

Targeted Mowing As a Weed Management Method To Increase Allelopathy in Rye (*Secale cereale*) Cover Crop, Part I

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Final Report

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Project Summary

Winter rye (*Secale cereale* L.) is an outstanding cover crop in its suppression of early season weeds. This suppression has been largely attributed to allelopathy; rye's allelochemicals inhibit weed germination and growth. However, its lack of suppression of late season weeds and its inconsistent results between years and regions hinder the use of rye as a cover crop. This study, which is Part I of a 2-year project, explores a management method that may be used to increase weed suppression of rye by manipulating its allelopathic activity. Increased knowledge of the mechanisms of allelopathic activity and weed suppression *in the field*, will allow farmers to manipulate these mechanisms to best suit their particular needs.

This study examined the effect of *targeted mowing* of rye in the fall and spring on its suppression of germination, growth, and reproduction of redroot pigweed (*Amaranthus retroflexus*). This targeted mowing allowed rye to re-grow before being killed by sickle bar mowing. The hypothesis being tested is that mowing induces an increase in allelopathic activity, which enhances rye's weed suppression ability. The design was a split-split plot complete block design with the presence or absence of rye root residue as whole plots; mowing treatments consisting of no mowing, fall mowing, and spring mowing as split plots; and rye shoot residue treatments consisting of no shoots, leached shoots, and unleached shoots as split-split plots. Rye shoot residues were bioassayed for allelopathic activity using lettuce seeds (*Lactuca sativa*). Redroot pigweed seeds were broadcasted in the split-split plots and covered with rye shoot residue. Pigweed emergence was monitored for four weeks, and cylindrical plant volume was used as a surrogate for above-ground biomass. As plants matured, seeds were collected and weighed.

Results showed that spring mowing significantly reduced redroot pigweed emergence compared to no mowing, or fall mowing treatments in rye root residue plots. This suggests that targeted mowing operates to increase weed suppression by increasing the allelopathic activity of rye roots. Indeed, the effect of spring mowing on emergence was most noticeable in no shoot residue split-split plots in which pigweed seeds were exposed to root residue only. Mowing did not have a significant effect on pigweed biomass or seed production.

In conclusion, targeted mowing in early spring may be used to manipulate the allelopathic activity of rye roots, thus decreasing weed emergence. Since redroot pigweed emergence was affected while its biomass and seed production were not, targeted mowing may be more effective when weed plants are most sensitive to allelopathic chemicals, namely at the seedling stage of growth. Currently I am conducting an experiment to determine if spring mowing can reduce weed emergence sufficiently for a tomato crop to be more competitive and thus produce higher yields.

Introduction

Winter rye (*Secale cereale* L.) is known to have many benefits as a cover crop, such as its ability to scavenge soil nutrients during the non-cropping season, enhance soil health, and control weed growth. Weed suppression by rye residue comes from the considerable biomass rye accumulates early in the growing season, which provides a physical barrier (Teasdale, 1998) as well as a chemical barrier (Barnes et al., 1987; Shilling et al., 1985; Creamer et al., 1996) against weed germination and growth. This suppression extends from 4 to 10 weeks (Masiunas et al., 1995; Putnam, 1986).

The effectiveness of rye as a weed suppresser is found in its ability to inhibit early season weed growth. However, the use of rye as a cover crop is hindered by several factors. Among them is the lack of weed suppression later in the cropping season, which is due to the short period in which allelochemicals are present in rye. This short-term weed suppression necessitates other means of weed control for the later part of the season. Another factor limiting the use of rye as a cover crop is inconsistent results between years and localities. This is due to limited knowledge of the mechanism of allelopathy in rye and why this mechanism varies. This project explores *Targeted Mowing* as a management method that may be used to increase weed suppression of rye by manipulating its allelopathic activity, and in the process, provide more mechanistic information on rye's allelopathy.

Allelopathy in rye is an environmental response to resource competition with neighboring plants. Since a plant cannot relocate when faced with adverse environmental conditions, such as a competing plant, an herbivore, or fungal hyphae, it relies on chemical defense compounds that can target these environmental threats at the same time. Rye is no exception; it produces hydroxamic acids that have been shown to be allelopathic against other plants (Barnes and Putnam, 1987; Yenish et al., 1995; Pérez and Ormeño-Núñez, 1993), insecticidal against aphids in wheat (Nicol and Wratten, 1997; and Thackray et al., 1990) and against the European corn borer in maize (Niemeyer, 1988), and antifungal against take-all, a crown and root-rot fungal disease of wheat and barley (Wilkes et al., 1999). Moreover, research shows that simulating an environmental threat, such as mechanically cutting leaves to simulate insect feeding, can induce an increase in the production of hydroxamic acids (Collantes et al., 1997, 1998; Morse et al., 1991). In a greenhouse study, Collantes et al., 1999 showed that clipping rye seedling shoots below the coleoptiles increased root and root exudate concentrations of hydroxamic acids, the allelochemicals implicated in allelopathy. Since defoliation increased root exudation of these allelochemicals in the Collantes study, it may also increase rye's allelopathic activity, and hence its weed suppression ability in the field. This current field study examines the effect of defoliation and regrowth of rye on its allelopathic activity and weed suppression ability. Targeted mowing provides a practical method of defoliating rye without considerable addition of labor to the farmer.

Objective

The objective of this study was to evaluate if mowing rye affects rye's weed suppression of *Amaranthus retroflexus*, redroot pigweed, and whether the time when rye is targeted for mowing alters its weed suppression ability. In order to examine the effect of mowing, some rye was targeted for mowing in November when it was beginning the tillering stage, and some rye was targeted for mowing in late March when it was beginning to elongate.

In order to better understand the mechanism of allelopathy, a secondary objective aimed to identify which part of the rye plant, shoot or root, was most affected by targeted mowing.

Collantes et al., 1999 suggested that roots would be most effected by mowing because cutting seedling leaves showed a much higher increase in hydroxamic acids in roots and root exudates than in the new leaves which grew out after cutting. However, much of the current research has established the allelopathic activity of rye by showing the toxicity of *shoot* residue (Burgos et al., 1999; Creamer et al., 1996; Yenish et al., 1995; Masiunas et al., 1995). Nevertheless, two studies have shown that rye root residue can suppress weeds more than shoot residue (Hoffman et al., 1996; Brecke and Shilling, 1996). This present study identified the relative roles of shoot and root residues in rye's allelopathic activity by creating field plots that differentiated between the allelopathic effect of roots and those of shoots.

Moreover, the project examined the effect of targeted mowing on the life cycle of redroot pigweed. In order to identify the stage in the life cycle of redroot pigweed most affected by targeted mowing, emergence, aboveground biomass accumulation, and seed production were monitored.

In addition, this study attempted to examine whether changes in redroot pigweed suppression in response to mowing correlated with changes in hydroxamic acid content of mowed rye.

Methods and Materials

This experiment was conducted in an organically managed field at the USDA Beltsville Agricultural Research Center in Beltsville, Maryland. The design of the experiment was a split-split plot complete block design with 4 blocks. Each block contained two whole plots, one seeded with rye (+ Roots) and the other not seeded (- Roots). These rye-seeded and unseeded whole plots were randomly assigned within each block and were 9m X 8m each. Each of these 2 whole plots contained 3 split plots for different mowing treatments. Each of these 3 mowing split plots contained 3 split-split plots for different shoot residue treatments (Fig.1).

Prior to cover crop planting, the field was chisel plowed and disked. On October 3, 2001, rye (*Secale cereale* cv. "Wheeler") was drilled into rye-seeded whole plots at a seeding rate of 67 kg/ha with 18cm row spacing. Unseeded whole plots were initially sprayed with vinegar to control winter annual weeds, and split-split plots within the unseeded whole plots were kept bare by hoeing and hand weeding throughout the season until June.

Mowing treatments applied to split plots consisted of fall mowing at the tiller stage (FM), spring mowing at the elongation stage (SM), and no mowing (NM) as a control. These split plots were randomly assigned within each whole plot. Mowing treatments were applied in November to FM plots, and late March to SM plots. These treatments were originally to be applied by mowing the rye with a lawn mower. However, due to draught conditions prevailing during Fall 2001 in Maryland, rye leaves tended to lie too flat on the ground for the effective use of a mower. Thus targeted mowing was applied by hand-cutting rye leaves, simulating mowing. As such, this report refers to *mowing* with the understanding that it is *simulated mowing* by hand-cutting rye. Leaves were cut to a height of 10 cm. This height was chosen because it would allow enough photosynthetic area for re-growth, and would prevent complete soil exposure over winter while allowing damage-induced increases in allelochemical production. This procedure of hand cutting was repeated in March for the spring mowing plots to provide uniformity in treatment application. Standing rye biomass was collected during this spring mowing.

After cutting the spring mowing plots in March, rye was allowed to regrow until it passed the heading stage in May at which time standing rye biomass was again collected and all plots were sickle bar mowed to kill the rye. Mowing rye when it is past the heading stage reduces the potential for regrowth (Teasdale and Abdul-Baki, 1998).

In early June, split-split plots designed for shoot residue treatments were randomly assigned within each mowing split plot. Shoot residue treatments applied to these split-split plots consisted of no shoots (NS), leached shoots (LS), and unleached shoots (S). Split-split plots within each mowing split plot located in unseeded whole plots received shoot residue from the corresponding mowing split plots in rye-seeded whole plots (Fig.1). For example, a fall mowing split plot in a rye-seeded whole plot provided shoot residue for itself and for the fall mowing split plot in the unseeded whole plot of the same block. Accordingly, five 0.25 m² quadrats of shoot residue were harvested from each of the mowing split plots located in rye seeded whole plots after sickle bar mowing. Two of these quadrats provided leached and unleached shoot residue for split-split plots within each mowing split plot located in rye whole plots, while two other quadrats provided leached and unleached shoot residue for the corresponding split-split plots located in unseeded whole plots. The fifth quadrat harvested remained without shoot residue for the No Shoot treatment (NS).

Next, all rye shoot residue removed from each of the 5 quadrats was oven dried at 60 °C. Unleached shoot residue was set aside while shoot residue assigned to the leached treatments underwent the leaching process. Leaching was carried out in a manner similar to Creamer et al., 1996 by leaching the residue in water at room temperature for 24hrs. After the initial 24hrs., the residue was drained, supplied with fresh water, and placed at 5°C for another 24hrs. This procedure was repeated a third time before the leached shoot residue was oven dried again at 60 °C. The leaching procedure was carried out for each leached shoot treatment in separate 5 gal. pails equipped with 80gal/ hr aquatic pumps to circulate the water.

Before returning leached and unleached shoot residue to the field, 200 redroot pigweed seeds were broadcasted in each 0.25m² split-split plot and covered with a thin layer of soil. Redroot pigweed seeds in split-split plots were then covered with leached or unleached residue according to design.

Emerging pigweed seedlings were counted weekly for 4 weeks after planting (WAP). These counts were cumulative in that the first week's count was recounted in the following weeks. As such, a reduction in the number of seedlings over time indicated seedling mortality.

Six weeks after planting redroot pigweed, up to 4 seedlings within each 0.25m² split-split plot were assigned for above ground biomass measurements while the remaining seedlings were removed to reduce seedling competition. Redroot pigweed biomass measurement followed Williams et al., 1998 with weekly recorded measurements of height and canopy diameter of plants. From these data, cylindrical plant volume was calculated and used as a surrogate of above ground plant biomass. This method allowed a non-destructive measure of plant growth over time. Finally, seeds were collected as plants matured by tapping seed heads into coin envelopes and then weighing the seeds.

Figure 2 shows weather conditions over the period of the experiment, collected from a USDA weather station located at the field. Precipitation was unusually low throughout the experimental period and it affected treatment application, namely fall mowing, as well as redroot pigweed germination and growth. Figure 2 also shows the time line for treatment applications and data collection.

Bioassay of Allelopathic Activity:

Samples of leached and unleached shoot residue were assayed for allelopathic activity using "Simpson Black-seeded" lettuce seeds (*Lactuca sativa*). Leachates collected during the leaching procedure were also bioassayed for activity. Twenty mls. of leachate were centrifuged at 16,300xg for 25 min. at 2°C. 3mls of the supernatant were added to a petri dish containing filter paper and allowed to evaporate. A control consisted of 3mls of distilled water added to a

petri dish containing filter paper and allowed to evaporate. For testing residue activity, 7.5 mg of shoot residue was placed under the filter paper in a dish and 3 mls of water added to the top. After all solutions had evaporated, filters were moistened with distilled water and 5 lettuce seeds were added to each dish. Dishes were then covered and placed in a growth chamber at 25 °C with a 16hr. light/8 hr. dark photoperiod. Seeds were scored for germination once the control seeds in distilled water had germinated, which took 2-3 days. Results are thus presented as percentages of the control.

Statistical Analysis:

Redroot pigweed data and lettuce bioassay data were analyzed by Analysis of Variance (ANOVA) as split-split complete block analysis with roots as the main factor, mowing as the subplot factor and shoot residue as the sub-sub plot factor with degrees of freedom adjusted by the Kenward-Rogers adjustment method. Graphic testing for homogeneity of variance indicated that square-root transformation was necessary for pigweed emergence and biomass data, and log transformation was necessary for seed mass data. Bioassay data were arcsine transformed before analysis. The means and standard errors shown in the graphs presented are those of untransformed data. Rye biomass data was analyzed by ANOVA with Mowing as the main factor. Comparisons between treatments were done using the Least Square Differences (LSD) method. All analyses were carried out using the Proc Mixed procedure of SAS version 8.2, SAS Institute Inc., Cary, NC, USA.

Chemical Analysis of Allelochemicals:

In order to assess the effect of mowing on allelochemical synthesis in roots and shoots of rye, mesh bags filled with root-free soil were planted in the field at the same time as rye planting to allow rye roots to grow into them. These bags were harvested over time along with the rye leaves above them for quantification of hydroxamic acids by High Performance Liquid Chromatography (HPLC). However, poor separation of these chemicals was achieved under current HPLC conditions using methods provided in the literature. As such, chemical extraction and quantification by HPLC was not possible as originally proposed. Extraction and quantification of these chemicals will be attempted using Thin Layer Chromatography (TLC) to verify the results of weed suppression obtained in the field.

Results and Discussion

Due to the draught conditions in Maryland during the experiment and the time at which *A. retroflexus* seeds were planted (in June), germination, biomass, and seed production were lower than typically seen with redroot pigweed plants in agricultural fields. On average, only 10% of the weed seeds germinated, and plants headed at a very small size.

The removal of allelochemicals from rye through leaching shoot residue was confirmed by testing the effect of the residue on germination of lettuce seeds (*Lactuca sativa*) (Fig. 3). 'Leached shoot' residue showed significantly higher germination of lettuce seeds than 'unleached shoots' ($t_{92.9} = 10.08$, $P < 0.001$), confirming that leached shoot residue was less allelopathic than unleached shoot residue in the field.

In rye seeded whole plots (+Roots), 'spring mowing' (SM) plots had significantly less redroot pigweed seedlings than either 'no mowing' (NM) plots or 'fall mowing' (FM) plots at 4 WAP (Fig.4, $t_{48} = 2.08$, $P = 0.042$; $t_{48} = 2.03$, $P = 0.048$, respectively). Since the opposite trend was seen in unseeded whole plots (-Roots), the decrease in weed emergence due to spring

mowing in the rye whole plots suggests that weed suppression of spring mowing was a result of the effect of mowing on roots.

In order to confirm this effect of spring mowing on roots, each shoot residue treatment (no shoot, leached shoot, and unleached shoot) was compared across mowing split plots located in rye seeded whole plots only (Fig.5). Results showed that in 'no shoot' plots in which pigweed seeds were subject to root residue only, seedlings in 'spring mowing' plots were significantly less than those in 'no mowing' plots ($t_{48} = 3.38$, $P = 0.001$), and 'fall mowing' plots ($t_{48} = 2.26$, $P = 0.028$). This confirms that the effect of mowing on seedling emergence is primarily through the effect of mowing on roots. Results also showed that in 'unleached shoot' residue plots, there was a decrease in seedling numbers in 'spring mowing' plots compared to 'no mowing' and 'fall mowing' plots, but the decrease was not statistically significant (Fig. 5). This suggests that shoot residue may alleviate the suppressive effect of roots when rye is mowed.

In addition, differences in the effect of mowing treatments on redroot pigweed emergence were not the result of differences in rye biomass in the various mowing treatments (Fig. 6). There was no significant difference between the mowing plots in standing rye biomass at the time of sickle bar mowing in May. This suggests that mowing in fall or spring did not compromise or enhance the ability of rye to accumulate dry matter.

Unlike the effect of mowing on redroot pigweed emergence, spring mowing did not significantly decrease pigweed biomass and seed mass (Fig. 7). Nevertheless there was a general stepwise decrease in pigweed biomass in response to mowing in 'no shoot' and 'unleached shoot' plots in rye whole plots, but it was not a statistically significant decrease (Fig. 7a). In addition, seed production appears to have been less affected by mowing than biomass (Fig. 7b), and differences seen at the onset of seed production disappear as time progresses.

Conclusion

Targeted mowing may be used in manipulating the allelopathic activity of rye roots to enhance weed suppression. Results of this study show that mowing in early spring when rye is breaking dormancy is more effective in redroot pigweed suppression than mowing in fall before winter dormancy has set in. This may be due to the growth stage at which rye was mowed. Rye may be more plastic in its response to mowing at the elongation stage than at the tillering stage, and thus can induce allelochemical production more effectively at the elongation stage. However, this explanation contradicts prevailing research showing that plants generally respond to stress more effectively at a younger growth stage. The more plausible explanation for the effectiveness of spring mowing over fall mowing is the duration that rye was given between allelochemical induction and kill time. Fall mowing allows rye to readjust and decrease its endogenous levels of allelochemicals to provide more energy for maintaining winter dormancy and resuming growth in the spring. In contrast, spring mowing provides less time for this adjustment to occur.

Furthermore, the effect of targeted mowing on weed life cycle varies; spring mowing is more effective against redroot pigweed seedling emergence than against growth and seed production. This suggests that redroot pigweed is most sensitive to allelochemicals at the seedling stage of growth. However, this study was unable to confirm that the effects of mowing on weed suppression were specifically due to an increase in the production of hydroxamic acids.

The lack of effect of mowing on rye shoot residue's allelopathic activity may result from the processing that all shoot residues received after rye kill. As mentioned earlier, unleached shoot residue was removed from the field and oven dried. In order to maintain uniformity in treatment application between leached and unleached shoot residue, unleached shoots were not

returned to the field until leached shoots were ready to be returned, which took approximately 14 days. The oven drying of unleached shoots may have altered the residue in such a way that it prevented full allelopathic activity in the field. In addition, while the shoots were being processed, root residues were degrading naturally and thus releasing allelochemicals in a more consistent manner.

The question that remains to be answered is whether targeted mowing in the spring will sufficiently decrease weed emergence and growth in order to provide an increase in crop yield. An experiment in a tomato production system is currently being conducted to address this issue. Of interest is whether spring mowing can reduce the competitiveness of weeds sufficiently for the crop to be more competitive, and whether rye shoots that are not removed from the field after rye kill will respond to mowing in a similar pattern to rye roots.

Outreach

The results of this study along with those of the current tomato study are expected to be shared with organic farmers through the Chesapeake Alliance for Sustainable Agriculture (CASA), and The Maryland Organic Food and Farming Association (MOFFA), as well as published in a peer-reviewed journal.

Acknowledgments

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Addenda

Included in this final report are photos of the experimental design and methods. Photos were taken at the experimental site, which is an organically managed plot at the USDA,

Beltsville Agricultural Research Center, Maryland, and at the University of Maryland at College Park.

Figures

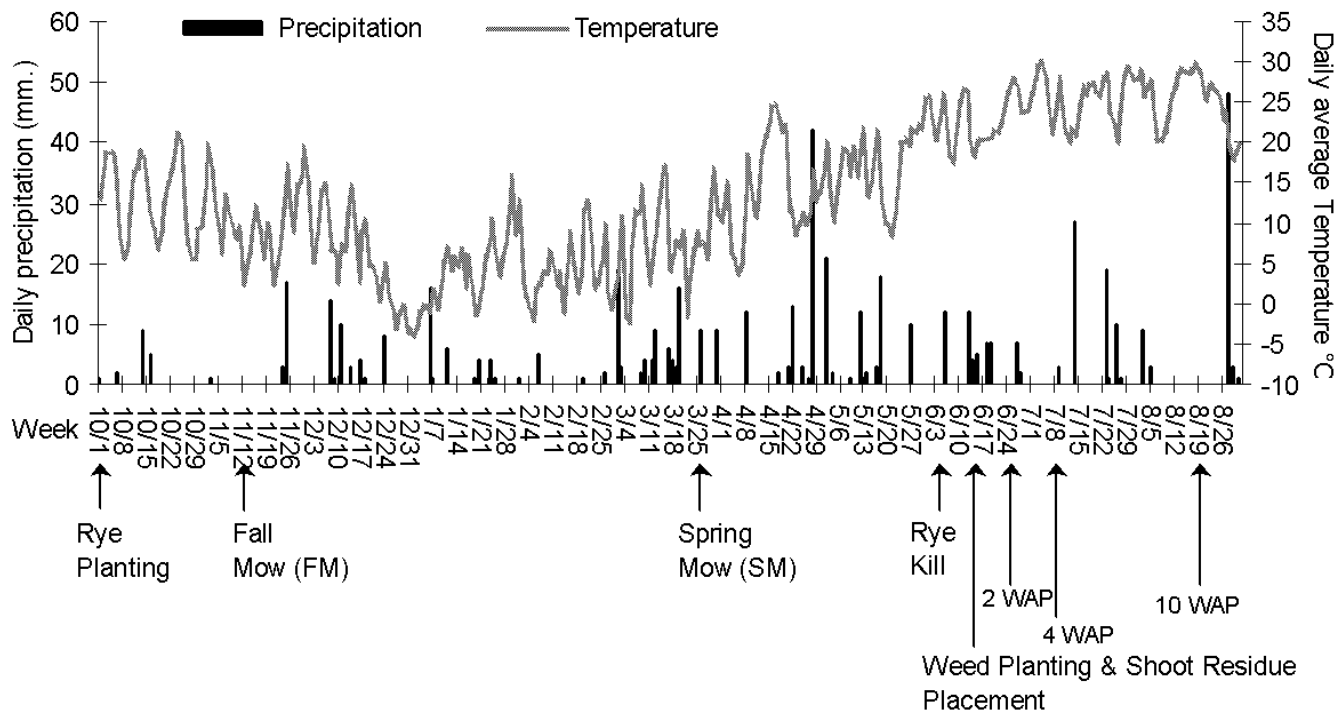
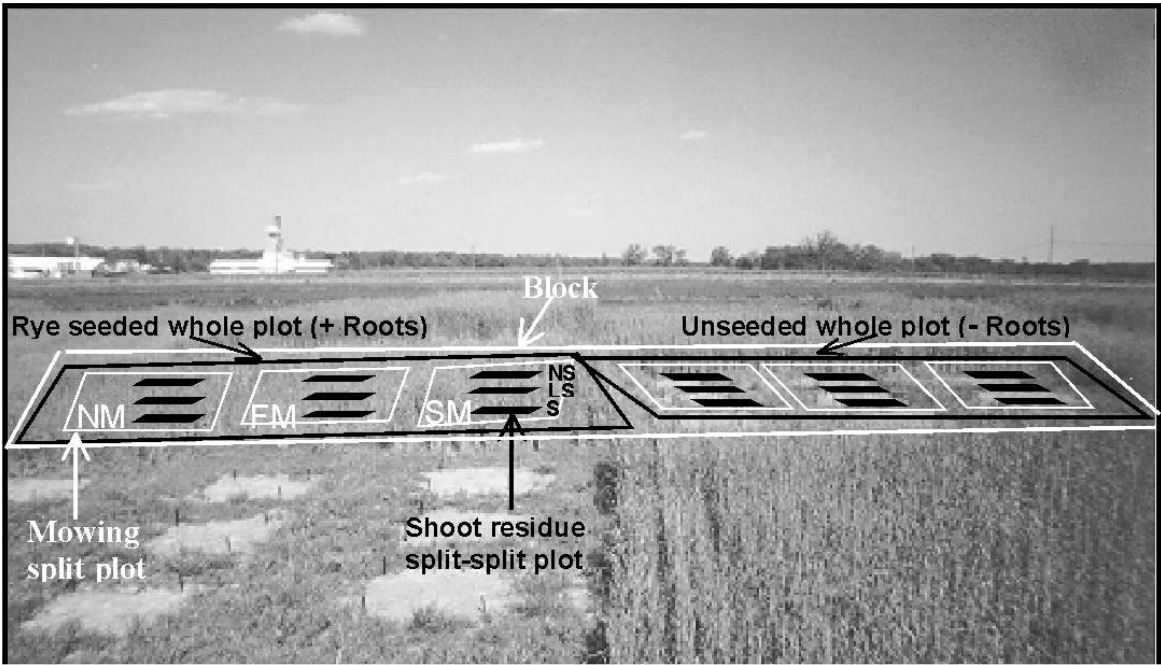


Figure 2: Precipitation and Temperature at the Experimental plots in Beltsville, Maryland, and timeline for treatment application

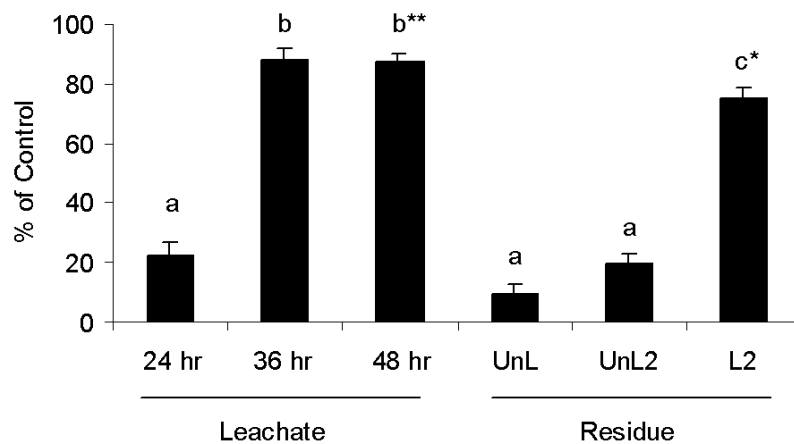


Figure 3: Effect of rye residue and leachate on germination of lettuce seeds. Treatments: Leachate after 24hrs., 36hrs., and 48 hrs. of leaching, unleached residue at the time of leaching (UnL), unleached residue from the field 2 weeks after placement in the field (UnL2), leached residue from the field 2 weeks after placement in the field (L2). Means \pm SE with the same letter are not significantly different at * $P < 0.01$, ** $P < 0.0001$.

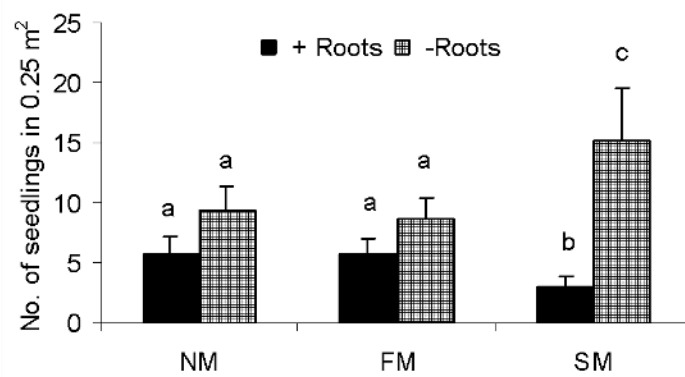


Figure 4: Effect of mowing on emergence of redroot pigweed in rye-seeded (+Roots) and unseeded plots (-Roots) at 4 WAP. Mowing plots: no mowing (NM), fall mowing (FM), and spring mowing (SM). Means \pm SE with the same letter are not significantly different at $P \leq 0.05$.

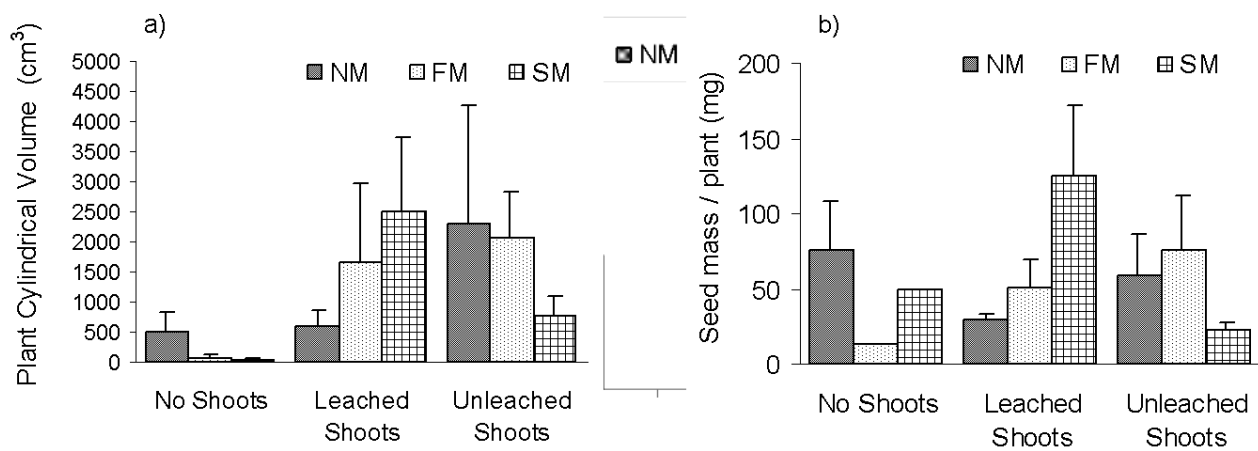


Figure 6: Means \pm SE of live dry weight in no

Figure 7: Effect of mowing on redroot pigweed a) biomass at 10 WAP, and b) seed production at 13 WAP in each shoot residue plot across mowing plots in rye-seeded whole plots.

Mowing plots: no mowing (NM), fall mowing (FM), spring mowing (SM)

