, final height, fresh biomass, leaf area index (LAI), and SPAD meter readings were taken. Emergence counts, height measurements, and leaf counts were not taken throughout the growth chamber study as a result of slow emergence. SPAD meter measurements were not taken in the growth chamber study due to the small leaf size. Dry biomass was weighed after being dried at 50°C for 48 hours. For the growth chamber study emergence counts, height, fresh biomass, and leaf area index were recorded at harvest. Dry biomass was weighed after being dried at 50°C for 48 hours.

Data were analyzed using mixed model analysis in SAS (SAS 9.4, SAS® Institute Inc. Cary, NC). Replicate was considered a random effect, while treatment was a fixed effect. Models appropriate for repeated measures were used to analyze data that was collected over time. After initial ANOVA, orthogonal contrasts were used to compare effects of factors. To determine significance, alpha was set at 0.05.

## RESULTS

### Greenhouse Studies

**Common Waterhemp.** The interaction of treatment and DAP was significant for common waterhemp emergence, height and leaf counts (Table 3). Mean number of plants emerged ranged from 1.5 to 5.5 at 11 DAP to 3.75 to 10 at 42 DAP. Generally, treatments of leachate of the cereal rye and winter pea had the least emergence throughout the study. Treatments of the dried leached residue of the cereal rye monoculture had the most emergence (Figure 1). Pots that were treated with dried leached residue of the cereal rye and winter pea mixture resulted in the least amount of plants emerged while the most plants emerged were in pots treated with dried leached residue of the cereal rye monoculture at 42 DAP (Figure 1). Common waterhemp height responded similarly in that the shortest plants were in pots treated with dried leached residue of the cereal rye and winter pea mixture. However, the tallest plants were in pots treated with the leachate from the cereal rye, winter pea, and radish mixture (Figure 2). Similarly, leaf counts were least following applications of dried leached residue of the cereal rye and winter pea mixture. The most leaves were seen after applications of leachate of the cereal rye, winter pea, and radish mixture (Figure 3).

Results of orthogonal contrast are listed in Table 4. No differences were observed among cover crop mixtures; however, differences in emergence were observed among residue types. Emergence following applications of dried residue was greater than emergence following fresh residue. Emergence following leachate application was intermediate and similar to emergence following application of both types of residue. Orthogonal contrasts did not identify differences between application treatments for leaf count or height. In a comparison among application



methods, the greatest number of common waterhemp plants emerged in pots treated with applications of dried residue (Figure 4). This suggests that the allelochemicals contained in cover crop mixtures contributed to weed control more than physical mechanism of weed suppression. It is possible that a difference wasn't seen between the leachate and dried residue applications because all the allelochemicals weren't extracted during the extraction process. While there wasn't a statistical difference among the application methods for plant height and leaf counts, similar trends were observed. In regard to height, the tallest plants grew in pots treated with dried leached residue while the shortest plants were in pots treated with fresh residue (Figure 5). The same was true for the leaf counts as well (Figure 6).

Analysis of variance indicated no differences among common waterhemp harvest measurements (Table 5), but trends were observed. The greatest reduction in emergence occurred following applications of the cereal rye and winter pea mixtures, while the least reduction came from the cereal rye, winter pea, and radish mixture applications (Figure 7). The shortest plants were found in pots treated with applications of fresh residue of the cereal rye and winter pea mixtures, while the tallest plants were treated with dried leached residue of cereal rye, winter pea, and radish (Figure 8). Common waterhemp LAI was least in pots treated with cereal rye and winter pea mixtures and greatest in pots treated with cereal rye monoculture, across all application methods (Figure 9). Similarly, the smallest SPAD readings within each application method were recorded in pots treated with the cereal rye and winter pea mixture, while the largest readings came from pots treated with the cereal rye, winter pea, and radish mixture (Figure 10). Lastly, the smallest dry weights were observed in pots that had been treated with the cereal rye and winter pea mixture with the largest in the control (Figure 11).

**Large Crabgrass.** The interaction of treatment and DAP was significant for large crabgrass emergence but not for height and leaf count (Table 6). The mean number of plants emerged ranged from 5.5 to 9.75 at 11 DAP and 6.5 to 10.5 42 DAP. Generally, application of leachate of the cereal rye monoculture resulted in the least emergence while treatments of dried leached residue for the cereal rye monoculture had the most (Figure 12). Despite lack of significance, trends in height and leaf counts were observed throughout the study. The shortest plants were in pots treated with leachate of cereal rye and winter pea while the tallest were treated with leachate of the cereal rye monoculture (Figure 13). In regard to leaf counts, little variation is seen among treatments throughout the study (Figure 14).

Results of orthogonal contrasts are listed in Table 7. No differences were observed among cover crop mixtures, but differences were observed in leaf counts among residue types. More leaves were seen on plants treated with applications of leachate than dried residue and fresh residue (Figure 15). While orthogonal contrasts did not indicate any differences between applications for emergence or height, trends were observed. Pots with the greatest emergence were treated with dried leached residue while the least emergence was in pots treated with leachate (Figure 16). At 42 DAP, all applications resulted in similar height measurements for all plants (Figure 17). A decrease in height and leaf count was observed around 23 DAP, likely due to insect damage in the greenhouse.

Analysis of variance showed there was not a treatment effect for harvest measurements (Table 5); however, trends were observed. The greatest emergence was seen in pots treated with dried leached residue from the cereal rye monoculture with the least amount of emergence in pots treated with the leachate from the cereal rye monoculture (Figure 18). Trends for height indicate that the tallest plants were treated with the dried leached residue of the cereal rye, winter

pea, and radish mixture with the shortest plants being treated with the dried leached residue of the cereal rye monoculture (Figure 19). LAI was greatest in pots treated with applications of fresh residue of the cereal rye and winter pea while it was least in the non-treated pots (Figure 20). SPAD meter readings were least in pots treated with applications of fresh residue of the cereal rye and winter pea while the greatest readings were in pots treated with leachate of the cereal rye monoculture (Figure 21). The lowest dry weights were in pots treated with applications of fresh residue of the cereal rye monoculture and the plants with the highest dry weights being treated with leachate of the cereal rye monoculture (Figure 22).

### **Growth Chamber Studies**

**Common Waterhemp.** Analysis of variance of common waterhemp measurements at yielded no significance differences. No treatment means were different than zero (Tables 8 and 9). This a result of poor emergence during the study.

**Large Crabgrass.** Analysis of variance shows that there was no treatment effect for harvest measurements (Table 8). Similar to the greenhouse studies, trends were observed among application methods. Overall, the least emergence was seen in pots treated with cereal rye monoculture and the greatest emergence was observed in pots treated with the cereal rye, winter pea, and radish mixture (Figure 23). Final height trends suggest that the shortest plants were treated with leachate of the cereal rye and winter pea and tallest were treated with fresh residue of the cereal rye (Figure 24). LAI trends were similar to height trends (Figure 25). Lastly, dry weight trends show that a majority of the applications yielded similar dry weights with the control having the heaviest plants (Figure 26).

## DISCUSSION

Differences between the greenhouse and growth chamber results are likely the result of differences in study conditions. The greenhouse experiment allowed the plants to experience more natural growth tendencies with natural lighting and temperatures that were similar to that of the current growing season. This is also similar to the season in which cover crops would be terminated for soybean production. They were also planted into bigger pots, allowing for more surface that needed to be covered by applications. Cover crops have the ability to impact weed populations due to their proximity of residue to the site of germination at the soil surface (Teasdale et al. 1991). These remaining residues on the surface have the ability to alter the growing environment by changing light availability, soil moisture, and soil temperature (Creamer et al. 1996). Amount of ground cover in a field setting is variable, depending on the density the producer plants at, making both situations applicable. Growing medium in the greenhouse was a potting medium commonly used by horticulture producers. It is possible that the chemical and microbial compositions interacted with the allelochemicals, altering their degradation. Allelochemicals have varying half-lives as they degrade into their metabolites (Macías et al. 2005). The length of half-life can be impacted by many soil characteristics including soil pH and microbial activity (Cipollini et al. 2012, Woodward et al. 1978, Fomsgaard et al. 2012). Pots used in the greenhouse had openings in the bottom while the growth chamber pots were solid bottoms. Openings in the bottoms of pots in the greenhouse allowed for leaching of solution while the closed bottoms in the growth chamber prevented this.

The growth chamber was a more controlled environment that was manipulated to simulate conditions during a spring-established field experiment. Seeds were planted in smaller

pots, decreasing the surface area and depth of the pot. The growing medium used in the growth chamber was 100% sand. This was picked to decrease the potential for allelochemicals to become sorbed. The use of straight sand as a growing medium resulted in the need to use fertilizer more frequently to make sure seeds had the appropriate nutrients for growth (Landis et al. 2014).

When comparing the two weed species, common waterhemp had slower emergence and growth in both studies. This resulted in seed being exposed to the allelochemicals for a longer period, which could have had a greater effect on the plants. Small amounts of common waterhemp emergence in the growth chamber also suggests that it is possible the seed had decreased in viability since initial germination tests conducted or that conditions were not ideal for germination. Leon et al. (2004) found that common waterhemp needed a minimum of 10°C to achieve 50% germination. In future studies common waterhemp emergence should be greater with a higher night temperature set around 20°C and new seed. Large crabgrass had more rapid growth, resulting in development of unaffected new tissue, possibly allowing it to grow out of the effects that the allelochemicals had on development. In the growth chamber, the environment was one more suitable for the growth of the large crabgrass.

The mixture consisting of cereal rye, winter pea, and radish resulted in the worst weed control, based on trends. The cereal rye monoculture had varying effects based on the measurement. The greatest effect was seen in the common waterhemp measurements in the greenhouse. This suggests that the growing environment was more suitable than the growth chamber for the common waterhemp. The applications of dried leached residue were least effective. This suggests that weed control by cover crops is more than a result of physical interference and is enhanced by allelochemicals. This in turn means that cover crops give

producers multiple means of control to incorporate into their integrated weed management program.

While this study did not fully identify the best cover crop mixture for weed control, modifications to this study and more replications would be beneficial. Future replications of this study could be modified as discussed previously to yield better results. Complementary field studies evaluating the effects of planting into an early terminated cover crop versus planting into a living cover crop could be conducted to verify the likelihood that our controlled-environment studies could be replicated in field conditions.

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## TABLES

**Table 1.** Treatment applications used for each study and the rate at which they were applied.

Treatment	Application Rate	
	Greenhouse	Growth Chamber
Cereal rye fresh residue	1.2g	2.1 g
Cereal rye dried leached residue	0.07g	0.13g
Cereal rye leachate	50mL	50mL
Cereal rye+winter pea fresh residue	1.2g	0.13g
Cereal rye+winter pea dried leached residue	0.09g	0.1g
Cereal rye+winter pea leachate	50mL	50mL
Cereal rye+winter pea+radish fresh residue	1.2g	0.1g
Cereal rye+winter pea+radish dried leached residue	0.06g	0.09g
Cereal rye+winter pea+radish leachate	50mL	50mL

**Table 2.** Cover crop mixtures used and the composition of each mixture.

Cover Crop Mixtures	Biomass weight (g)	% Composition
Cereal rye monoculture	14.4 g	100%
Cereal rye+winter pea	14.4 g	50% per species
Cereal rye+winter pea+radish	14.4	33% per species

Effect <sup>a</sup>	Emergence		Height		Leaves	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
DAP	17.71	<.0001	53.94	<0.0001	79.4	<0.0001
tmt	2.08	0.0312	1.19	0.2983	1.65	0.1012
DAP*tmt	1.81	0.0002	1.35	0.0411	1.67	0.0022

<sup>a</sup>DAP = days after planting; tmt = treatment.

**Table 4.** Orthogonal contrasts of emergence, leaf counts, and height for common waterhemp during greenhouse study ( $\alpha=0.05$ ).

Comparison	EMERGENCE		LEAF COUNT		HEIGHT	
	estimate	p value	estimate	p value	estimate	p value
Rye monoculture vs mixtures	4.3192	0.4034	1.2361	0.8258	1.9952	0.7892
Rye vs rye+pea	0.8971	0.7611	3.0326	0.3431	3.3222	0.4343
Rye vs rye+pea+rad	3.4221	0.2466	-1.7964	0.574	-1.327	0.7544
Cover crop vs nontreated	-12.4082	0.2624	-3.0878	0.7946	-12.2478	0.4361
Rye monoculture vs nontreated	-1.7645	0.2838	1.0227	0.5617	-0.2869	0.9022
Fresh residue vs dried, leached residue	-6.675	0.0197	-3.3846	0.2678	-6.4167	0.1133
Fresh residue vs leachate	2.9029	0.3255	-1.0604	0.74	-3.0097	0.4787
Dried,leached residue vs leachate	2.525	0.3757	-4.829	0.1143	-4.6492	0.2507

**Table 5.** Analysis of variance for measurements taken at harvest in the greenhouse study for common waterhemp and large crabgrass.

	Emergence		Height		SPAD		LAI		Dry Weight	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
<b>Common Waterhemp</b>										
<b>Effect</b>										
tmt <sup>a</sup>	1.77	0.120	1.13	0.377	0.49	0.866	1.61	0.162	0.86	0.568
<b>Large Crabgrass</b>										
<b>Effect</b>										
tmt <sup>a</sup>	0.49	0.869	0.26	0.979	0.83	0.598	0.83	0.594	0.42	0.915

<sup>a</sup>tmt = treatment.

**Table 6.** Large crabgrass analysis of variance for repeated measures in the greenhouse study. Significance seen between interactions between DAP and TMT for emergence ( $\alpha=0.05$ ).

Effect <sup>a</sup>	Emergence		Height		Leaves	
	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
DAP	2.77	0.004	192.04	<0.0001	107.23	<0.0001
tmt	0.35	0.9578	0.59	0.801	1.1	0.3641
DAP*tmt	2.57	0.0038	1.26	0.0878	1.24	0.113

<sup>a</sup>DAP = days after planting; tmt = treatment.

**Table 7.** Orthogonal contrasts of emergence, height, and leaf count for large crabgrass during greenhouse study ( $\alpha=0.05$ )

Contrast	Emergence		Height		Leaves	
	estimate	p value	estimate	p value	estimate	p value
Rye monoculture vs mixtures	-5.6245	0.454	4.1865	0.6052	2.25	0.1859
Rye vs rye+pea	-4.175	0.3247	1.9695	0.6664	1.0833	0.2586
Rye vs rye+pea+rad	-1.4495	0.7322	2.217	0.6275	1.1667	0.2238
Cover crop vs nontreated	-7.0505	0.6444	9.942	0.5462	4.25	0.2191
Rye monoculture vs nontreated	-1.625	0.4731	-0.84	0.7308	0.2222	0.6643
Fresh residue vs dried, leached residue	1.6505	0.6739	-4.27	0.3245	-0.5556	0.5311
Fresh residue vs leachate	1.95	0.6453	-6.732	0.1413	-2.1944	0.0226
All residue vs leachate	2.2495	0.7645	-9.294	0.2516	-3.8333	0.0247
Dried, leached residue vs leachate	2.7255	0.4872	0.2475	0.9533	0.08333	0.9251

**Table 8.** Analysis of variance for measurements taken at harvest in the growth chamber study for common waterhemp and large crabgrass.

Emergence	Height	SPAD	LAI	Dry Weight
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<b>Common Waterhemp</b>										
<b>Effect</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>
tmt <sup>a</sup>	1.14	0.3699	1.24	0.3084	0.99	0.468	0.85	0.5802	1.14	0.369

<b>Large Crabgrass</b>										
<b>Effect</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>	<b>F Value</b>	<b>Pr &gt; F</b>
tmt <sup>a</sup>	2.12	0.0607	2.21	0.0513	1.52	0.188	0.85	0.5797	2.12	0.061

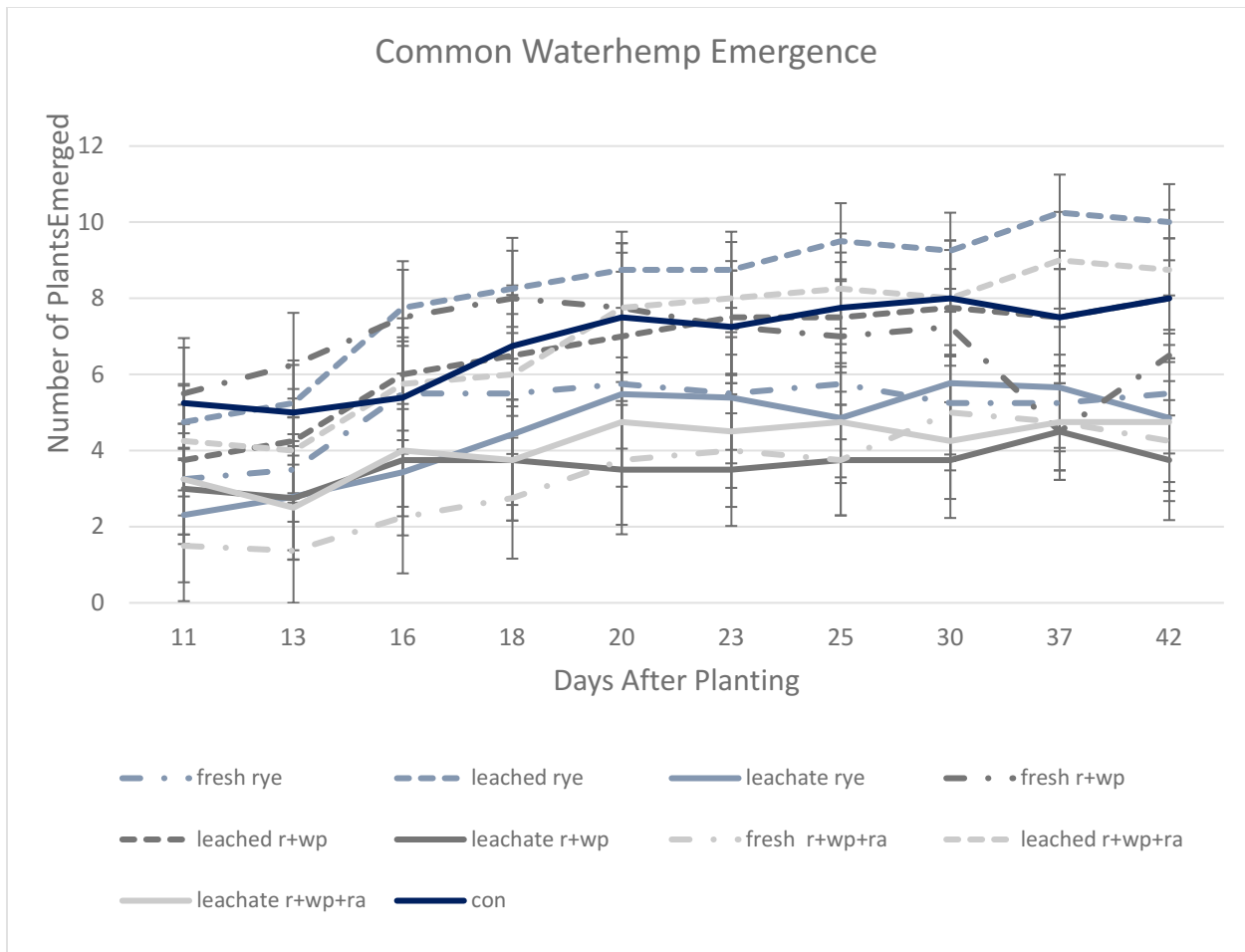
<sup>a</sup>tmt = treatment

**Table 9.** Least square means for at harvest measurements of common waterhemp plants in the growth chamber.

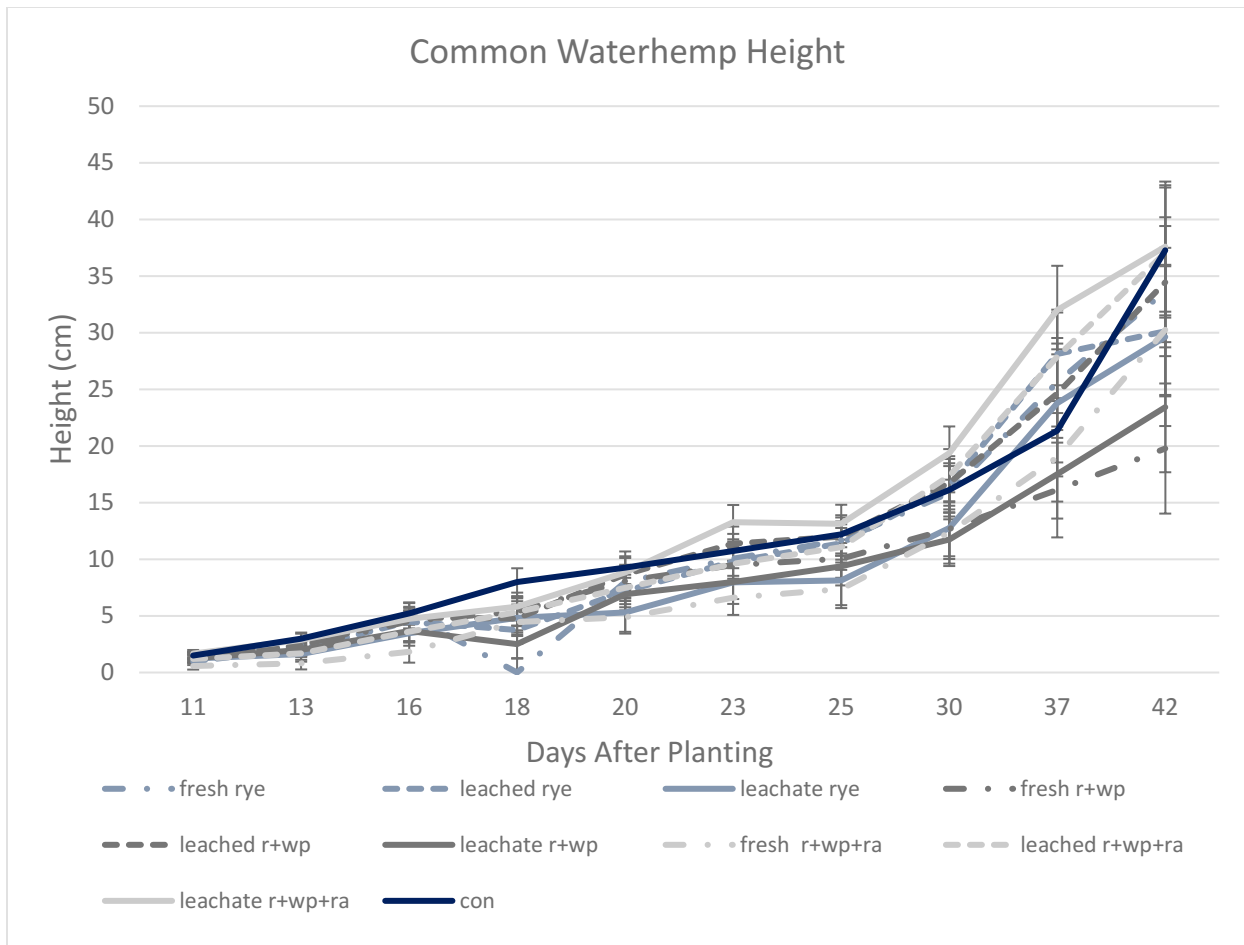
<b>tmt</b>	<b>Emergence</b>		<b>Height</b>		<b>Dry Weight</b>		<b>LAI</b>	
	<b>LSM</b>	<b>SE</b>	<b>LSM</b>	<b>SE</b>	<b>LSM</b>	<b>SE</b>	<b>LSM</b>	<b>SE</b>
1	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191
2	0.1886	0.3138	0.5185	0.7068	-0.00294	0.007529	0.006702	0.01191
3	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191
4	0.4386	0.3138	1.0185	0.7068	0.002056	0.007529	0.01145	0.01191
5	0.1886	0.3138	0.3935	0.7068	0.01206	0.007529	0.0182	0.01191
6	-0.06142	0.3138	-0.3565	0.7068	0.01206	0.007529	-0.00355	0.01191
7	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191
8	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191
9	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191
10	-0.3114	0.3138	-0.7315	0.7068	-0.00294	0.007529	-0.0088	0.01191

Abbreviations: LAI= leaf area index, LSM= least square mean, SE= standard error

## FIGURES

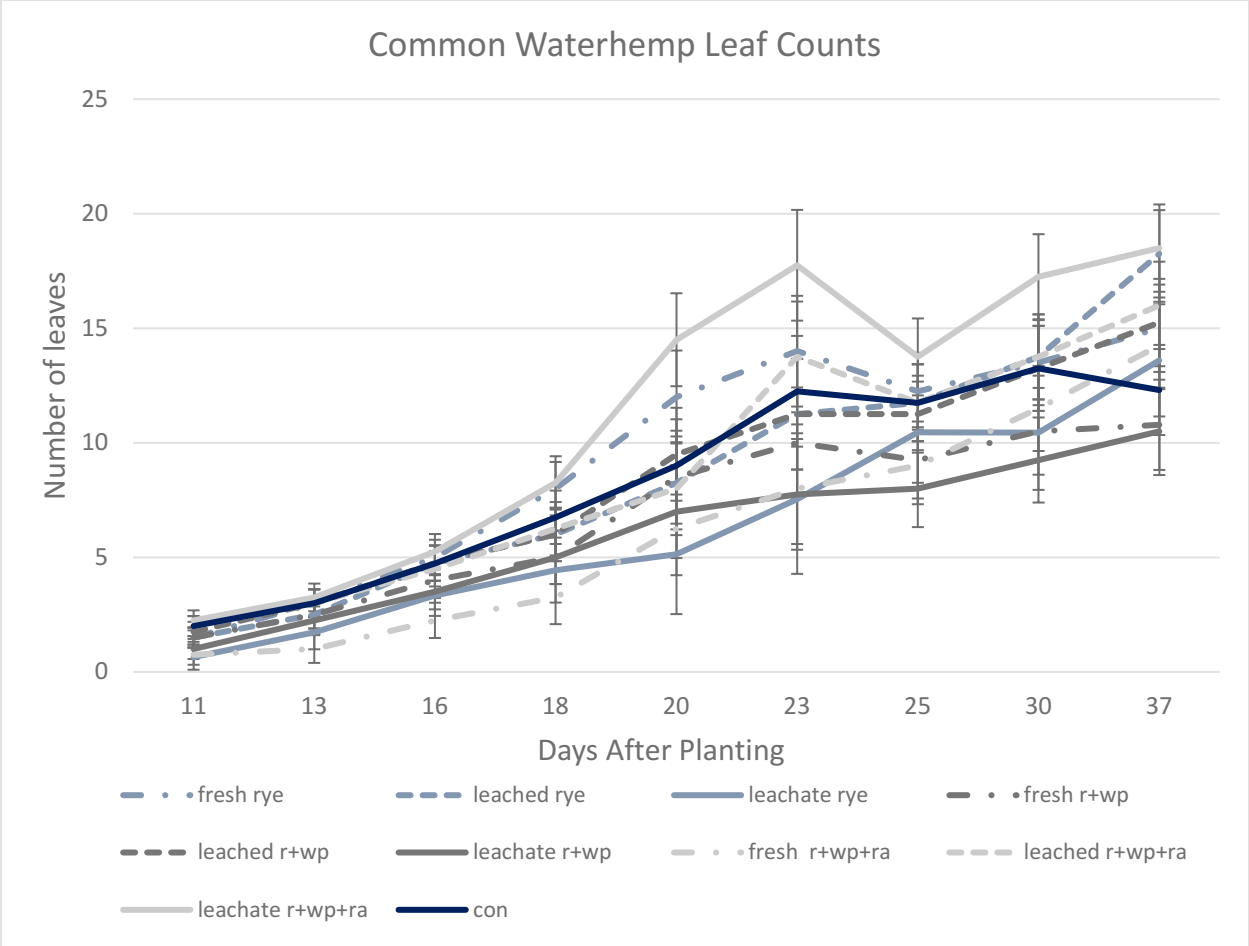


**Figure 1.** Emergence of common waterhemp plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

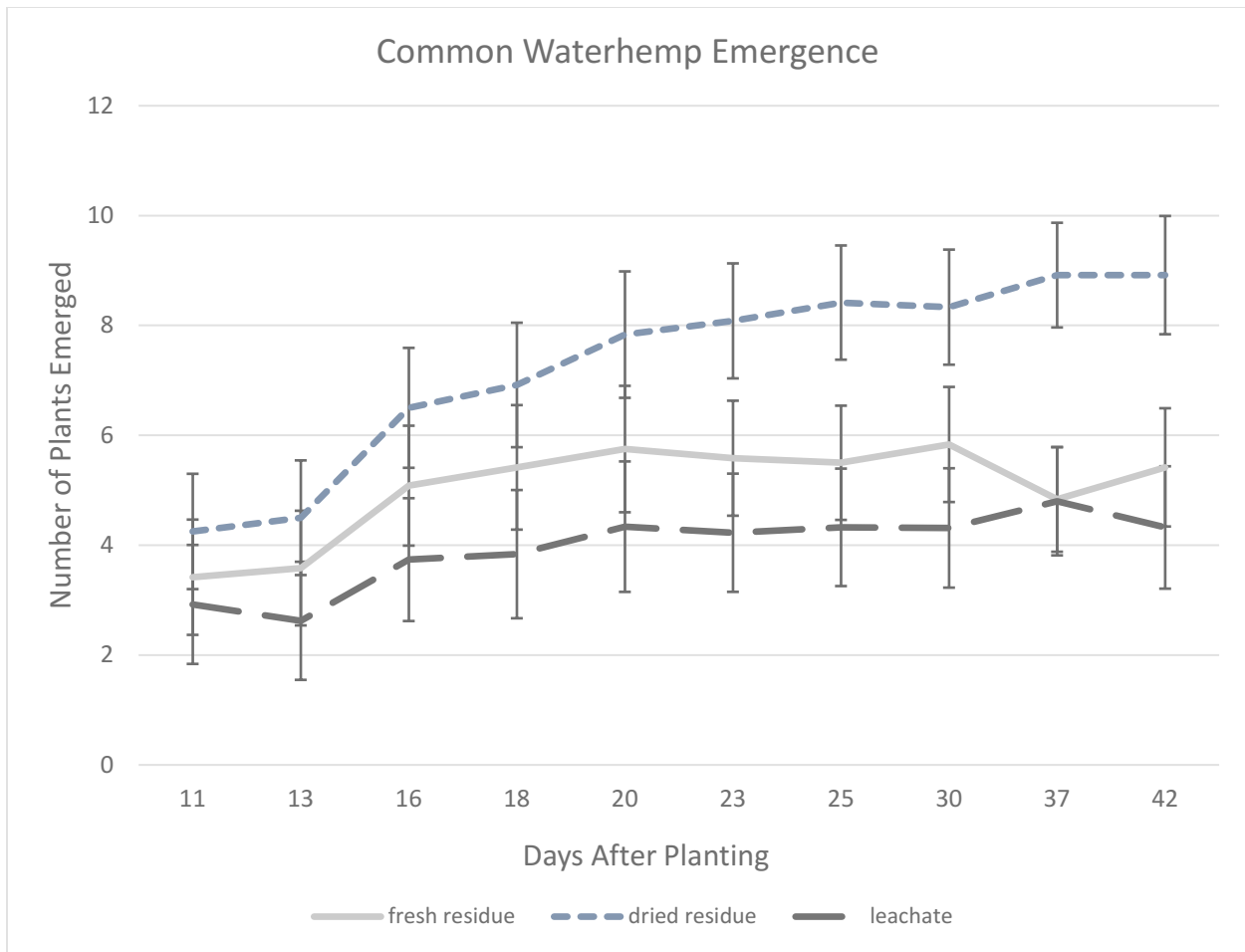


**Figure 2.** Height of common waterhemp plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

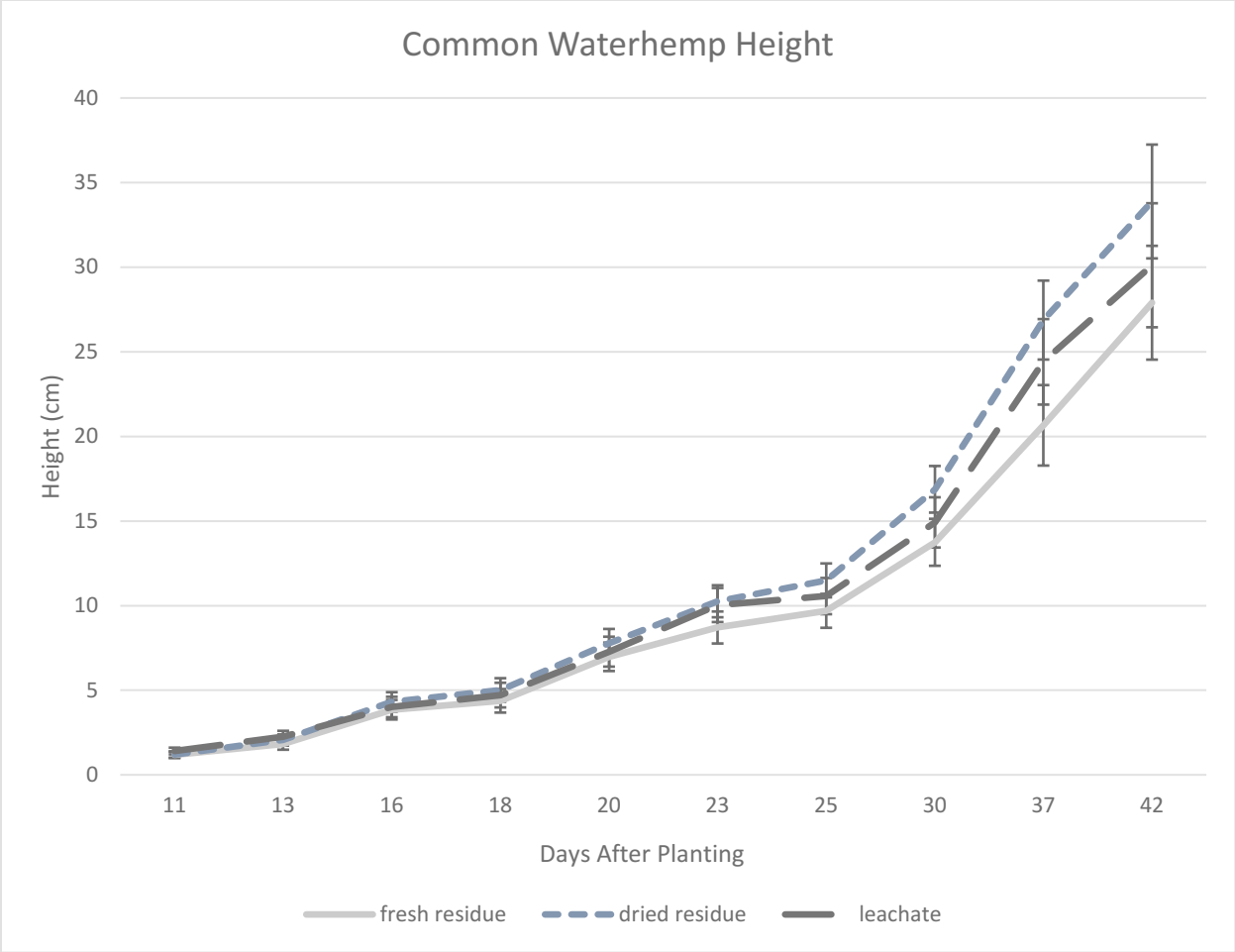




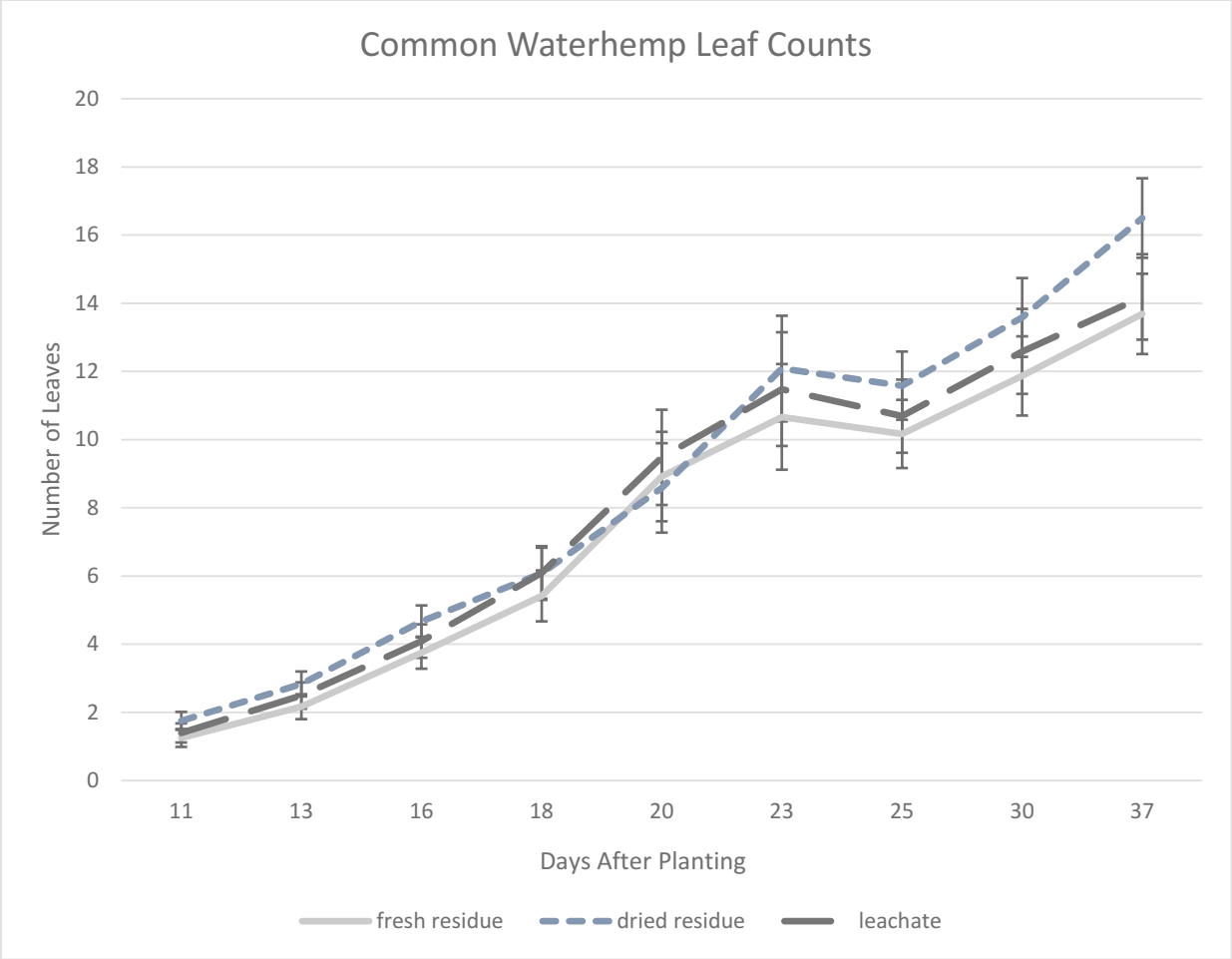
**Figure 3.** Leaf counts of common waterhemp plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



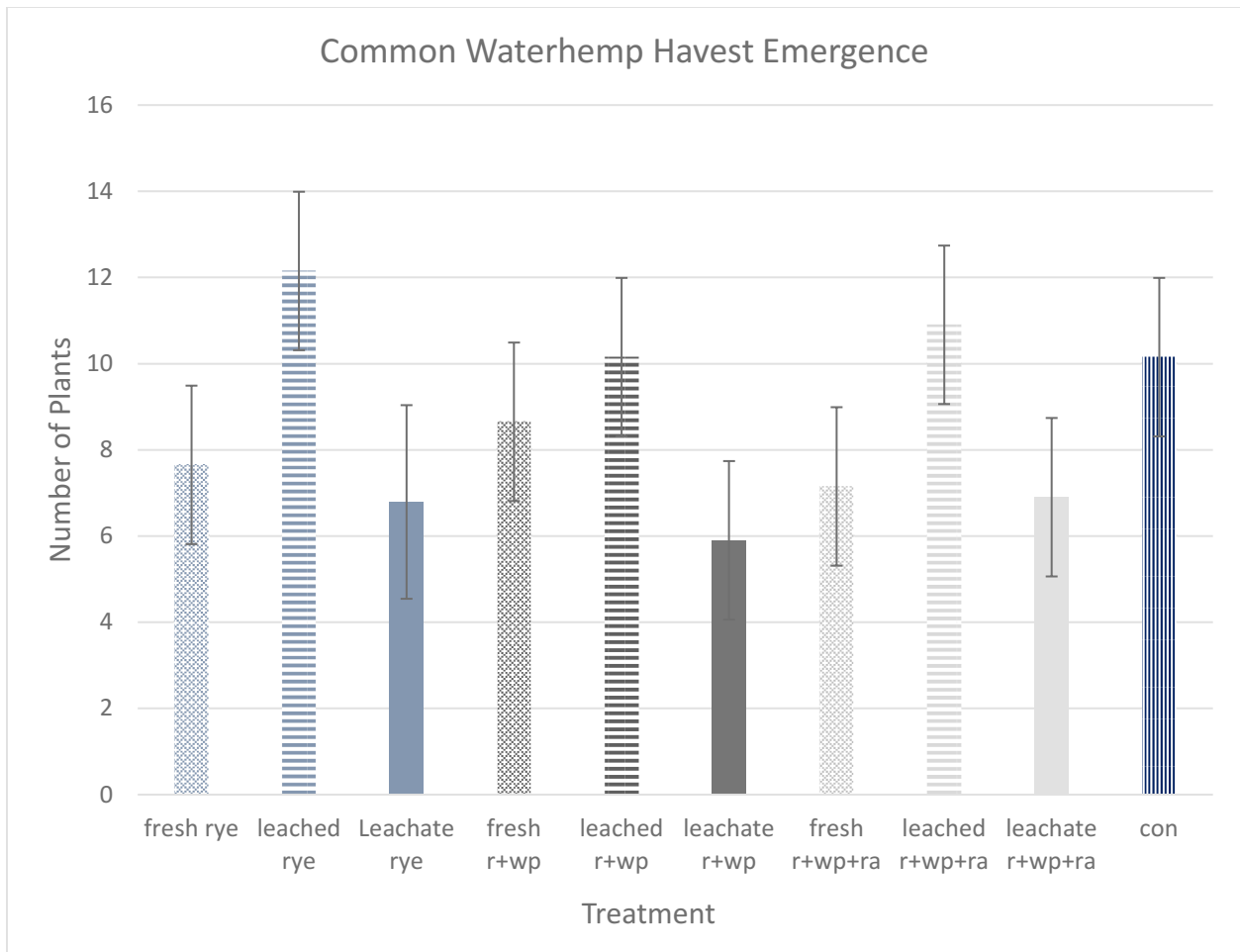
**Figure 4.** Emergence of common waterhemp throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.



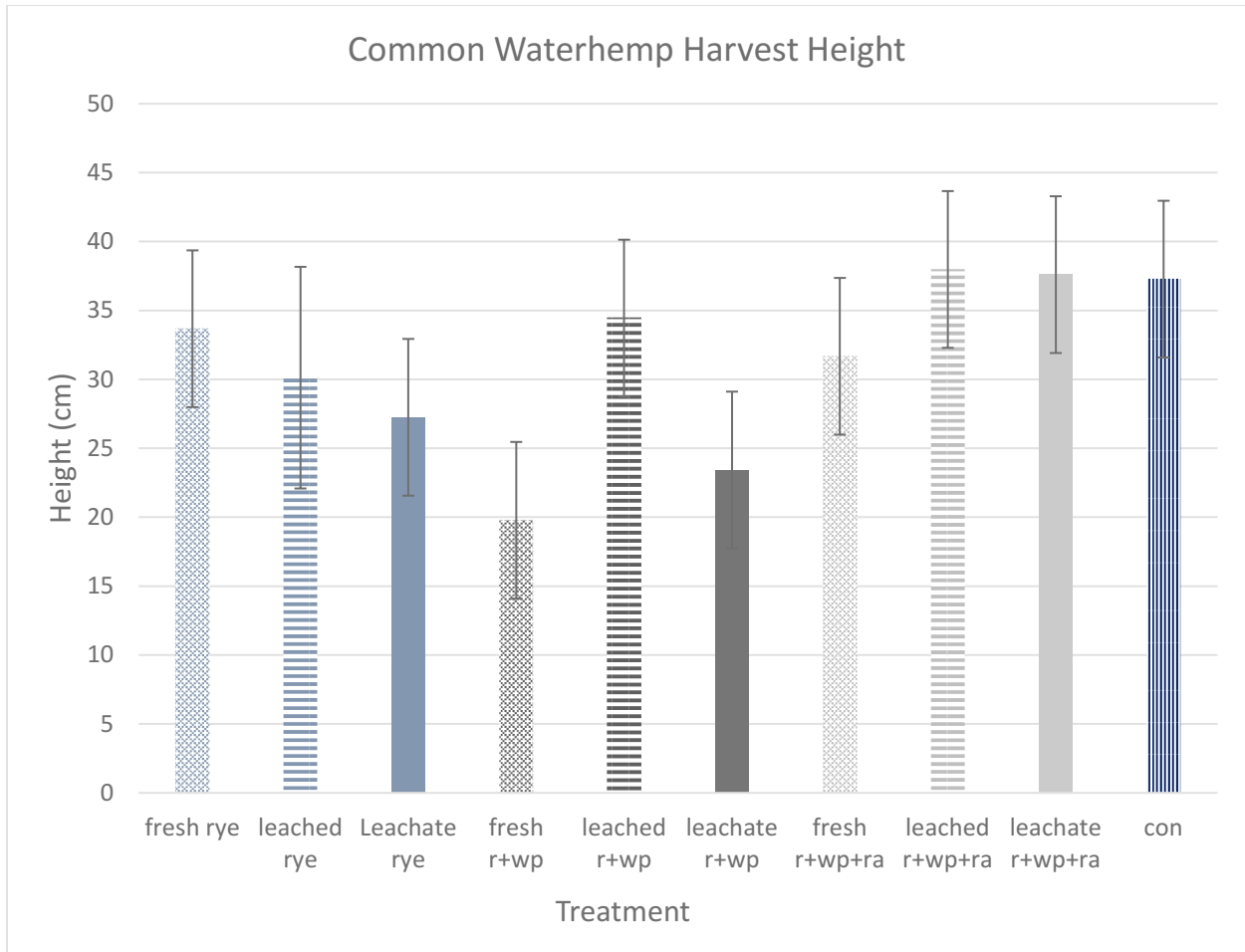
**Figure 5.** Height of common waterhemp throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.



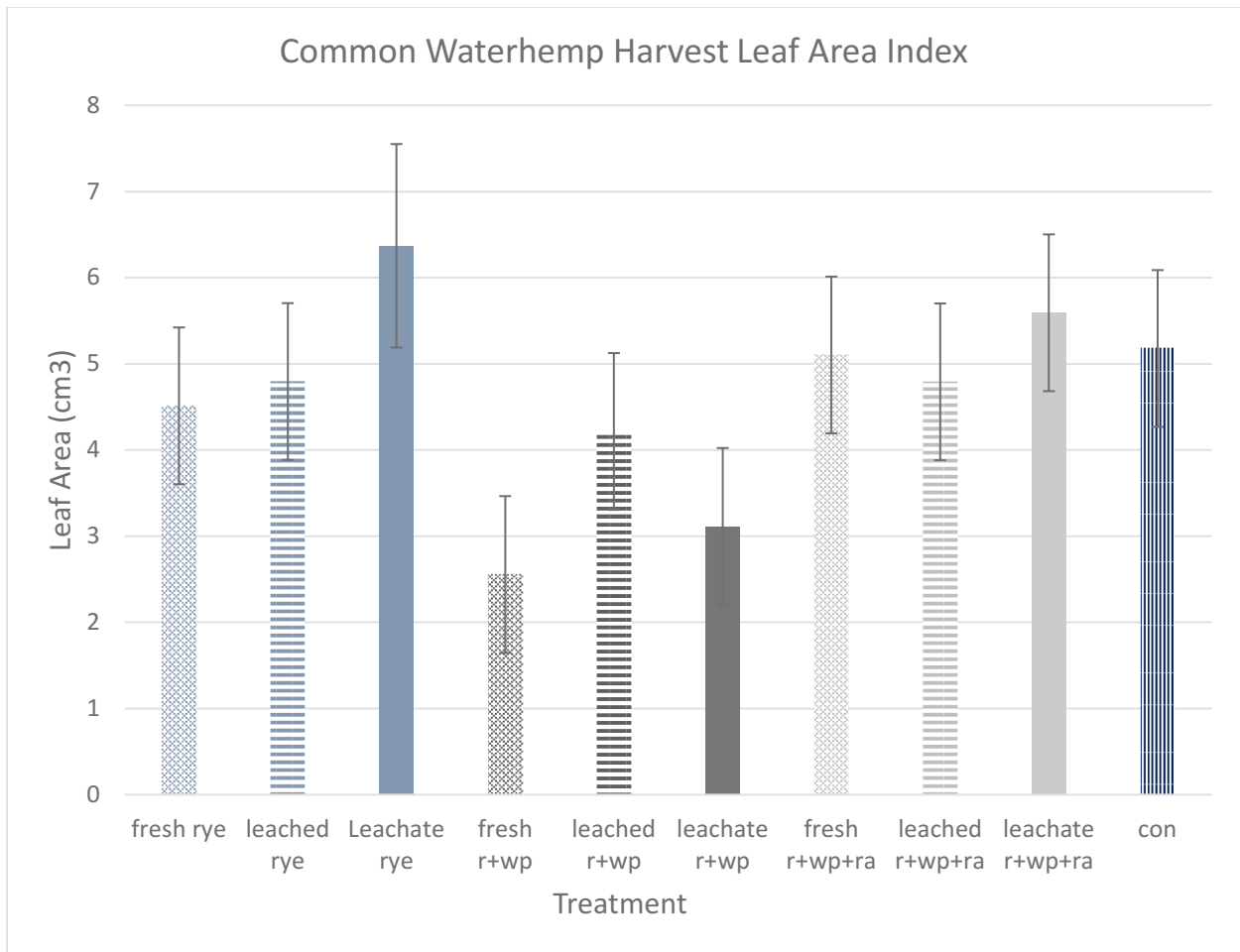
**Figure 6.** Leaf counts of common waterhemp throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.



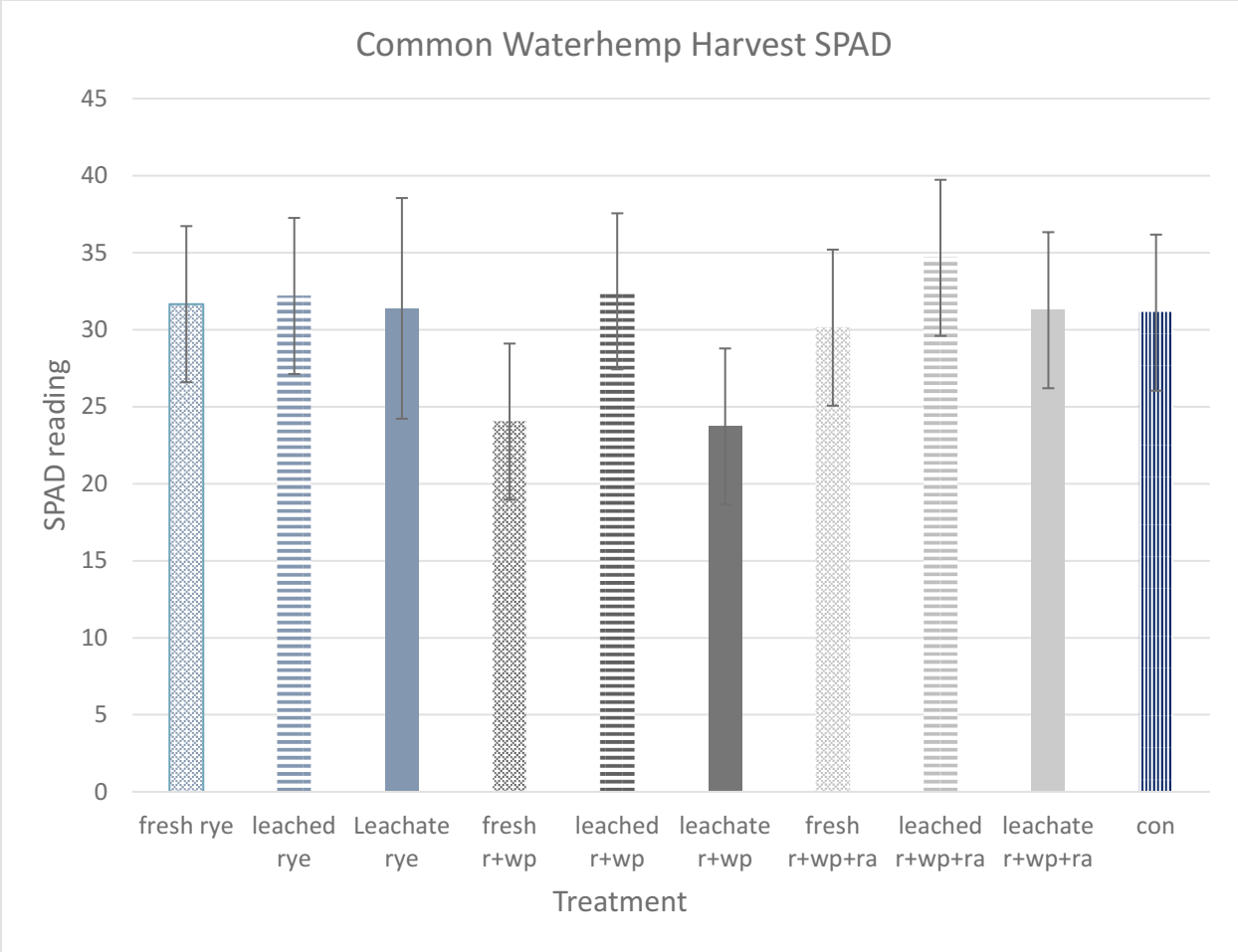
**Figure 7.** Total emergence at harvest of common waterhemp in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



**Figure 8.** Final height at harvest of common waterhemp plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

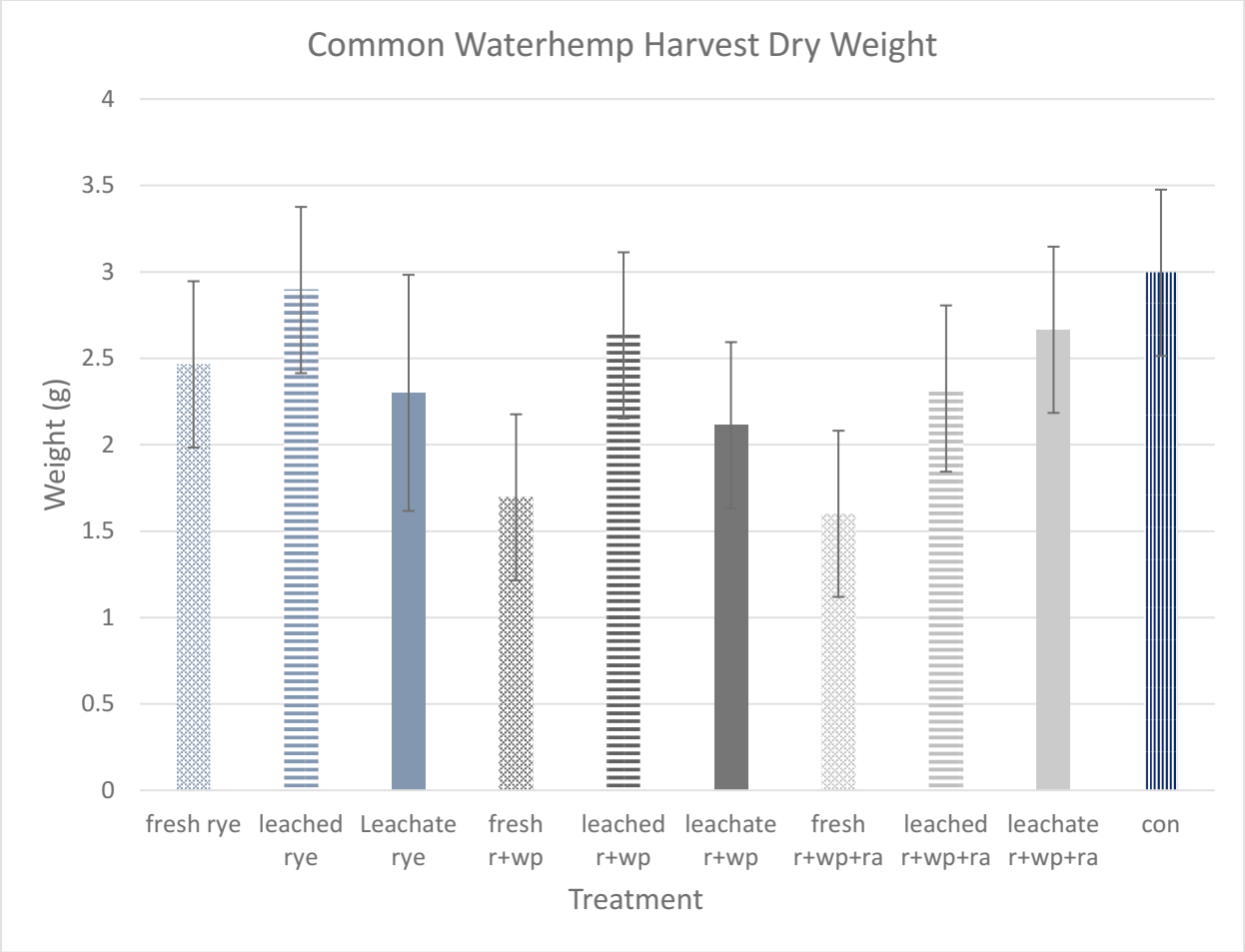


**Figure 9.** Leaf area index at harvest of common waterhemp plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

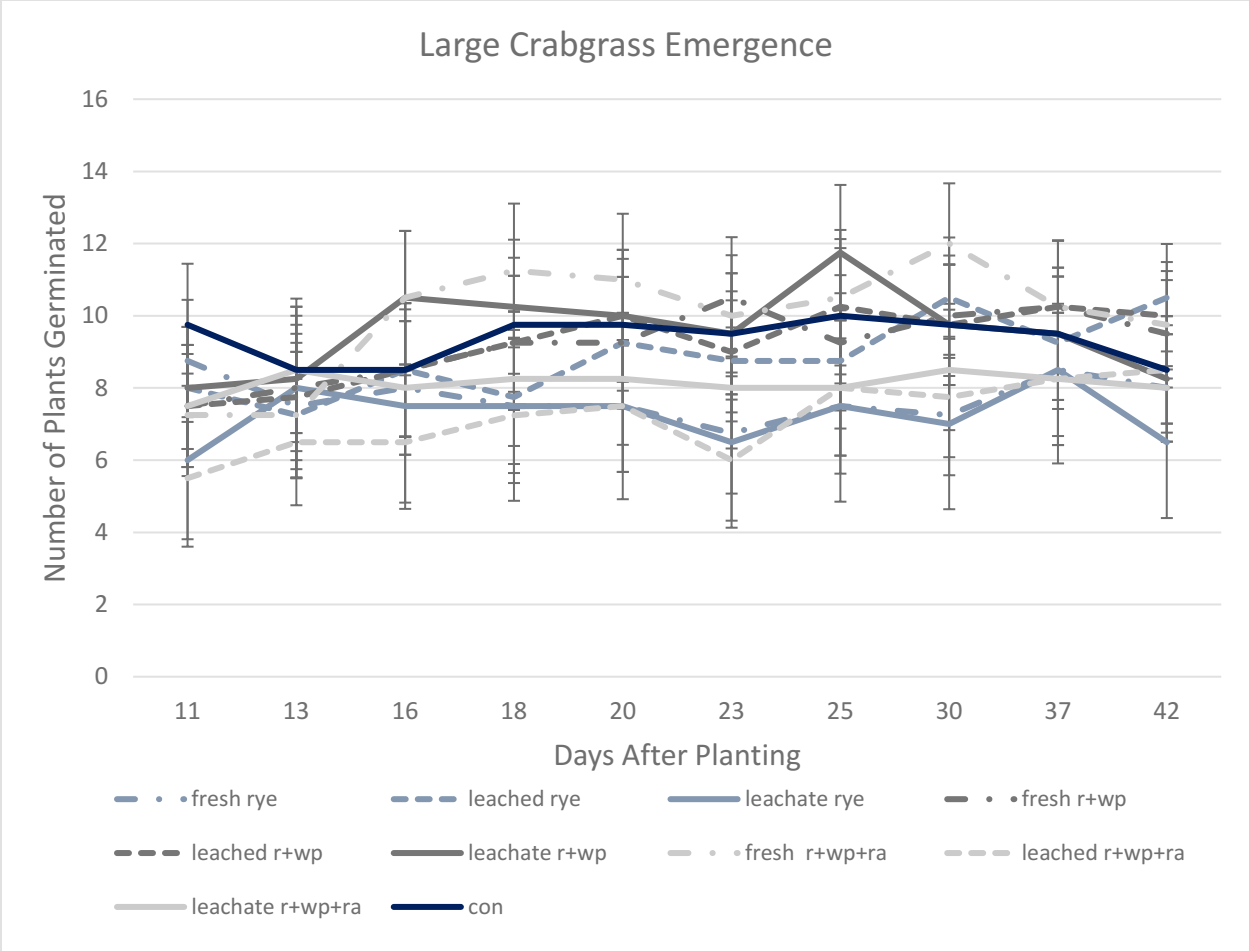


**Figure 10.** SPAD meter readings at harvest of common waterhemp plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

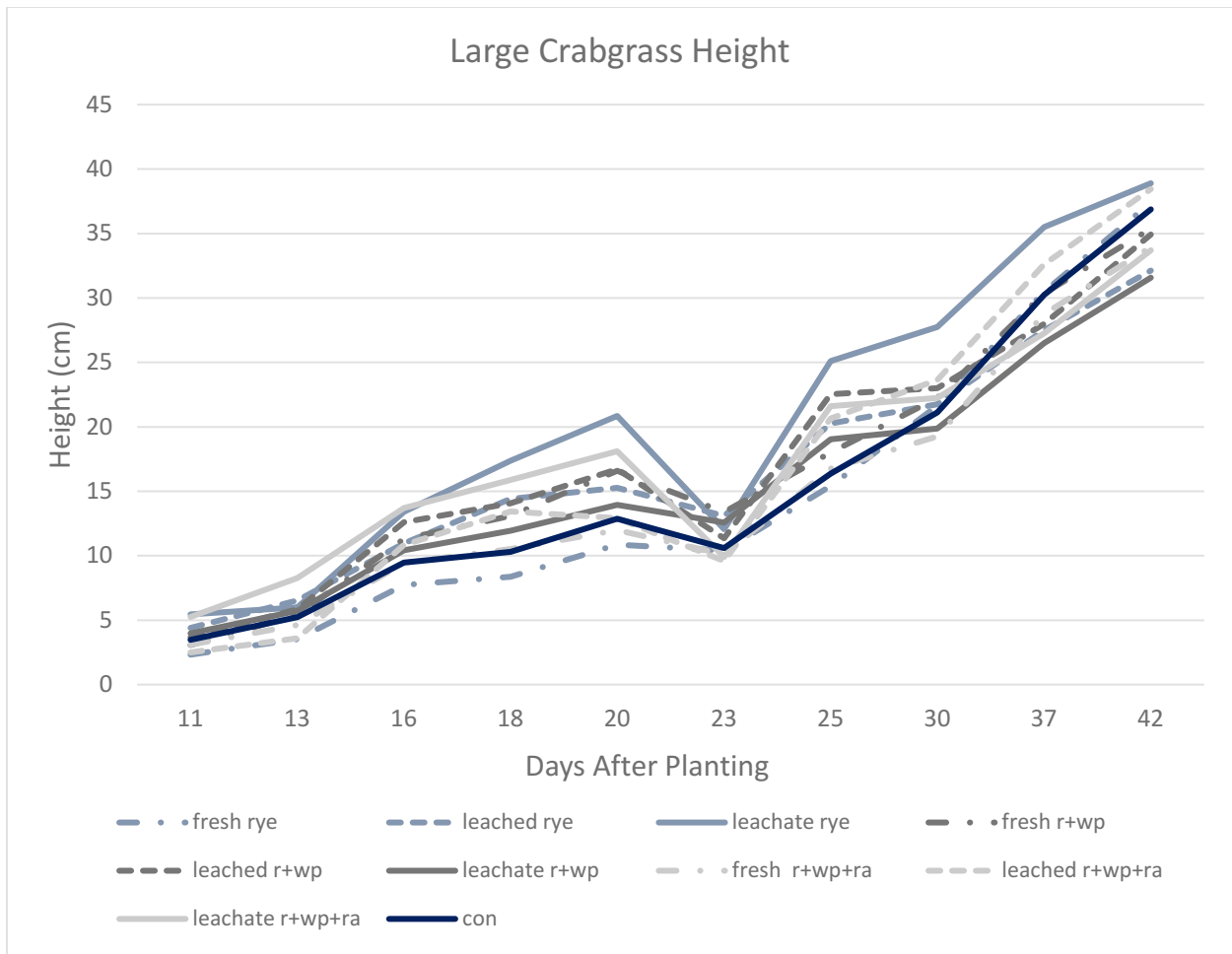




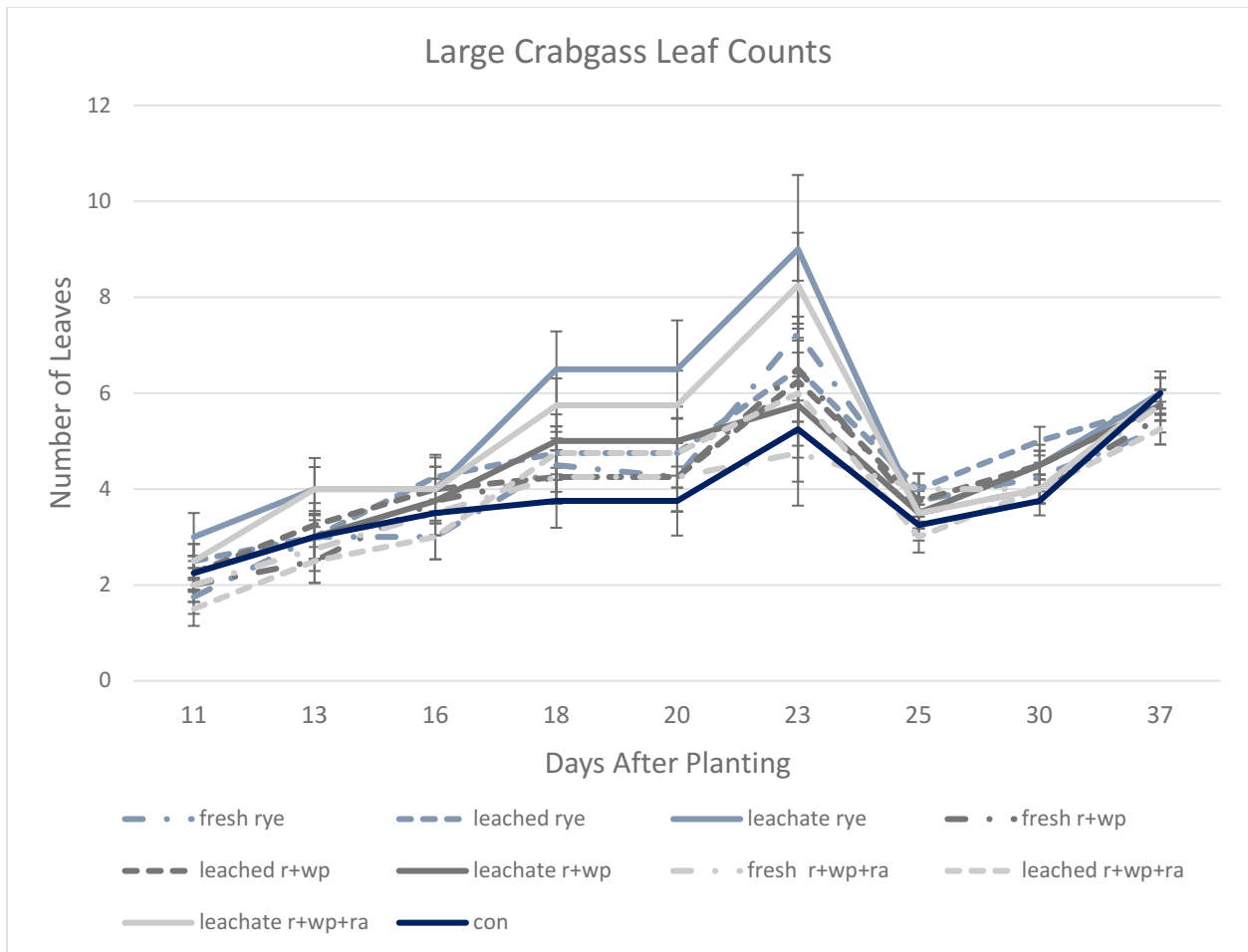
**Figure 11.** Dry weight 48 hours after harvest of common waterhemp plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



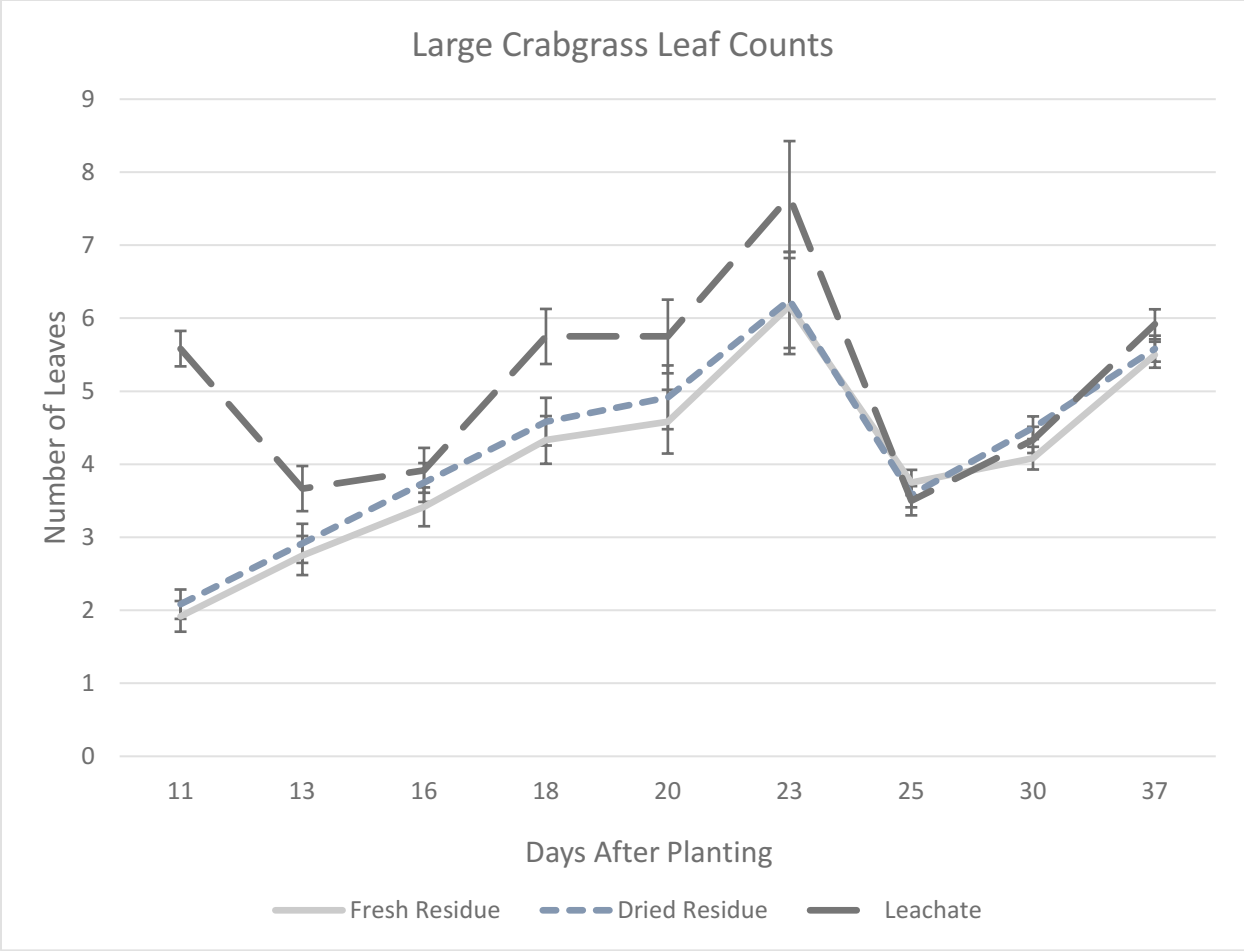
**Figure 12.** Emergence of large crabgrass plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



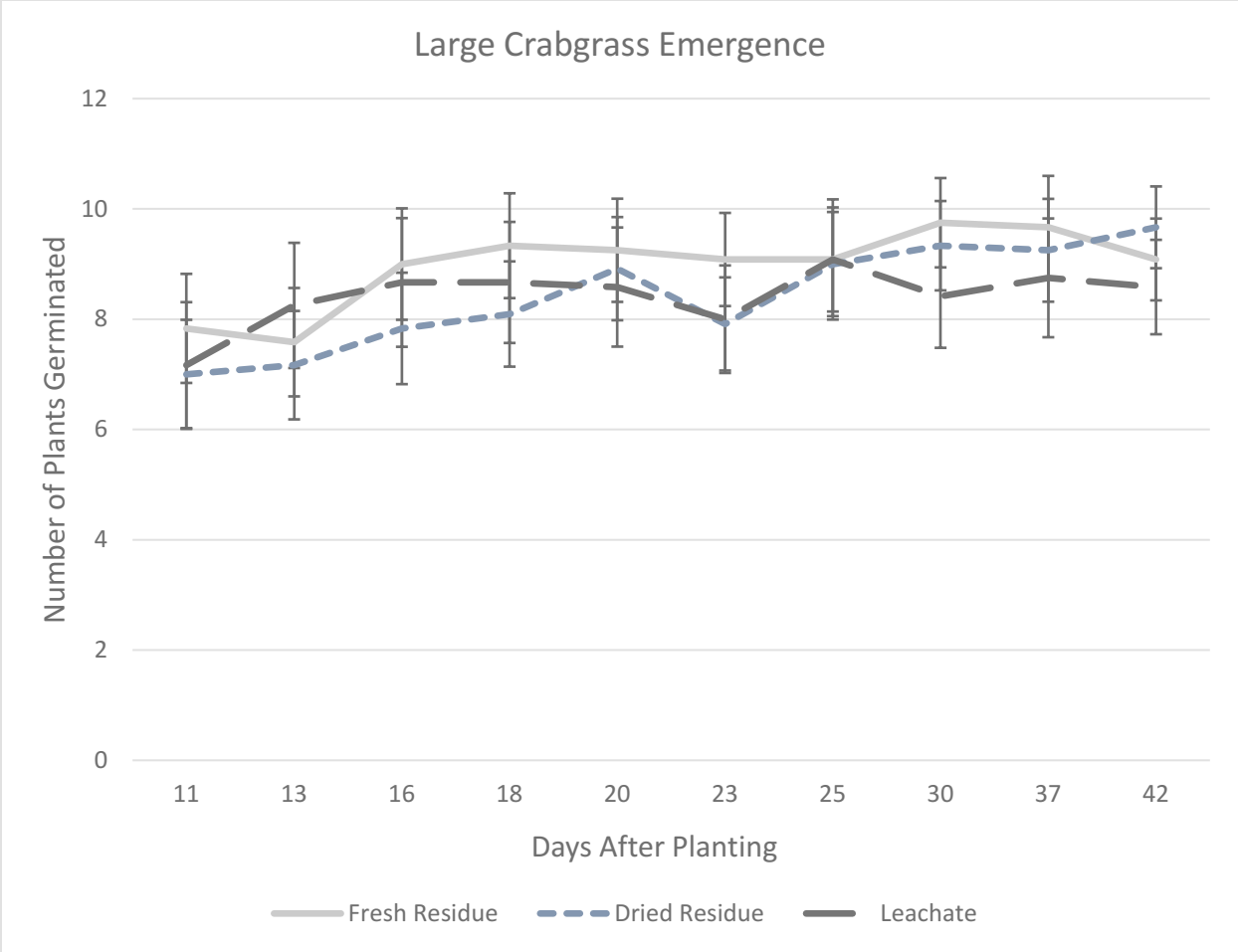
**Figure 13.** Height of large crabgrass plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



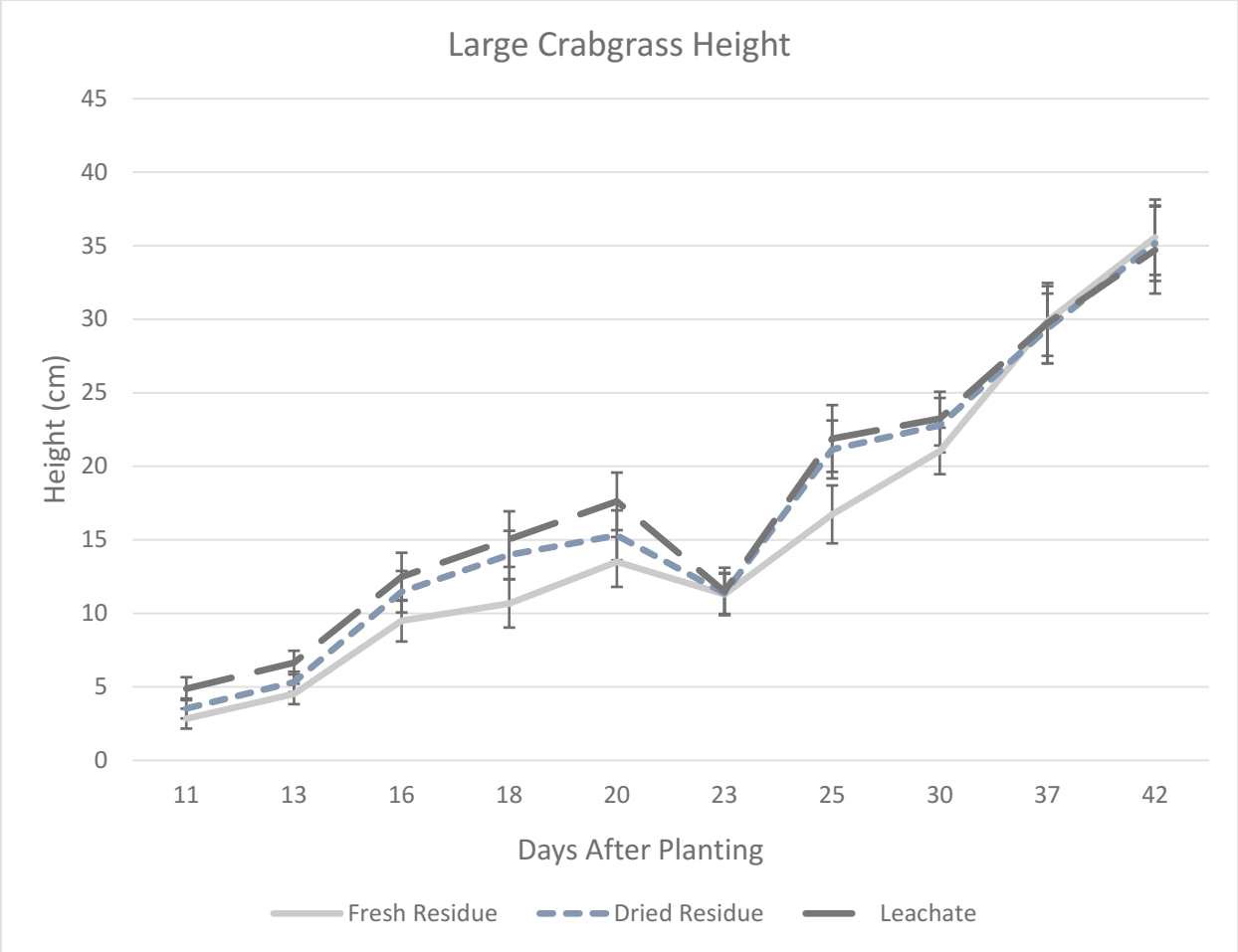
**Figure 14.** Leaf count of large crabgrass plants throughout the greenhouse study based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



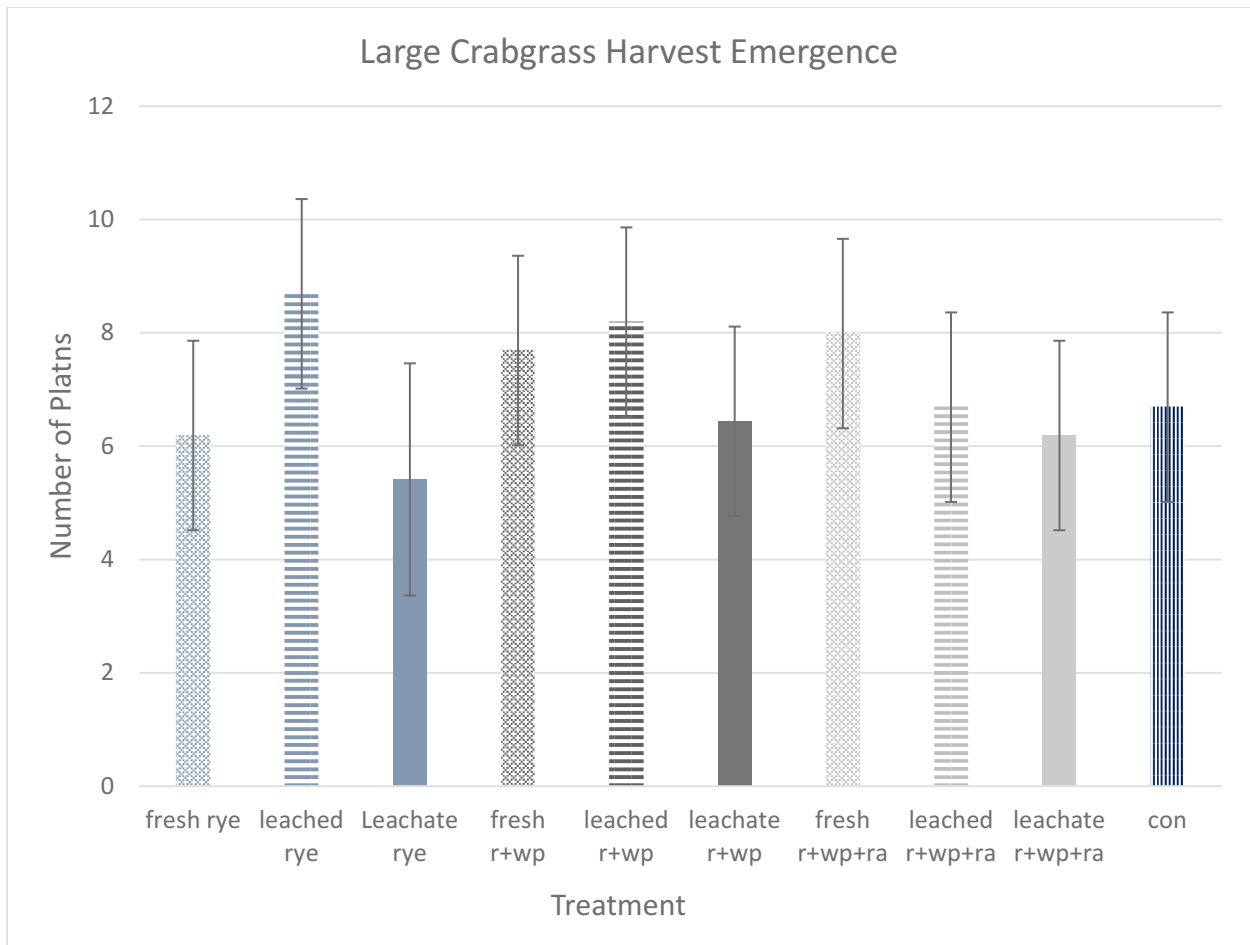
**Figure 15.** Leaf counts of large crabgrass throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.



**Figure 16.** Emergence of large crabgrass throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.

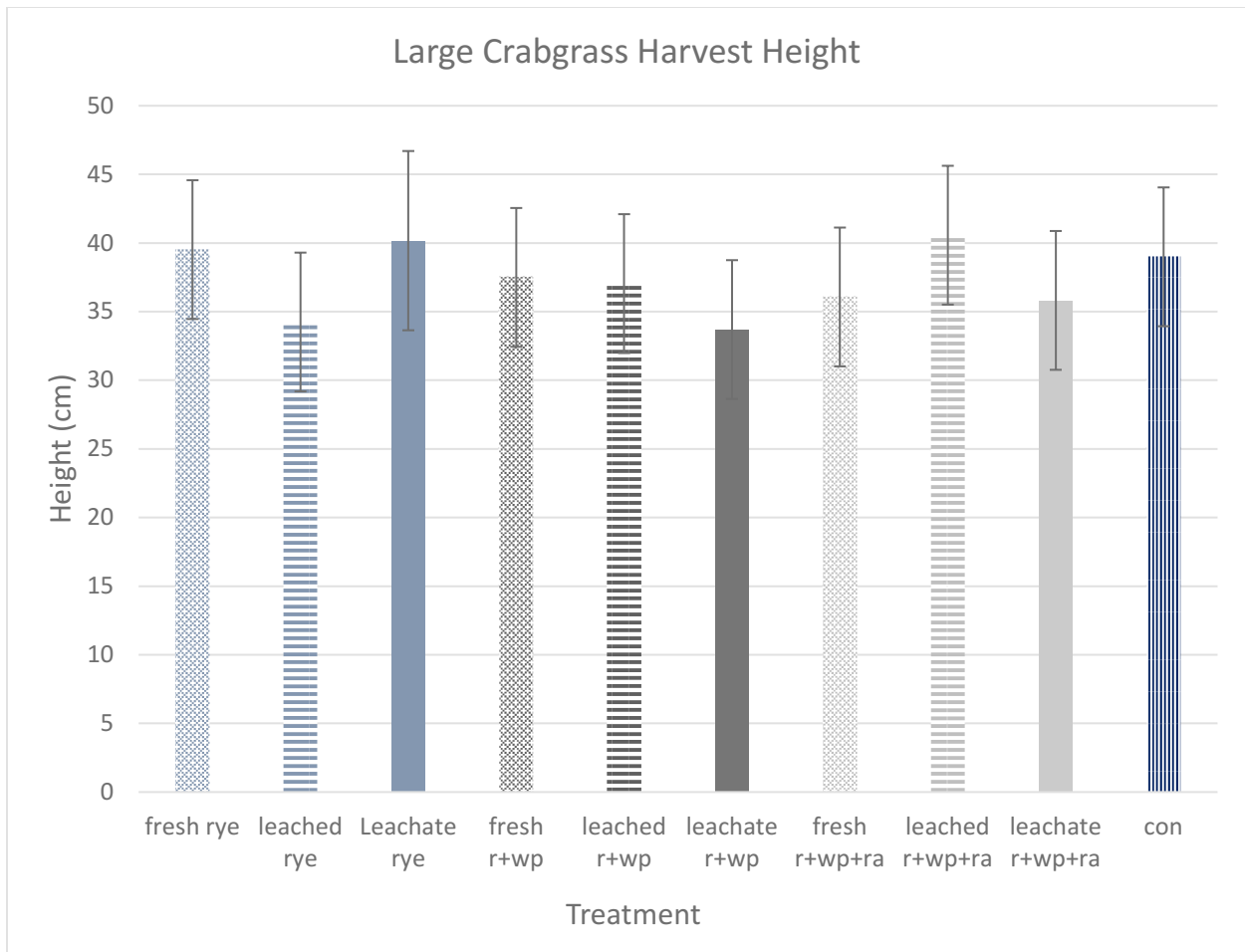


**Figure 17.** Height of large crabgrass throughout the greenhouse study based on applications of fresh residue, dried residue, and leachate.

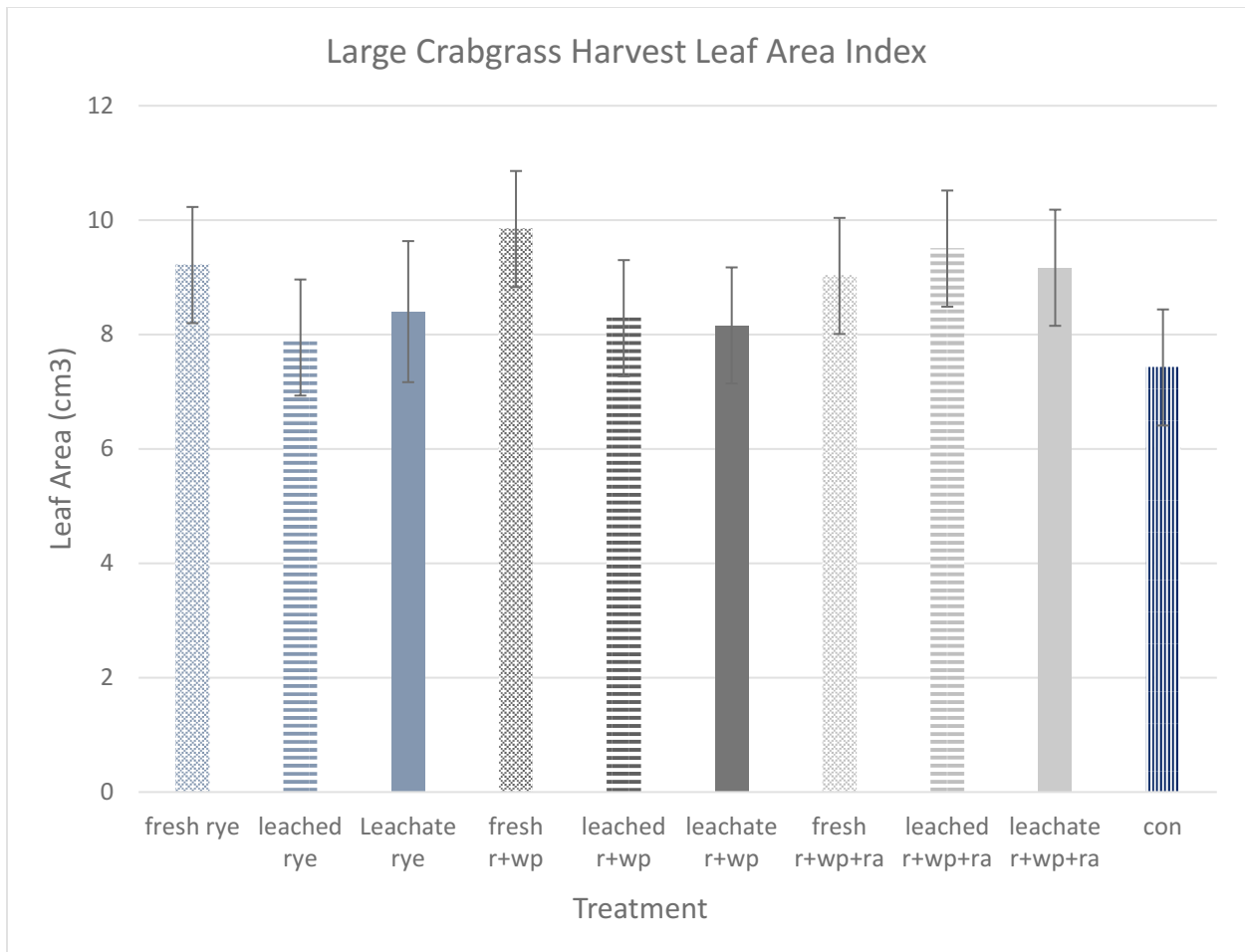


**Figure 18.** Total emergence at harvest of large crabgrass plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control

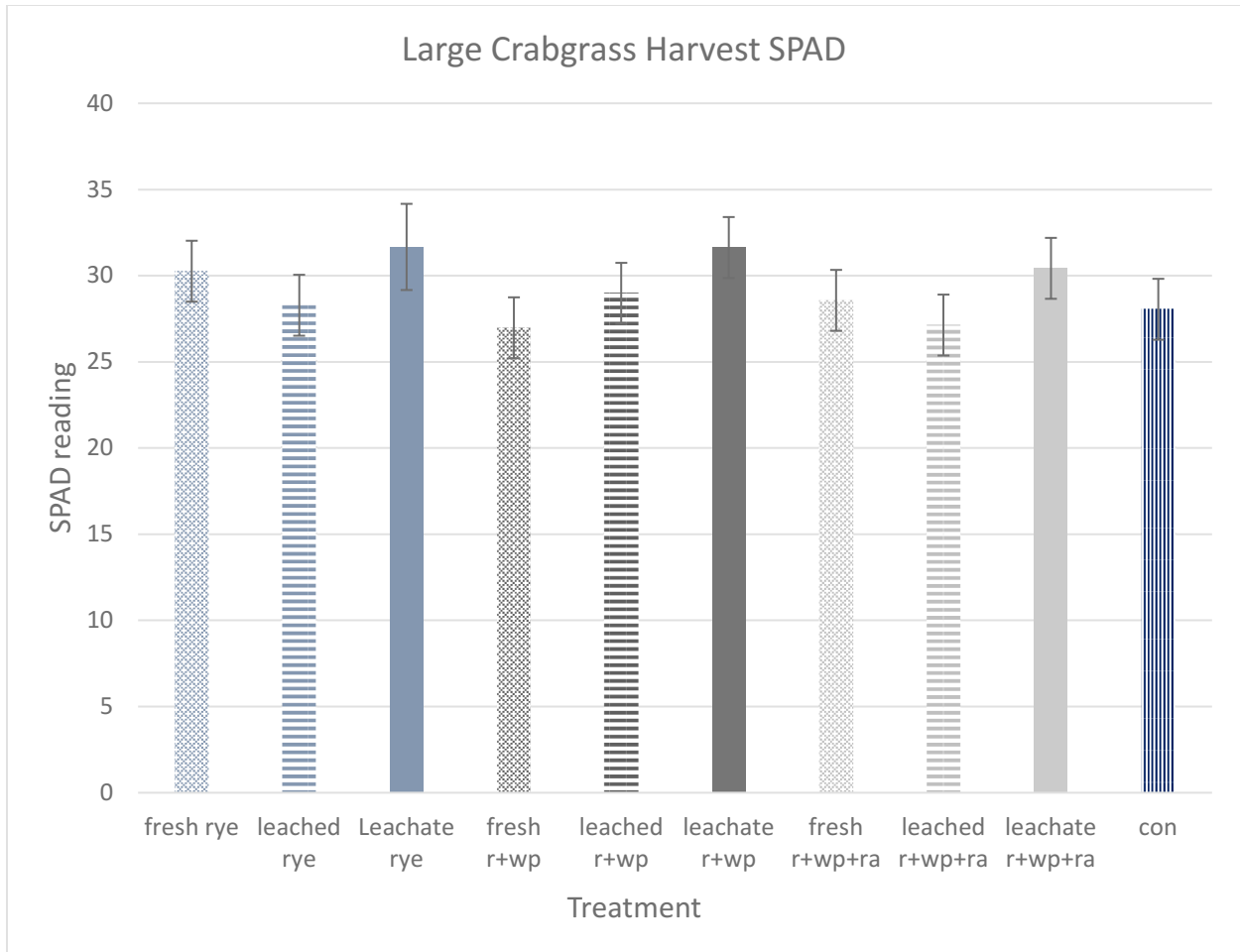




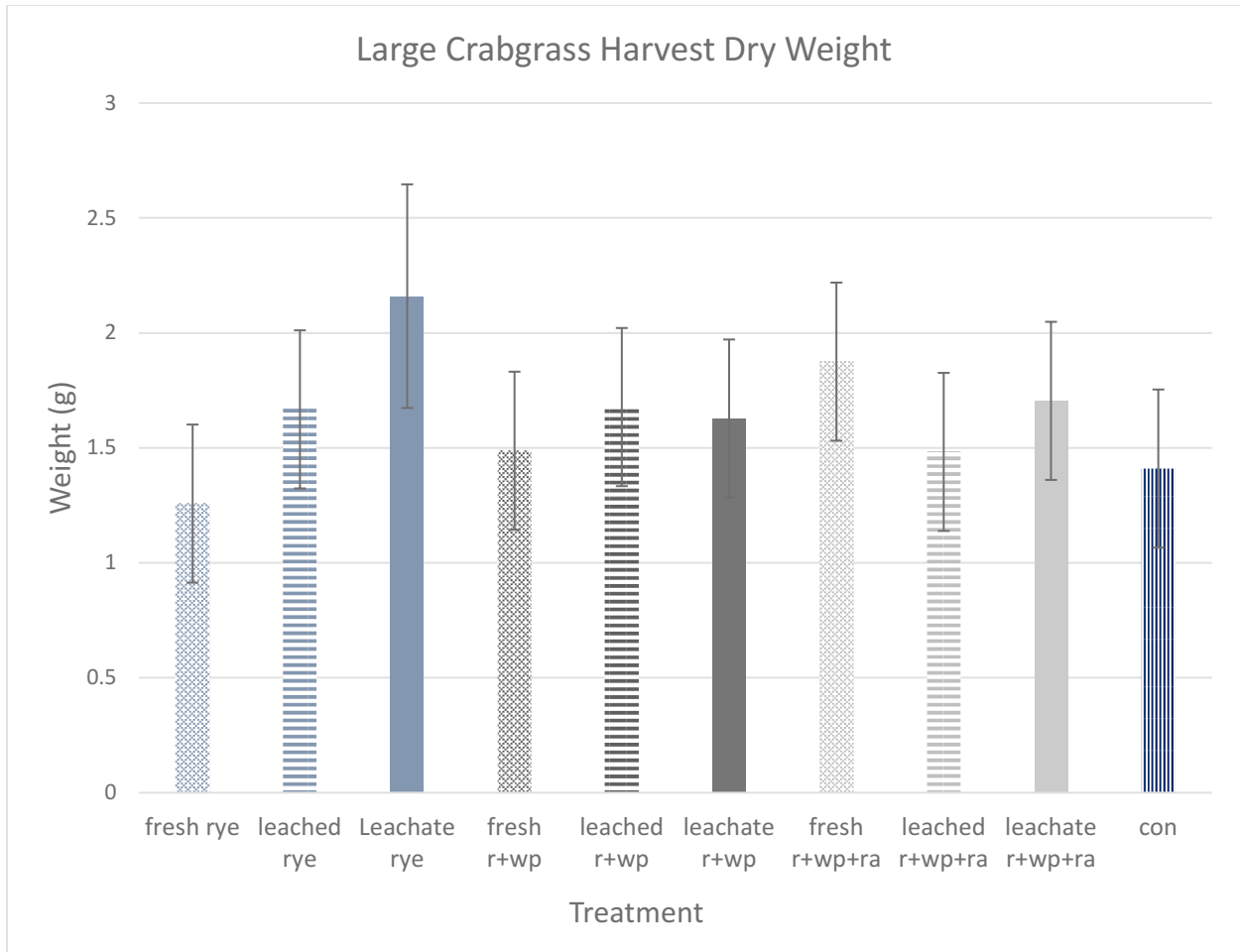
**Figure 19.** Final height at harvest of large crabgrass plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



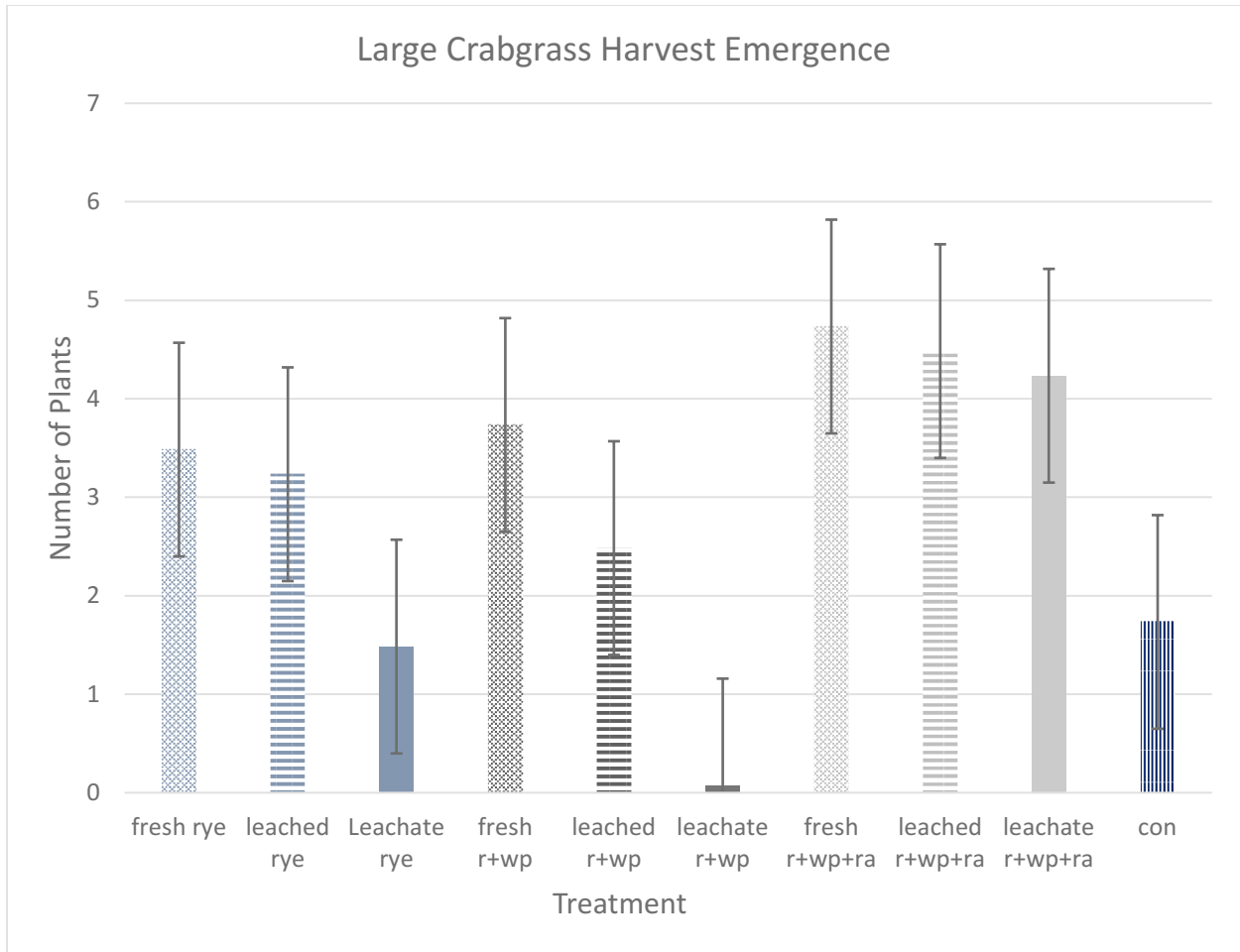
**Figure 20.** Leaf area index at harvest of large crabgrass plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



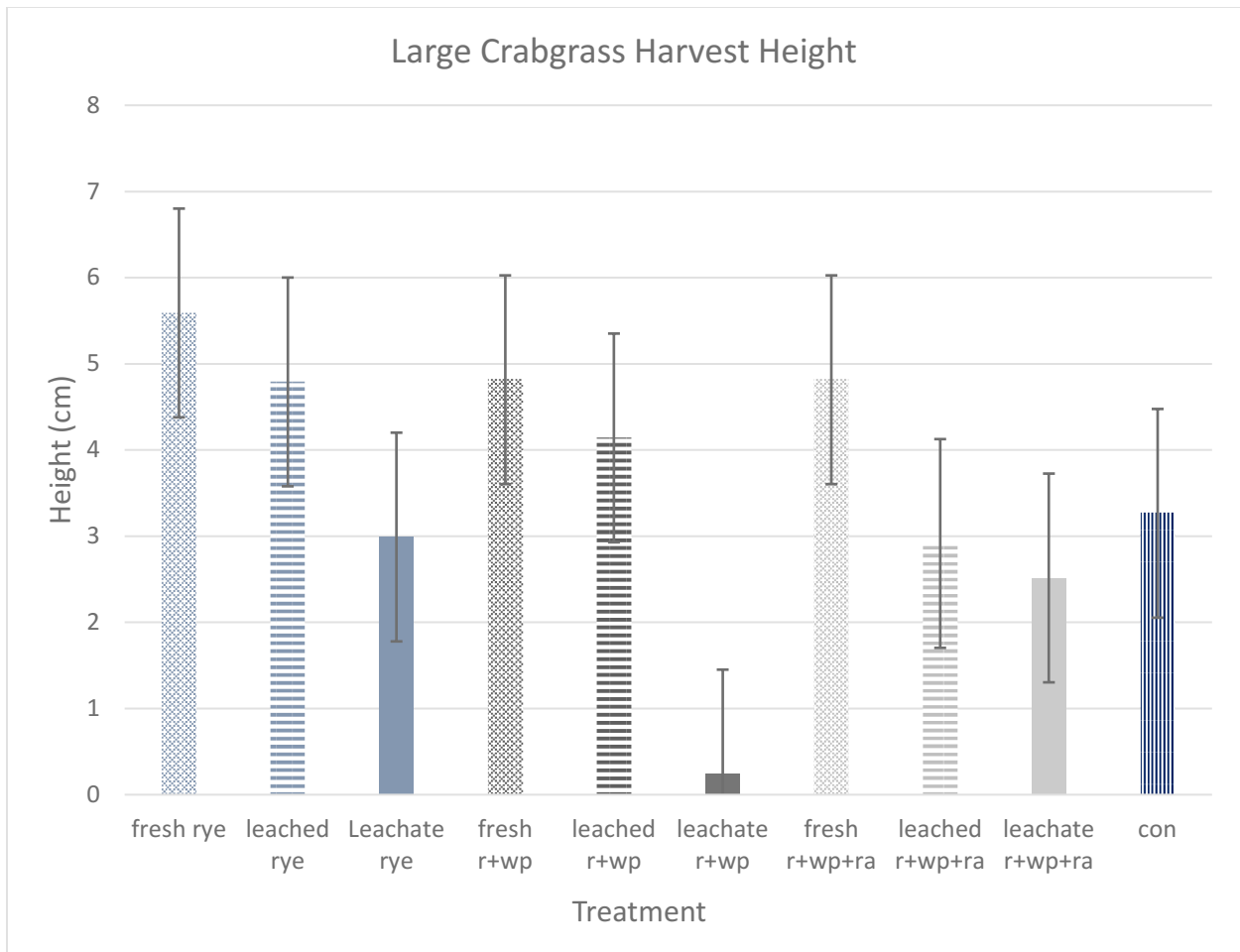
**Figure 21.** SPAD meter readings at harvest of large crabgrass in the greenhouse plants based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



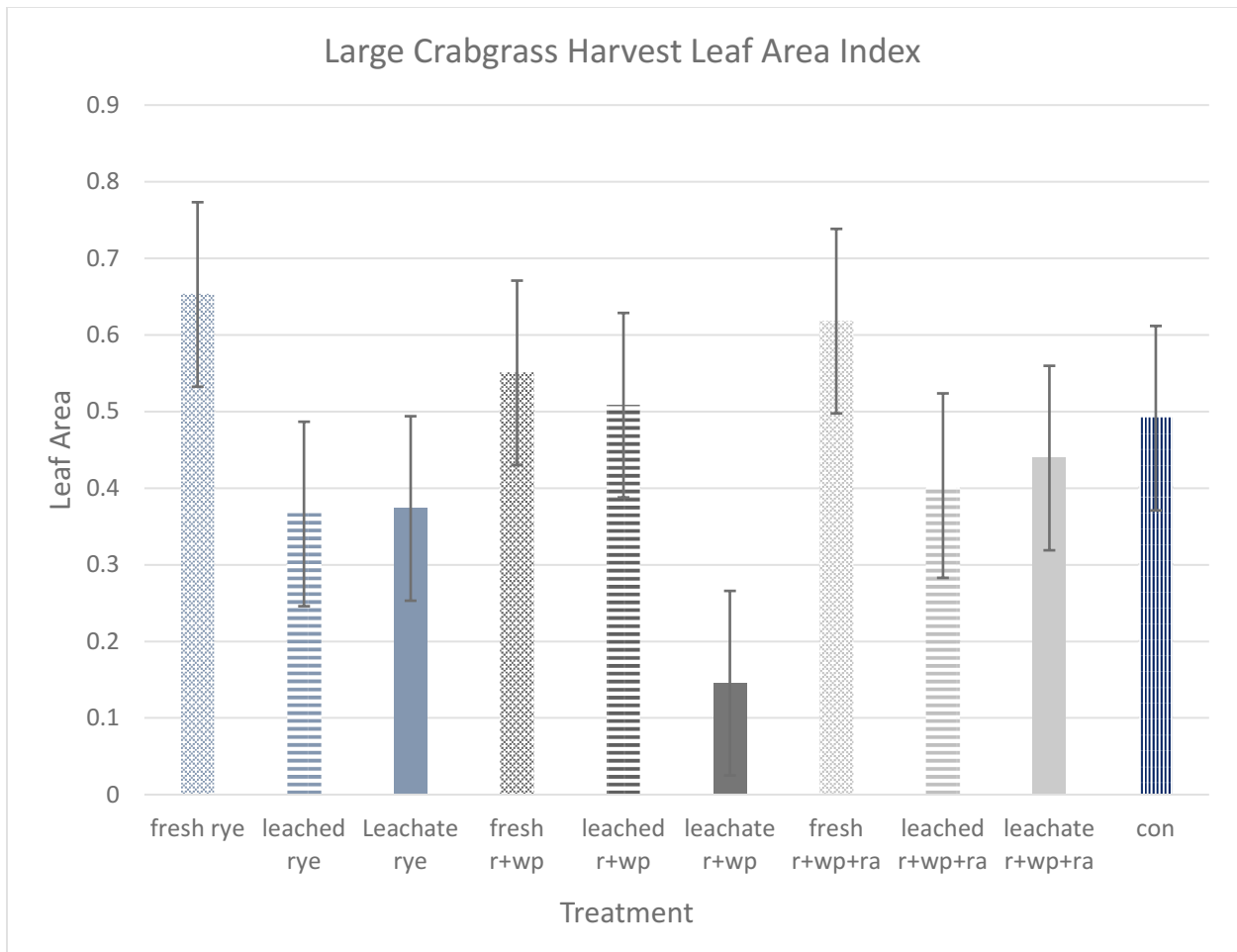
**Figure 22.** Dry weight 48 hours after harvest of large crabgrass plants in the greenhouse based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



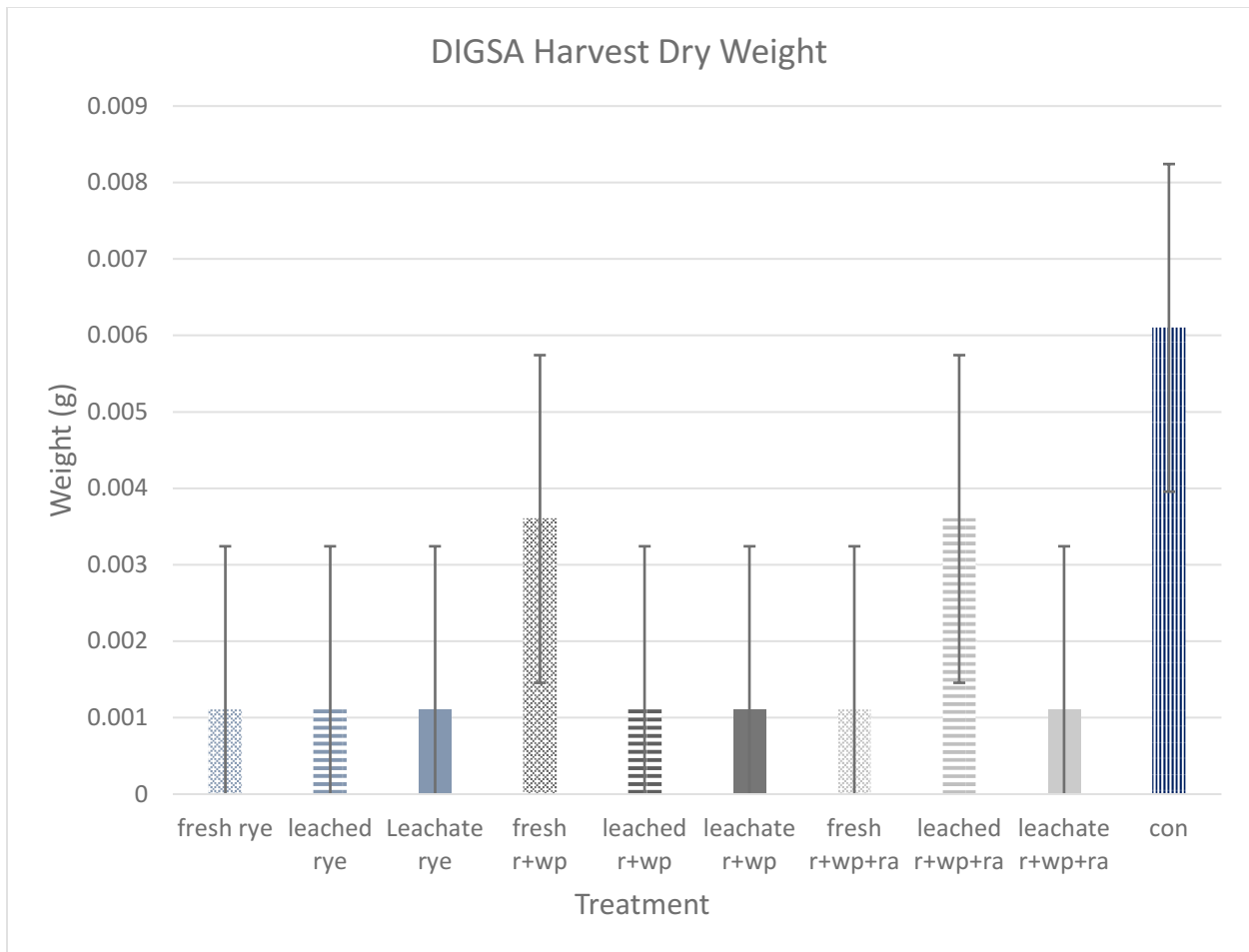
**Figure 23.** Total emergence at harvest of large crabgrass plants in the growth chamber based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



**Figure 24.** Final height at harvest of large crabgrass plants in the growth chamber based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



**Figure 25.** Leaf area index at harvest of large crabgrass plants in the growth chamber based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control



**Figure 26.** Dry weight 48 hours after harvest of large crabgrass plants in the growth chamber based on treatment. Abbreviations: r+wp= rye + winter pea, r+wp+ra= rye+winter pea+radish, con= control