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INTERSEEDING COVER CROPS TO SUPPRESS WEEDS IN CORN-SOYBEAN ROTATIONS IN KENTUCKY

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INTERSEEDING COVER CROPS TO SUPPRESS WEEDS IN
CORN- SOYBEAN ROTATIONS IN KENTUCKY

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THESIS

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A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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ABSTRACT OF THESIS

INTERSEEDING COVER CROPS TO SUPPRESS WEEDS IN
CORN- SOYBEAN ROTATIONS IN KENTUCKY

Cover crops are typically sown between cash crops and can suppress weed emergence and growth. If cover crops are sown after cash crop harvest the system is left susceptible to weed emergence while they establish. Interseeding cover crops into a standing cash crop may limit this bare period by allowing cover crops to become established, go into dormancy, and then revive around cash crop senescence. Studies were conducted in Princeton and Lexington, KY, to determine (i) which corn pre-emergent herbicides and mixtures of herbicide active ingredients commonly used by Kentucky growers would impact interseeded cover crop density and biomass, (ii) which grass entries that are adapted to Kentucky would be best to interseed in corn, and (iii) if interseeded cover crops would suppress weeds similar to a cover crop planted after cash crop harvest. There were few reductions in interseeded cover crop density and biomass from the pre-emergent herbicides tested. Among the entries interseeded in four site-years, the tall fescue pre-cultivars generally performed the best but none were consistently able to survive the summer when interseeded into corn. Compared to a cereal rye cover crop seeded after corn harvest, interseeded cover crops produced less biomass and therefore suppressed fewer weeds.

KEYWORDS: interseeding, cover crops, weed suppression

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July 17, 2018

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1.1. Introduction

In Kentucky, winter cover crops are traditionally planted between the summer annual crops of corn (*Zea mays* L.) and full-season soybeans (*Glycine max* (L.) Merr.). Over the winter, the cover crops are covering the ground which helps prevent erosion, suppresses weeds, and depending on the species, adds nutrients to the soil (Norsworthy et al. 2012). Many of these benefits are maximized with more cover crop biomass (Vencill et al. 2012). Depending on when the main crop is harvested, cover crops planted after harvest may not have favorable growing conditions to accumulate biomass. Interseeding the cover crops may help increase biomass production, particularly during the fall period.

When cover crops are interseeded, they are planted between the rows of a main crop while that main crop is growing. Given that the interseeded cover crops will be alive under the main crop in low light conditions, they need to be shade tolerant. They should also be able to survive in the hot, dry summer conditions found in Kentucky while still winter hardy to provide ground cover into the following spring. Cover crops could be interseeded in several ways such as aerially broadcast or using a drill. Interseeding timing varies too from planting the cover crops with the main crop to planting just before main crop senescences. A balance between planting when light will reach the ground so the seeds can germinate and minimizing competition needs to be found. If planted when conditions are right, interseeded cover crops will already be established by the time the main crop is harvested.

1.2. Interseeding vs planting after harvest

Cover crops are often planted after harvesting a main crop when growing conditions are changing. Oftentimes the growing conditions for cover crops are not favorable for a long enough period to accumulate sufficient biomass before winter. Given that interseeded cover crops will have more time to grow in favorable conditions they are thought to provide more ground coverage and weed suppression.

Some studies have shown how interseeding will allow cover crops to produce more biomass and ground coverage (Hively and Cox 2001; Scott et al. 1987). In one study all interseeded species except for medium red clover (*Trifolium pratense* L.) and barrel medic (*Medicago lupulina* L.) had more ground cover and biomass the following spring than the cereal rye (*Secale cereale* L.) planted after cash crop harvest (Hively and Cox 2001). During one year of that study there was more precipitation than average and cold temperatures during the fall which hindered the post-harvest planted cereal rye from establishing and highlighted the benefits of interseeding (Hively and Cox 2001). Another study found that ground cover was generally higher when cover crops were interseeded when the corn was 0.15- 0.30 m compared to those planted after harvest (Scott et al. 1987). They also report that fall ground cover was higher for all treatments with cover crops, interseeded or post-harvest planted, than the no cover control (Scott et al. 1987). These findings show that merely having cover crops will increase the ground cover and suggest interseeding may lead to higher ground cover compared to post-harvest planted cover crops.

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1.3. Main crop yield impacts

1.3.1. Interseeding timing effects on main crop yield

Cover crops need to be interseeded before the canopy closes so there is light for germination but planting too early can result in unacceptable levels of competition between the interseeded cover crop and the main crop. If cover crops are interseeded too early, during the critical weed free period, they could compete heavily with the main crop thus reducing yields. One study saw the negative effects of planting too early (Uchino et al. 2009). When hairy vetch (*Vicia villosa* Roth.) was planted 14 days before the corn, all the corn had died by 77 days after it was planted resulting in no yield (Uchino et al. 2009). They attribute this to the rapid growth of the hairy vetch causing senescence of the corn (Uchino et al. 2009). However, they found increases in corn yield when hairy vetch was interseeded 21 days after planting corn compared to planting 14 days before the corn, with the corn, or no cover indicating this may be an optimal time to interseed (Uchino et al. 2009). By delaying hairy vetch planting to 21 days after the corn, the corn had time to establish and grow without competition from the cover crop. In that same study, they also examined how interseeding affected soybean yield. They interseeded cereal rye into soybeans and found an increase in soybean yield when the cereal rye was interseeded the same day as planting the soybeans or 21 after planting compared to having no cover or interseeding 14 days before the soybeans were planted (Uchino et al. 2009). Again, by delaying the interseeding, the main crop could grow without added competition. Other studies have shown no difference in yields due to interseeding time (Abdin et al. 1998; Belfry and Van Eerd 2016; Mohammadi 2010), indicating environmental variables and species may play a role in how competitive interseeded cover crops will be.

1.3.2. Positive yield impacts

Interseeded cover crops may positively impact the main crop by providing nutrients or suppressing weeds (Mohammadi 2010; Scott et al. 1987). One study saw an 8% increase in average corn yield when hairy vetch was interseeded compared to no cover (Mohammadi 2010). There were no differences in corn yield based on when interseeding occurred (at corn planting or ten days after corn emergence; Mohammadi 2010). While there was no difference in corn yield if hairy vetch was interseeded at a lower (25 kg ha^{-1}) or higher (50 kg ha^{-1}) seeding rate, the leaf area index and chlorophyll values of the corn did increase as hairy vetch seeding rate increased (Mohammadi 2010). The increased seeding rate may have provided more nutrients (as hairy vetch is a legume that can fix atmospheric nitrogen, thus increasing nitrogen supply to the soil) or suppressed more weeds, thereby helping the corn to be less stressed than the lower seeding rate. However just having interseeded cover crops provided benefits to the corn regardless of seeding rate. Another study also saw increases in corn yield when other legumes were interseeded (Scott et al. 1987). Corn yield was increased when medium red clover, mammoth red clover (*Trifolium pratense* L. var. *perenne* Host), white clover (*Trifolium repens* L.), and medium red clover + annual ryegrass (*Lolium multiflorum* L.) were interseeded compared to plots with no cover and no added nitrogen (Scott et al. 1987). For the no cover control plots to have higher yield than every interseeded plot, 39 kg ha^{-1} or more nitrogen had to be added (Scott et al. 1987). This shows that the legume species added nitrogen to the soil which may have contributed to an increased corn yield. The corn had access to the added nitrogen because the legumes were interseeded while

the corn was still growing. If legumes are interseeded, it may allow for less added

nitrogen to be applied and still maintain consistent yields.

1.3.3. Neutral yield impacts

Many studies have found no difference in the main crop yield when cover crops

are interseeded (Baributsa et al. 2008; Belfry and Van Eerd 2016; Scott et al. 1987;

Uchino et al. 2012). In one experiment there was no difference in sweet or seed corn

yield when alfalfa, oilseed radish (*Raphanus sativus* L.), or a mixture were interseeded at

either V4-V6 or V10-V12 compared to no cover (Belfry and Van Eerd 2016). They also

found no difference in seed corn yield when 18 cover crop treatments were hand sown at

V4-V6 (Belfry and Van Eerd 2016). Both experiments indicate these species and

mixtures did not compete enough with the corn to reduce yield. A study by Uchino et al.

(2012) found no reductions in corn yield when cereal rye and hairy vetch were

interseeded 3-5 weeks after corn seeding compared to plots with no cover. Similar results

were found when red clover and chickling vetch (*Lathyrus sativus* L.) were interseeded

into corn and no corn yield reductions were found (Baributsa et al. 2008). Scott et al.

(1987) saw no reductions in corn silage yield when cover crops were seeded at two

different planting dates in two of their experiments. Cover crops provide many benefits

(reduce erosion, suppress weeds, add nutrients) so even if the current main crop yield is

not increased the net gain from interseeding cover crops could still be positive.

Yields have also been studied when cover crops are interseeded into soybeans.

Compared to the no cover control, there was no difference in soybean yield when oat

(*Avena sativa* L.), cereal rye, and a cereal rye-oat mixture were overseeded (Johnson et

al. 1998). In this study overseeding is similar to interseeding but was done later in the

main crop growing season and the seeds were broadcast onto the soil instead of drilled. There is still potential for the cover crops to compete with the main crop and cause yield loss. However they only saw a 5% yield loss in one year (Johnson et al. 1998). That year, they had to replant all the cover crop treatments due to weather conditions (Johnson et al. 1998). Having to drive through the field a second time may have contributed to the yield loss they saw that year (Johnson et al. 1998).

1.3.4. Negative yield impacts

If interseeded cover crops are competing with the main crop for resources, there may be a reduction in main crop yields. The goal of interseeding is to get the benefits of cover crops without reducing the yields of the main crop. Some studies have found reductions in the yields of the main crop (Abdin et al. 1998; Fakhari et al. 2015; Scott et al. 1987). In one study plots with interseeded cover crops including cereal rye, hairy vetch, and berseem clover (*Trifolium alexandrinum* L.) had lower forage corn yields compared to the weed free no cover treatment (Fakhari et al. 2015). Between the treatments with interseeded cover crops, the hairy vetch treatment had the highest yield followed by berseem clover and cereal rye had the lowest yield (Fakhari et al. 2015). Reductions were also found in the average five year silage corn yield when annual ryegrass was interseeded compared to plots without nitrogen or cover (Scott et al. 1987).

Competition between the cover crop and the main crop is not the only concern. If the weeds are not controlled prior to planting, then weeds can compete with both the cover and main crop. Abdin et al. (1998) saw this at one location where the weed pressure was high causing poor establishment of the interseeded cover crops. The weeds competed with both the cover crops as well as the corn and the corn yields of all

interseeded treatments were 20% lower than the no cover weed free treatments (Abdin et al. 1998). They conclude a combination of interseeded cover crops and chemical control of weeds prior to interseeding should be utilized (Abdin et al. 1998). Chemical control could help reduce the competition from the weeds giving the corn and interseeded cover crops time to establish and grow. Another study also saw reductions in yield due to high weed pressure (Mohammadi 2010). In plots where weeds were not controlled, there was 30% lower corn yield as well as 33% lower interseeded hairy vetch dry weight compared to plots where weeds were controlled by hand weeding (Mohammadi 2010). This supports what Abdin et al. (1998) concluded: an integration of both chemical or physical measures and interseeding will provide the best weed control.

1.3.5. Impacts of interseeded cover crop on the following rotational crop

While it is of interest how the current main crop yield is affected, it is also important that the following rotational crop's yield is not diminished. Many studies have found either increases or no change in main crop yields following interseeded cover crops (Hively and Cox 2001; Scott et al. 1987). In one year, when establishment of the interseeded cover crops was good, corn yield was 21% higher following an interseeded white clover cover crop and 15% higher following an interseeded medium red clover cover crop compared to plots with no cover (Hively and Cox 2001). Both interseeded treatments had higher corn yield than following treatments with a post-harvest seeded cereal rye, 22% and 16%, respectively (Hively and Cox 2001). That same year there was no increase or decrease in corn yield following interseeded annual ryegrass and creeping red fescue (*Festuca rubra* L.) cover crops (Hively and Cox 2001). The following year when establishment of the cover crops was not as good, they also found no effect on corn

yields in plots following legume species (alfalfa (*Medicago sativa* L.), white clover, medium red clover, barrel medic) compared to plots with no cover (Hively and Cox 2001). No reductions in the following corn yield were observed after 11 different cover crop species were seeded at three planting dates (when the corn was 0.15-0.30 m tall, at midsilk, or after corn harvest; Scott et al. 1987). These results show the benefits of interseeding cover crops can carry over into the next main crop or at minimum may not hurt the following crop.

Other studies have found reductions in the yield of the following rotational crop (Hively and Cox 200l; Johnson et al. 1998; Scott et al. 1987). Following a fall with increased precipitation, corn yield was lower in plots that had annual ryegrass and creeping red fescue interseeded in the previous soybean crop compared to plots with no interseeded cover crop (Hively and Cox 2001). Of all the interseeded cover crops, annual ryegrass and creeping red fescue had the most biomass in the spring prior to planting the corn which may have contributed to the lower corn yield (Hively and Cox 2001). Johnson et al. (1998) found that cereal rye alone and an oat- cereal rye mixture overseeded in soybeans resulted in lower corn yields the following season compared to oat alone and a no cover control. They attribute the lower yields to reduced corn height in those plots which may be because of lower soil temperatures, allelopathy, or lower nutrient availability (Johnson et al. 1998). Another study showed reduced corn yields in plots that previously had a mixture of medium red clover + perennial ryegrass (*Lolium perenne* L.), perennial ryegrass alone, and cereal rye (Scott et al. 1987). The mixture of medium red clover + perennial ryegrass as well as the perennial ryegrass alone caused yield reductions when they were seeded when the corn was 0.15-0.30 m tall (Scott et al. 1987).

The cereal rye was only seeded at two later times (midsilk and post-harvest) and caused reductions at both (Scott et al. 1987).

1.4. Weed management influences

1.4.1. Positive effects on weed management

One goal of interseeding cover crops is to help suppress weeds that could emerge during the main crop growing season and directly after. Several studies have found reductions in weed biomass (Fakhari et al. 2015; Mohammadi 2010; Uchino et al. 2009; Uchino et al. 2012) as well as weed density (Uchino et al. 2015) when cover crops were interseeded compared to having no cover.

In one study, the total weight of weeds plus main crop was calculated. In the plots where hairy vetch was interseeded 21 days after corn planting, not only was the total weight higher, but weeds only comprised 2% of the total weight. In comparison, in plots without interseeded cover, weeds made up 47% of the total weight (Uchino et al. 2009). They also observed that the interseeded cover crops competed with the weeds at early growth stage enough to prevent those weeds from overtaking the main crop (Uchino et al. 2009). In the plots without cover crops they observed weeds that were taller than the main crop and therefore competing for light (Uchino et al. 2009). The decrease in competition from weeds in plots with interseeded cover crops lead to significantly higher yield in those plots compared to plots without cover crops (Uchino et al. 2009). This shows that interseeded cover crops can compete with the weeds therefore reducing the total weed biomass and benefiting the main crop without the use of herbicides. Another study found weed dry weight decreased roughly 50% as interseeded hairy vetch planting rate increased from 0 to 50 kg ha^{-1} (Mohammadi 2010). There was a negative correlation

between hairy vetch dry weight and weed dry weight showing that interseeding hairy vetch may help reduce weed growth (Mohammadi 2010). In this study, irrigation was used so moisture was not a limiting factor which may have allowed the cover crops to better compete with weeds.

Another study found the no cover treatment had a higher weed density and weed dry weight than any of the treatments with an interseeded cover crop (Fakhari et al. 2015). The no cover control had 30 weeds m^{-2} while the average weed density in the cover crop treatments was only 10 weeds m^{-2}. Between the treatments with an interseeded cover crop, the weed dry weight was similar but the weed density was higher in the berseem clover treatment than in the hairy vetch or cereal rye (Fakhari et al. 2015). Berseem clover had the lowest cover crop biomass of the three so that may explain the increased weed density (Fakhari et al. 2015). This also supports Mohammadi's (2010) finding that more cover crop biomass will correlate to less weed biomass.

Oftentimes, interseeding cover crops will be part of a rotation instead of just in one crop. Uchino et al. (2012) concluded that interseeding could suppress weeds in a potato (*Solanum tuberosum* L.)- soybean- corn rotation and since there were no differences between weed growth over the four years, they suggest interseeding can suppress weeds in different environmental conditions. The weed number and dry weight in the corn plots was 26% and 38%, respectively, lower in the treatments with cover crops than without (Uchino et al. 2012). In soybean, the weed density was 58% lower in the interseeded plots compared to the no cover plots however the dry weight of the weeds was the same (Uchino et al. 2012). Weed density was also reduced when cereal rye was

interseeded in soybeans and hairy vetch was interseeded into corn (Uchino et al. 2015). In these studies, weed biomass and density was reduced when cover crops were interseeded.

Uchino et al. (2012) also looked at the VCR (vegetation cover ratio) which is the "percentage of area covered by vegetation to unit soil surface area" when the main crop had reached maximum height. In the potato- soybean- corn study mentioned above, they found a negative correlation between the VCR of the main crops plus the cover crops and the weed dry weight (Uchino et al. 2012). They also found the VCR of main crops plus the cover crops was increased in the interseeded treatments (Uchino et al. 2012). This suggests that interseeding cover crop would help to reduce the weed dry weight because the VCR would be higher.

These studies show that interseeding cover crop can help reduce weed density and biomass. If interseeded cover crop have more biomass that may help suppress weeds as well if the vegetation cover ratio is higher with interseeded cover crops then less light will infiltrate and weeds will have more difficulties emerging.

1.4.2. Cover crops as weeds

Cover crops, if not controlled, can become weeds, set seed, and reduce yields. Over half (50.5%) of non-cover crop users who participated in SARE's Annual Cover Crop Survey said their main concern was that a cover crop would become a weed (CTIC 2017). Annual ryegrass is of specific concern because it is naturally tolerant to many herbicides. One study found acceptable control using glyphosate but other herbicides they tested, such as paraquat and glufosinate, provided little control (Cornelius and Bradley 2017b). As different herbicides and mixes are being used to control herbicide resistant weeds, control of annual ryegrass may become more of an issue.

1.4.3. Complications in weed management with interseeding

While some studies have suggested integrating chemical control with interseeding will help the success, it is challenging to find chemicals that can be used with cover crops. In many production systems, it is common to apply an herbicide with residual activity before or at planting of the main crop. This means herbicides will be in the soil when the cover crops are interseeded. Another complication is that once the cover crops have been interseeded, herbicides cannot be applied that would kill the cover crop. This means that even if a field is weedier than desired, herbicides cannot be used to control the weeds. Some studies have looked at how certain pre-emergent herbicides or herbicide mixes will influence the establishment of interseeded cover crops. Due to different selectivity of herbicides, grasses and legumes might respond differently to the same herbicide.

1.4.3.1. Grass species

Grass species have tolerance to some herbicides but other herbicides have been shown to cause reductions in biomass if they are still present when interseeding occurs (Cornelius and Bradley 2017a; Tharp and Kells 2000; Wallace et al. 2017). In one study pyroxasulfone and s-metolachlor reduced annual ryegrass (interseeded at V5) late fall biomass by more than 80% compared to the untreated check in the first year (Wallace et al. 2017). The second year they found pendimethalin at the 1x rate and pendimethalin + atrazine at 0.5x rate resulted in 65% and 64% lower late fall annual ryegrass biomass compared to the untreated check (Wallace et al. 2017). The experiment was repeated for a third time and they found reductions in annual ryegrass biomass in the pendimethalin (32%), s-metolachlor (32%), and s-metolachlor plus mesotrione plus atrazine (36%)

treatments compared to the untreated check (Wallace et al. 2017). Overall, they conclude that s-metolachlor and pyroxasulfone cause too much injury to be used with interseeded annual ryegrass due to their persistence in the soil (Wallace et al. 2017). They attribute the variability between years to different location effects which are discussed below.

Another study, looking at different herbicides, found injury and reduction in annual ryegrass stand as well (Tharp and Kells 2000). This study did not utilize interseeding but had corn planted adjacent to the experiment that was used to determine when to seed the cover crops. The herbicide treatments (EPTC, metolachlor, and pendimethalin) were applied and annual ryegrass was seeded at different times based on the corn planted adjacent to the field (not emerged, 1-2 collars, 3-4 collars, and 5-6 collars; Tharp and Kells 2000). The annual ryegrass was most affected by metolachlor, which caused a minimum of 91% injury, based on a visual rating, at all seeding dates and at least an 88% reduction in density at all planting dates (Tharp and Kells 2000). Pendimethalin caused injury and reduced density at all planting dates except in 1996 when the last planting date did not result in injury (Tharp and Kells 2000). That year had more rainfall than the previous year which may explain the differences in the years (Tharp and Kells 2000). Of the three herbicides, plots with EPTC resulted in densities similar to the untreated check at the earliest planting date; in 1995 densities of annual ryegrass were the same as the check at the fourth planting and in 1996 at the third planting (Tharp and Kells 2000). These two studies agree that metolachlor and pendimethalin can cause injury even if cover crops are planted at V5 when herbicides were applied at corn planting.

A third study, which also did not use interseeding but still shows the susceptibility of cover crops to injury, found similar results (Cornelius and Bradley 2017a). Cover crops were seeded in the fall after corn or soybean harvest and herbicides had been applied to the corn and soybeans around V2. They found a 38% stand reduction and a 41% biomass reduction when wheat (*Triticum aestivum* L.) was planted in the fall following soybeans which had s-metolachlor or a mixture containing s-metolachlor applied (Cornelius and Bradley 2017a). When Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot) was planted following soybeans, there was a 27% biomass reduction following s-metolachlor in one year and a 20% reduction in stand following a mixture of fomesafen + s-metolachlor in another year (Cornelius and Bradley 2017a). They also studied pyroxasulfone and found reductions in oat (Cornelius and Bradley 2017a). Oat following pyroxasulfone in soybean had a 45% reduction in stand and a 68% reduction in biomass in one year (Cornelius and Bradley 2017a). Another year there was a 33% reduction in stand (Cornelius and Bradley 2017a). Following corn that had pyroxasulfone applied, oat had an 81% reduction in stand and a 67% reduction in biomass (Cornelius and Bradley 2017a). Italian ryegrass also showed decreases in stand and biomass following pyroxasulfone (Cornelius and Bradley 2017a). In one year, following soybeans that had pyroxasulfone applied there was a 68% reduction in stand and an 82% reduction in biomass compared to the untreated control (Cornelius and Bradley 2017a). Another year, there was a 57% and 67% reduction in stand and biomass, respectively (Cornelius and Bradley 2017a). Following pyroxasulfone in corn, both Italian ryegrass stand and biomass were reduced 95% (Cornelius and Bradley 2017a). Pyroxasulfone reduced Italian ryegrass stand and density the most of all herbicide

treatments (Cornelius and Bradley 2017a). These studies highlight the challenge of combining herbicides with interseeding grasses.

1.4.3.2. Legume species

Legumes are commonly used a cover crops because of their ability to fix nitrogen which may help the following crop so it is important to see how legumes respond to commonly applied herbicides. Some studies have shown reductions in stand or biomass (Cornelius and Bradley 2017a; Tharp and Kells 2000; Wallace et al. 2017) but generally the injury to legumes is less than that to grass species. One study showed no significant reductions in red clover biomass due to commonly used corn herbicide treatments in two out of three years (Wallace et al. 2017). The following year however, mesotrione and s-metolachlor + mesotrione + atrazine reduced red clover biomass 98% and 97%, respectively (Wallace et al. 2017). Looking at single active ingredient across sites, only mesotrione caused reductions in red clover biomass (Wallace et al. 2017). Another study found a reduction in winter pea (*Pisum sativum* L.) and hairy vetch biomass following corn that had a mesotrione application (Cornelius and Bradley 2017a). The mixture of glyphosate + mesotrione + s-metolachlor + atrazine applied to corn reduced the following hairy vetch stand 26% and biomass 33% (Cornelius and Bradley 2017a). Following soybeans, the s-metolachlor treatment reduced winter pea and hairy vetch biomass 43% and 31%, respectively (Cornelius and Bradley 2017a).

Another study found crimson clover (*Trifolium incarnatum* L.) could be planted earliest following pendimethalin compared to EPTC or metolachlor (Tharp and Kells 2000). The treatments with pendimethalin showed no injury or stand reduction when the clover was planted at the second planting date (Tharp and Kells 2000). Although for all

herbicide treatments there was no reduction in stand or biomass if planting was delayed

to 28 days after herbicide application (Tharp and Kells 2000). Cornelius and Bradley

(2017a) had crimson clover in their study as well. They planted the cover crops between

61-97 days after the herbicide application and saw reductions in crimson clover biomass

after s-metolachlor in one year (Cornelius and Bradley 2017a). Several other herbicides

(metribuzin, sulfentrazone + cloransulam, fomesafen, imazethapyr, chlorimuron +

thifensulfuron, acetochlor, clopyralid, nicosulfuron, clopyralid + acetochlor +

flumetsulam + atrazine, atrazine, tembotrione, and isoxaflutole) reduced either the stand

or biomass of crimson clover (Cornelius and Bradley 2017a).

While these studies show that herbicides can reduce the establishment of cover

crops that are interseeded, not all locations will be affected the same. In the study by

Wallace et al. (2017) there was wide variability between fall cover crop biomass across

site-years in the nontreated check plots. They suggest there was an environmental factor

or planting factor that had an effect that was not assessed in this study and may make

herbicide treatment differences difficult to detect (Wallace et al. 2017). There was also

one location that had differences in both red clover and annual ryegrass biomass the other

locations did not have (Wallace et al. 2017). They suggested these differences could

potentially be due to different soil texture and lower organic matter and cation exchange

capacity (CEC) than the other locations (Wallace et al. 2017). This allowed for more

herbicide to be available for cover crop uptake and resulted in more injury. The location

with lower organic matter and CEC showed reductions that other locations did not

(Wallace et al. 2017). They conclude that soil texture needs to be considered when

deciding which pre-emergence herbicides to use with an interseeding system but there are options that do not cause injury in most soils (Wallace et al. 2017).

These studies show that some herbicides will reduce establishment or biomass production mostly in grass species. However, there are options that will not cause injury and could be successfully used with interseeding. Soil factors and weather need to be considered at every location to make the best decision about which pre-emergence herbicides can safely be used.

1.5. Factors influencing interseeded cover crop success

1.5.1. Planting time

The earlier cover crops are interseeded, the more time they have to produce biomass but planting too early can cause unacceptable levels of competition between the cover crop and the main crop. The earlier cover crops are interseeded after the critical weed free period the more biomass they may produce (Abdin et al. 1997; Belfry and Van Eerd 2016; Scott et al. 1987). One study found cover crops interseeded when seed corn was V4-V6 accumulated 33% more biomass than the treatments sown when the corn was V10-V12 (Belfry and Van Eerd 2016). Another study saw an increase in ground cover in earlier seeded cover crops (Abdin et al. 1997). Twelve forage species were interseeded 10 and 20 days after corn emergence at two different locations (Abdin et al. 1997). On average earlier seeded treatments provided 41% more ground cover than the later seeded ones (Abdin et al. 1997). There was a general increase in percent ground cover when cover crop species were seeded into corn when it was 0.15-0.30 m tall compared to the same species that were seeded when the corn was at midsilk (Scott et al. 1987). This held true for two different experiments one with 11 species and the other with 13 species

(Scott et al. 1987). In this case, the increased ground cover did not reduce corn yields which highlights how interseeding can be beneficial to the cover crop without sacrificing main crop yields.

One study found that while seeding oat earlier than soybean leaf drop was beneficial, seeding too early left the oat growing in high temperature and competitive conditions which reduced oat shoot growth (Johnson et al. 1998). Oat seeded 26 days before soybean leaf drop had more oat shoot biomass than earlier and later seeding dates (Johnson et al. 1998). They suggest this seeding date was successful because it was sown before the soybeans dropped their leaves (Johnson et al. 1998). When the leaf drop did occur that provided the soil with cover to help hold in moisture and aid in oat growth (Johnson et al. 1998). This date was also not too early that the oat was stressed by high temperatures and resource competition for long (Johnson et al. 1998).

Other studies have found no increase in biomass production in earlier planted cover crops (Mohammadi 2010; Abdin et al. 1997). One study found hairy vetch biomass at corn maturity was the same whether hairy vetch was planted the same day as the corn or 10 days after the corn emerged (Mohammadi 2010). The extra time did not correspond to more biomass production. Another study found while one location did show earlier seeding could lead to more ground cover the other location had no significant difference between the two planting dates on ground cover or biomass production (Abdin et al. 1997).

These studies show that while planting earlier may result in increased biomass or ground cover it is not always consistent. There may be differences between crops and environments that need to be considered.

1.5.2. Species selection

Knowing when to plant is one factor but another factor is knowing what species to plant. Some cover crop species are better at producing biomass when interseeded than others. The overall rotation will also impact if a grass versus a legume or brassica should be planted.

Legume species, such as clover, are commonly planted because they can add nitrogen to the soil which can benefit the following crop. Legumes have been shown to produce the most biomass in many studies (Abdin et al. 1997; Baributsa et al. 2008; Belfry and Van Eerd 2016; Scott et al. 1987) which can help suppress weeds. In one study, crimson clover generally produced the most biomass followed by a mixture of red clover and ryegrass (Abdin et al. 1997). Another study found annual ryegrass and a mixture of annual ryegrass + medium red clover planted when the corn was 0.15-0.30 m tall provided the most ground cover in both the fall and spring (Scott et al. 1987). A different study by Scott et al. (1987) showed medium red clover, cereal rye, and perennial ryegrass provided the most ground cover the following spring. They also found yellow sweet clover (*Melilotus officinalis* (L.) Pall), as well as perennial rye, alfalfa, and cereal rye, was one of the most successful of the species planted when the corn was 0.15-0.30 m tall (Scott et al. 1987). Other legume species have also been shown to do well. One study found chickling vetch produced more biomass than red clover (Baributsa et al. 2008). Another study found oilseed radish and a mixture of oilseed radish + forage pea (*Pisum sativum* L.) produced the most biomass of the species and mixtures they interseeded in seed corn at the V6-V8 stage (Belfry and Van Eerd 2016).

While clover species are good biomass producers, they may not be ideal in all situations, such as before another legume species or in areas with excess nitrogen. Therefore, grass species have been compared to see which can cover the most ground and produce the most biomass in an interseeded setting. One study found no major differences between cereal rye, oat, and a mixture of cereal rye and oat (Johnson et al. 1998). In that study, oat produced more fall biomass than cereal rye or the mixture in one year but the average of the three years shows no differences between treatments (Johnson et al. 1998). There were no differences in the spring biomass or the residue left from the treatments (Johnson et al. 1998). While cereal rye did not do better than oat in that study, in another study cereal rye did produce more biomass than hairy vetch and berseem clover (Fakhari et al. 2015).

Ryegrass, both annual and perennial, has been found to provide the most ground cover compared to other cover crop species (Hively and Cox 2001; Scott et al. 1987). Perennial ryegrass provided the most fall ground cover when planted when the corn was 0.15-0.30 m tall or at midsilk compared to all other treatments planted at the early planting time (Scott et al. 1987). Perennial ryegrass covered 84% of the ground in the fall when planted early and 85% of the ground when planted at midsilk (Scott et al. 1987). Another study found annual ryegrass, as well as alfalfa, had the most fall biomass and ground cover in one year when broadcast into soybeans (Hively and Cox 2001). In one spring they also found annual ryegrass and creeping red fescue had the most biomass and ground cover (Hively and Cox 2001). Whether a legume or a grass species would fit best in the overall rotations, there are cover crop species that can produce biomass and cover the ground when interseeded.

1.5.3. Environmental factors

While interseeding may be successful in one study or location, interseeding will vary in different environments. Conditions such as precipitation, temperature, and main crop will influence the success of interseeding.

1.5.3.1. Temperature

A negative correlation between the vegetation cover ratio of the main crop plus the cover crop and the weed dry weight has been found (Uchino et al. 2012). By interseeding a cool season cover crop into a warm season main crop, even if there are fluctuations in temperature the vegetation cover ratio of the main crop plus cover crop can remain high which may correlate to lower weed biomass. In one study it was suggested that interseeding is successful across environmental conditions because in warm years the main summer annual crops do well and in cool years the winter annual cover crops do well (Uchino et al. 2012). In one year lower temperatures during the early growth stages of corn interseeded with hairy vetch favored the hairy vetch (winter annual) growth (Uchino et al. 2009). During this time the summer annual corn showed suppressed growth due to the cooler temperatures and eventually died (Uchino et al. 2009). In this case even though the corn died, the vegetation cover ratio for the corn plus the hairy vetch was high and resulted in low weed density and dry weight (Uchino et al. 2009). However the goal of interseeding is to maintain high main crop yields, so it is undesirable for the main crop to do poorly. Temperature needs to be considered when choosing species to interseed to ensure a high vegetation cover ratio but also to avoid years where the cover crop will rapidly grow and suppress the main crop.

1.5.3.2. Precipitation

Precipitation is another environmental factor that will greatly influence interseeded cover crops. Precipitation after interseeding could be used as a predictor of the aboveground biomass (Wilson et al. 2013). One study found the best way to predict fall aboveground biomass of cereal rye aerially seeded into corn and soybeans was by looking at the cumulative precipitation in the seven days after seeding (Wilson et al. 2013). This only accounted for 43% of the variation in biomass production but was the best model based on the factors they studied (Wilson et al. 2013). In one year of another study with limited precipitation, treatments planted when the corn was 0.15-0.30 m tall established well but lost vigor throughout the season (Scott et al. 1987).

Moisture content has been reported to be the biggest factor influencing germination of cereal rye (Wilson et al. 2013). The critical moisture content has been reported to be 0.083 g water g soil^{-1} (Wilson et al. 2013). Below this moisture content germination was reduced and below 0.051 g water g soil^{-1} germination was stopped (Wilson et al. 2013). This was supported in another study where there was delayed emergence of chickling vetch and red clover due to no precipitation for a two week period after interseeding (Baributsa et al. 2008).

If moisture is limiting, competition between the main crop, cover crop, and weeds will be increased. This was observed when low moisture availability benefitted the weeds and interseeded crimson clover (Abdin et al. 1998). This increased competition resulted in 14% lower corn yield than any other interseeded treatment and 19% lower yield than the weed free no cover plots (Abdin et al. 1998). They conclude crimson clover is too competitive when moisture is a limiting factor (Abdin et al. 1998).

1.5.4. Main crop

Interseeding will vary depending on the main crop. One study argued that interseeding is more compatible with soybeans than corn due to the different weed distributions they observed (Uchino et al. 2015). Corn had higher weed density in the corn row and soybeans had higher weed density between the rows (Uchino et al. 2015). Across both corn and soybeans having an interseeded cover crop reduced the weed density more between the crop rows than in the crop rows (Uchino et al. 2015). Since corn had more weeds in the corn row which may not be suppressed by interseeding and soybeans had more weeds between the rows where the interseeding would suppress weeds they conclude interseeding will better suppress weeds in soybeans (Uchino et al. 2015). Weed distributions may change over time or location but if there are trends then interseeding where weeds are more prevalent would help maximize the benefits of interseeding.

1.5.5. Interseeded cover crop survival

Many cover crops that have been studied with interseeding are winter annuals. One issue with planting a winter annual in the summer is that it may not have the right conditions in which to grow and therefore may die (Belfry and Van Eerd 2016; Uchino et al. 2009). In one study, while there was good germination and establishment for all treatments, the cover crops died when they reached the 3-5 leaf stage regardless of if they were interseeded when the corn was V4-V6 or V10-V12 (Belfry and Van Eerd 2016). They attribute this death to the lack of solar infiltration through the sweet corn canopy (Belfry and Van Eerd 2016). They suggest lack of solar infiltration caused early senescence in cereal rye as well (Belfry and Van Eerd 2016). They had another

experiment with more species and mixtures interseeded when seed corn was between V6-V8 (Belfry and Van Eerd 2016). Here they saw up to 54% ground cover provided by cereal rye before it senesced midseason (Belfry and Van Eerd 2016). Another study also saw interseeded cover crops senesce early (Uchino et al. 2009). They saw cereal rye and hairy vetch die before the main crop harvest regardless of planting date (Uchino et al. 2009). Early senescence of interseeded cover crops should be considered when choosing cover crop species and when to interseeded.

1.6. Conclusion

Interseeding has been shown to be capable of increasing cover crop biomass, therefore suppressing weeds, while not reducing main crop yield (Baributsa et al. 2008; Belfry and Van Eerd 2016; Fakhari et al. 2015; Hively and Cox 2001; Mohammadi 2010; Scott et al. 1987; Uchino et al. 2009; Uchino et al. 2012; Uchino et al. 2015). However, results are not consistent and interseeded cover crops do not always survive. These inconsistent results may be due to environmental conditions that will differ by year and location. Given that environmental conditions will likely influence interseeded cover crops, research needs to focus on the potential to interseed cover crops in Kentucky. Going forward, research should focus on if herbicides can be used prior to interseeding in Kentucky without causing injury, what species can be interseeded, and if interseeding can provide better weed suppression compared to post-harvest planted cover crops.

Chapter 2: Pre-emergent herbicide effects on interseeded cover crops

2.1. Introduction

Cover crops are typically planted after harvesting the main crop. In a summer annual crop rotation with winter annual cover crops, this leaves the soil bare and susceptible to erosion and winter annual weed emergence until the cover crop establishes and provides adequate ground cover. An alternative is to interseed the cover crops into the main crop while it is big enough to withstand competition (i.e. after the critical weed free period) but before the canopy closes. In corn (*Zea mays* L.) the critical weed free period ends around V5-V7 so interseeding could be targeted for this time frame. This would allow the cover crops to establish before the corn canopy closes, reducing light penetration to the soil surface, and start producing biomass as the corn senesces.

When cover crops are interseeded, they are planted between the rows of a main crop while that main crop is growing. Given that the interseeded cover crops will be alive under the main crop in low light conditions, they need to be shade tolerant. They should also be able to survive in the hot, dry summer conditions found in Kentucky while still winter hardy to provide benefits through the winter and the following spring. Cover crops could be interseeded in several ways such as aerially broadcast or using a drill.

One goal of interseeding cover crops is to help suppress weeds that could emerge during the main crop growing season and directly after. However, interseeded cover crops alone may not be able to control weeds to an acceptable level. Several studies have shown the importance of using weed management that combines at least two tactics (chemical, physical, biological, cultural) to control weeds instead of relying on one measure alone. One study found poor establishment of interseeded cover crop when the weed pressure was high before interseeding (Abdin et al. 1998). In this case the weeds

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competed with the main crop and the interseeded cover crops and reduced the effectiveness of both. They conclude a combination of interseeded cover crops and chemical control of weeds should be utilized (Abdin et al. 1998). Chemical control could help reduce the competition from the weeds giving the corn and interseeded cover crops time to grow. Another study also saw reductions in yield due to high weed pressure (Mohammadi 2010). Compared to plots where weeds were controlled by hand weeding, there was approximately a 30% reduction in both corn yield and interseeded cover crop biomass when weeds were not controlled (Mohammadi 2010). This supports what Abdin et al. (1998) concluded: an integration of both chemical or physical measures and interseeding will provide the best weed control. Given physical weed control is challenging and costly to do on a large scale and incompatible with in no-tillage systems, chemical measures would be ideal.

While studies have suggested that integrating chemical control with interseeding will help the success of the cover crop, it is challenging to find chemicals that can be used without injuring the interseeded cover crops (Cornelius and Bradley 2017a; Tharp and Kells 2000). In many production systems, it is common to apply a soil residual herbicide before or at planting of the main crop. This means herbicides may persist in the soil when the cover crops are interseeded and may reduce the establishment or growth of the cover crops. Due to differential selectivity of herbicide active ingredients, grasses and legumes might respond differently to the same herbicide product.

Tharp and Kells (2000) report that some herbicides may cause injury and stand reductions in annual ryegrass (*Lolium multiflorum* L.) even if planting is delayed to corn stage V5-V6, but if there is rainfall between the herbicide applications and cover crop

planting, injury can be minimized. This study however used different herbicides from the ones commonly used by Kentucky growers and did not utilize interseeding. Wallace et al. (2017) interseeded cover crops into corn and reported that, in the locations studied (Pennsylvania, Maryland, New York), annual ryegrass and red clover (*Trifolium pratense* L.) could be interseeded following applications of many soil residual herbicides. Although they note that there were differences in cover crop injury between the locations they studied, they attribute these to differences in soil texture and cation exchange capacity (CEC) in the different soil types (Wallace et al. 2017). A study conducted in Missouri, which has a more similar summer climate to Kentucky, reported some corn and soybean (*Glycine max* (L.) Merr.) herbicides may cause injury to the following cover crops (Cornelius and Bradley 2017a). This study however did not utilize interseeding and cover crops were planted after cash crop harvest which would give the herbicides more time to degrade than in an interseeding scenario.

Results will likely vary in different locations due to soil type and climate differences—an important distinction highlighted by authors of these studies (i.e. Wallace et al. 2017). CEC is determined by the clay and organic matter of the soil. Soils with higher CEC will retain herbicides longer than soils with lower CEC (Curran 2001). As long as herbicides are bound to the soil particles they will be unavailable for plant uptake but once they are released from the soil they will be able to be taken up by the plants growing at that time (Curran 2001). Climate factors such as rainfall and temperature will impact herbicide persistence and degradation as well. Higher temperatures will speed up the degradation of herbicides if moisture is available (Curran 2001). Given adequate temperature, increased precipitation will also increase degradation while drought

conditions will encourage herbicide persistence (Curran 2001). Given that different herbicides will respond differently based on climate conditions and even in similar climates soil types may cause differing responses, soil residual herbicide influence on interseeded cover crops should be examined in a variety of locations.

The objective of this study was to determine which corn pre-emergent herbicides and mixtures of herbicide active ingredients commonly used by Kentucky growers could be used prior to interseeding without causing reductions in interseeded annual ryegrass and red clover establishment and growth. We expected that treatments containing higher rates of active ingredients or multiple active ingredients would reduce establishment and growth more than lower rates or single active ingredient treatments. We also expected treatments with active ingredients that control broadleaves more than grasses (groups 5 and 14) to reduce red clover establishment and growth more that treatments containing active ingredients that control grasses (group 15) and vice versa for annual ryegrass.

2.2. Materials and methods

2.2.1. Plot establishment. This experiment was conducted at the Kentucky Agricultural Experiment Station Spindletop Research Farm near Lexington, KY, USA (38°8′7.4″ N, 84°29′57.6″ W). The soil type was Bluegrass-Maury silt loam (Fine/Fine-silty, mixed, active, mesic Typic Paleudalfs). The field used in 2016 was previously cropped to cereal rye (*Secale cereale* L.) with fallow over fall and winter and the field in 2017 was cropped to tobacco (*Nicotiana tabacum* L.) followed by a cover crop of wheat (*Triticum aestivum* L.). An initial burndown herbicide application to control winter annual weeds or the cover crop was completed on 29 March 2016 and 8 April 2017 using 0.84 kg ae ha^{-1} of glyphosate. Both fields were then tilled; on 7 May 2016 a disk was

used and on 20 May 2016 it was worked with a soil finisher. On 18 April 2017, a disk and soil finisher were used. Soil residual herbicide treatments were assigned to plots using a completely random design in both years. Plots were 3 m wide by 9 m long.

2.2.2. Corn planting and treatment establishment. Fertility was applied based on soil sampling and the University of Kentucky's recommendations; 155 kg ha^{-1} of nitrogen (as urea) and 58 kg ha^{-1} of potassium (as potassium chloride) were applied on 29 April 2016. On 25 April 2017, 168 kg N ha^{-1} (as urea) was applied but no additional potassium was needed. Each plot contained four rows of corn spaced 76 cm apart; Stine 'R9740VT3pro' was planted with a six-row planter (MaxEmerge 1755, John Deere) on 27 May 2016 and Stine '9714G' was planted with a four-row planter (MaxEmerge 1750, John Deere) on 3 May 2017. In both years the corn was seeded at a rate of approximately 74,000 seeds ha^{-1}. The soil residual herbicides were applied pre-emergence immediately following planting using a backpack sprayer calibrated to deliver 224 L ha-1 at 207 kPa using DG8004 nozzles (TeeJet Technologies). Weather conditions during application were 3-5 mph SSW wind, 29C air temp, 55% relative humidity, and 19C dew point in 2016 and in 2017, 3-7 mph NNE wind, 18 C air temp, and 8C dew point. Dates of other significant field events are shown in Table 2.1 and the herbicide treatments used are listed in Table 2.2.

2.2.3. Cover crop planting. Two cover crop species (annual ryegrass variety 'Marshall' and red clover variety 'Kenland') were interseeded in each plot. The cover crops were interseeded using a two-row interseeder unit (Interseeder Technologies) on 25 June in 2016 and 8 June in 2017. This unit is a high clearance drill designed to drill three cover crop rows spaced 19 cm apart between cash crop rows on a 76 cm row spacing.

The annual ryegrass was seeded at a rate of 22.4 kg ha^{-1} and the red clover at a rate of 11.2 kg ha^{-1}. Each species was interseeded in half of each plot so they could be evaluated independently. The corn was at V6 when interseeding occurred.

2.2.4. Other field operations. On 9 June 2016 a post emergent application of glyphosate (0.84 kg ae ha^{-1}) plus 1% AMS v/v was made to control emerged weeds; this application was not necessary in 2017. In 2017, drip irrigation was used in each plot to ensure there was adequate moisture. A total of 8.4 cm was applied during the growing season. Dates and amounts of irrigation are in Table 2.1.

2.2.5. Data collection.

Cover crop density and biomass. Cover crop density was measured periodically each year; collection dates are in Table 2.1. The number of cover crop plants was counted within two 0.25m^2 quadrats per plot with two rows of the cover crop included in each quadrat. Cover crop density was measured again in the spring before cover crop termination. In spring 2017, due to low density, density was measured between two rows of corn stubble (area 76 cm wide) and a length of 3 m. Density was not measured in spring 2018 since the red clover had good survival and the annual ryegrass had tillered so it was not possible to obtain an accurate density measurement. All cover crop density was standardized to consistent units for analysis. Cover crop biomass was collected from two 0.25m^2 quadrats per plot on the dates in Table 2.1. At cover crop termination in 2017, biomass of both species was collected between two corn rows (area 76 cm wide) and 3 m long in each plot due to low density. At cover crop termination in 2018, annual ryegrass biomass was collected in a similar manner. The red clover biomass was collected from

two 0.25m^2 quadrats per plot. The biomass was dried at 60C until consistent weight was achieved and then weighed to obtain dry weight.

2.2.6. Data analysis. Prior to analysis all data were checked for normality and homogeneous variances. When assumptions were not met, either a log or square root transformation was used. Once assumptions were met, data were subject to ANOVA using PROC GLIMMIX in SAS v 9.4 (SAS Institute 2012). When ANOVA indicated significant treatment differences ($P < 0.05$), estimates were used to compare pre-planned treatments. Years were analyzed separately due to differences in management (i.e. irrigation) and significant year * treatment interactions. Treatment was considered as a fixed factor, while replicate was considered a random factor. Due to low red clover survival in spring 2017, plots with less than 5 g m^{-2} of red clover dry biomass were not included in the analysis.

2.3. Results and Discussion

The untreated control did not consistently have the highest initial cover crop density or biomass prior to termination, for either species, in either year. There may be environmental conditions (see subsequent discussion) that influenced the establishment and growth of the interseeded species and masked the differences from the pre-emergent herbicides. Other studies have also reported variable establishment and growth in untreated plots (Curran et al. 2018; Wallace et al. 2017).

Total precipitation and average daily minimum and maximum temperatures 14 days prior to herbicide application, between herbicide application and interseeding and 35 days after interseeding as well as 10 year averages are given in Table 2.3. Conditions prior to herbicide application will give an indication of soil moisture when the herbicides

were applied. Conditions between herbicide application and interseeding may impact how much herbicide is left in the soil when the cover crops are interseeded. Conditions after interseeding may give an indication of how quickly the cover crops establish and therefore start up taking up herbicides from the soil solution, if they are still present. As soil moisture and temperature increase, herbicide degradation will increase (Curran 2001). Increased precipitation after herbicide application may also result in herbicide leaching (Curran 2001).

2.3.1. Interseeded annual ryegrass

2016: The initial annual ryegrass density, measured three weeks after interseeding, was affected by the pre-emergent herbicide treatments ($P < 0.001$; Table 2.4). The only pre-emergent herbicide that resulted in a density significantly lower than the untreated control at this time was dimethenamid -P (0.84 kg ai ha^{-1}) + atrazine (1.12 kg ai ha^{-1}; Table 2.4). This combination resulted in a 29% decrease in density compared to the control.

Some pre-emergent herbicide combinations resulted in lower initial densities than other combinations in 2016 (Table 2.5). Atrazine combined with a higher rate (2.28 kg ai ha^{-1}) of acetochlor resulted in lower stand than atrazine combined with a lower rate (1.39 kg ai ha^{-1}) of acetochlor. The higher rate of acetochlor with atrazine also had lower density than a medium (1.96 kg ai ha^{-1}) rate of acetochlor + tembotrione + thiencarbazone. However, the lower rate of acetochlor + atrazine had a higher initial density than dimethenamid -P combined with either rate (1.12 or 1.68 kg ai ha^{-1}) of atrazine. The combination of dimethenamid -P with either rate of atrazine had lower initial density than dimethenamid -P + saflufenacil.

While initial density averaged 174 plants m^{-2} over all treatments, by early August 2016 the annual ryegrass stand had decreased to about 36 plants m^{-2}. By corn harvest in fall 2016 there were few surviving interseeded annual ryegrass plants (<2 plants m^{-2}). Prior to spring termination in 2017, there was not sufficient annual ryegrass biomass to analyze statistically. Since all treatments showed a decline in annual ryegrass survival over the summer, there is likely an environmental factor that outweighed the herbicide treatment effects.

2017: There were no differences in annual ryegrass density two weeks after interseeding (P > 0.05) in 2017 (Table 2.4). The average initial density was 97 plants m^{-2}. While there was less rainfall prior to the herbicide applications in 2017 compared to 2016 (Table 2.3), there was more time and rainfall between the herbicide applications and interseeding as well as more rainfall after interseeding in 2017. That extra rainfall may have degraded the herbicides in the soil so no differences were detected. Herbicides generally degrade quicker when soil moisture is higher (Curran 2001). With more moisture in the soil, microbial activity is increased which is one of the main ways herbicides are degraded (Curran 2001).

Annual ryegrass density declined over the summer, though less dramatically than in 2016. The density remained high through early July when the density averaged 93 plants m^{-2}. By early August the density had reduced to 79 plants m^{-2} and by corn harvest there were 38 plants m^{-2}.

At spring termination, herbicide treatment impacted annual ryegrass biomass (P < 0.05; Table 2.6). None of the herbicides reduced biomass significantly below the control (data not shown). There was only one comparison that was significantly different for both

initial annual ryegrass density in 2016 and final biomass in 2018 but the reduction was opposite for the two times. This suggests environmental factors could be more responsible for the reductions than the herbicides.

Spring biomass was reduced when acetochlor (1.96 kg ai ha^{-1}) was combined with tembotrione (0.08 kg ai ha^{-1}) + thiencarbazone (0.01 kg ai ha^{-1}) or tembotrione (0.09 kg ai ha^{-1}) compared to just tembotrione itself or in combination with thiencarbazone (Table 2.6). Dimethenamid -P (0.84 kg ai ha^{-1}) also reduced biomass when it was combined with tembotrione + thiencarbazone compared to just tembotrione + thiencarbazone. However, dimethenamid -P + tembotrione + thiencarbazone had similar biomass as just tembotrione (0.09 kg ai ha^{-1}). Dimethenamid -P (0.56 kg ai ha^{-1}) also reduced biomass when combined with saflufenacil compared to dimethenamid -P + atrazine (1.68 kg ai ha^{-1}). That combination of dimethenamid -P + atrazine also had more biomass than acetochlor (2.28 kg ai ha^{-1}) + atrazine (1.12 kg ai ha^{-1}). Dimethenamid -P + atrazine (1.68 kg ai ha^{-1}) had more biomass than dimethenamid -P + atrazine (1.12 kg ai ha^{-1}) again suggesting an environmental factor that is influencing the cover crops more than the herbicides.

The results from this study suggest that these herbicides will not reduce the initial density or spring biomass of interseeded annual ryegrass compared with a no herbicide control. Some active ingredients and combinations of active ingredients resulted in higher densities and biomass than others. Establishment of annual ryegrass was sometimes reduced when a high rate of acetochlor or dimethenamid-P was used. Spring biomass was also reduced when a high rate of acetochlor or dimethenamid-P was used but the results were not consistent. Dimethenamid -P alone has been shown in other studies to slightly reduce annual ryegrass biomass measured in the fall compared to a control (Wallace et al.

2017). In that study they reported about 16% reduction in annual ryegrass biomass in the fall following a dimethenamid -P application which they suggest is not enough of a reduction to prevent use. However, combining dimethenamid -P with another herbicide may increase reduction as is shown in this study. Another study found no reduction in Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot), a close relative of annual ryegrass, stand 28 days after emergence but the biomass was reduced 37% by atrazine and 50% by tembotrione + thiencarbazone (Cornelius and Bradley 2017a). In this study tembotrione + thiencarbazone only caused reductions when used in combinations with other herbicides and atrazine caused reductions but not consistently.

2.3.2. Interseeded red clover

2016: The initial density of red clover, measured three weeks after interseeding, was not influenced by the pre-emergent herbicide treatments (P > 0.05; Table 2.4). The average initial density was 183 plants m^{-2}. The interseeded red clover density in 2016 declined over time but not as severely as the annual ryegrass. In early August, the red clover density averaged 74 plants m^{-2}. At corn harvest, density declined to 23 plants m^{-2} and by termination the following spring there were <5 plants m^{-2}. Of the treatments that were included in the analysis, there were no differences in red clover biomass prior to termination due to pre-emergent herbicide treatments (P > 0.05).

2017: Red clover initial density two weeks after interseeding was not impacted by the pre-emergent herbicides (P > 0.05; Table 2.4). The average initial density was 163 plants m^{-2}. Unlike 2016, red clover in 2017 survived through the summer and maintained a good stand through the winter. By spring termination, there were no treatment differences (P > 0.05) and on average the biomass was between 247-335 g m^{-2}.

Red clover establishment and biomass was not reduced by any herbicides used in this study. A previous study has reported that only mesotrione and mesotrione + s-metolachlor + atrazine reduced red clover fall biomass whereas dimethenamid -P, saflufenacil, acetochlor, and atrazine did not (Wallace et al. 2017). Another study reported a 35% and 38% reduction in crimson clover (*Trifolium incarnatum* L.) biomass when atrazine and tembotrione, respectively, were applied prior to planting (Cornelius and Bradley 2017a). These studies highlight the variability in herbicide impacts that are observed with cover crops. The results from this study suggest that these herbicides can be safely used with interseeded red clover in central Kentucky given the weather conditions are similar to the two years of this study.

Both species saw a decline in density in 2016 and annual ryegrass saw a decline in 2017 though not as sharp as 2016. It is likely environmental factors influenced both species causing the declines since even the untreated control showed declines. One possible reason there was better survival in 2017 is more light infiltration through the corn canopy. In 2017, a shorter corn hybrid was used which may have benefitted the interseeded species. Red clover has been shown to produce significantly less aboveground biomass when grown in 50% shaded conditions compared to full sun. Biomass was reduced even further when red clover was grown in 80% shade (Lin et al. 1999). This could explain why red clover maintained a high density in 2017 since the corn was shorter and therefore the clover had more sunlight. Perennial ryegrass (*Lolium perenne* L.), a close relative of annual ryegrass, was also examined in that study. It only showed significant biomass reductions when grown in 80% shade (Lin et al. 1999). It

may be possible that the annual ryegrass was not limited by sunlight but by another environmental factor.

Another possible reason the annual ryegrass and red clover did better in 2017 compared to 2016 is the average temperature between June-August in 2016 was 1.2C higher than the 30-year average and in 2017 it was 0.5C lower than the 30-year average. Annual ryegrass has been shown to start showing severe signs of stress as temperatures reach 38C (Richardson 2004). While average temperatures in 2016 were well below that, it is likely temperatures reached 38C several times during the growing season. Red clover aboveground biomass decreased as temperatures increased from 15.5 to 32C but it still produced biomass after being at 32C for 45 days (Gist and Mott 1957). This demonstrates that red clover could survive over a month with temperatures constantly higher than average daily temperatures in Kentucky. However it is unclear how red clover would respond to temperatures higher than 32C even for short periods.

Insufficient rainfall was not likely to cause the reductions in stand. In both years, there was about two cm more precipitation between June-August than the 30-year average and in 2017 drip irrigation was used ensuring adequate moisture was available for the cover crops. In this study moisture was not limiting but should be considered with interseeding red clover. One study found root growth decreased as light intensity decreased. They conclude that red clover under shaded conditions will not have the root growth necessary if moisture is limiting (Gist and Mott 1957).

These results suggest the commonly used pre-emergent corn herbicides used in this study would not consistently or severely reduce interseeded annual ryegrass or red clover establishment or growth. A bigger influence was the environment which should be

studied further to determine what factors caused declines in density and how to alleviate those complications.

Table 2.1. Dates of major field events and data collection.

Event	2016	2017
Initial burndown	29 March	8 April
Fertilized	29 April	25 April
Corn planted	24 May	3 May
Applied pre-emergent herbicide treatments	27 May	3 May
Interseeded	25 June	8 June
Weed management	9 June	N/A
Irrigated (cm applied)	N/A	24 July (1.8) 26 July (3.1) 31 July (3.5)
Corn harvest	4 October	5 October
Spring cover crop sampling prior to termination	20 April 2017	25 April 2018
Data collected		
Cover crop density	15 July 1 and 3 August* 4 October 20 April 2017	22 June 7 July 8 August 21 September
Cover crop biomass	20 April 2017	25 April 2018

* Due to rainfall during data collection, data were collected over several days.

Table 2.2. Herbicide active ingredients and rates used.

Trt Number	Trade Name	Formulation	Active Ingredient	Rate kg ai ha^{-1}	Manufacturer and Manufacturer Location
1	untrt	NA	NA		NA
2	Harness Xtra	5.6 L	acetochlor + atrazine	1.39 + 1.12	Monsanto St. Louis, MO monsanto.com
3	Degree Xtra	4.04 ME	acetochlor + atrazine	2.28 + 1.12	Monsanto St. Louis, MO monsanto.com
4	Harness	7 E	acetochlor	2.28	Monsanto St. Louis, MO monsanto.com
4	Aatrex	4 L	atrazine	1.12	Syngenta Greensboro, NC syngenta.com
5	Harness	7 E	acetochlor	1.96	Monsanto St. Louis, MO monsanto.com
5	Capreno	3.45 SC	tembotrione + thiencarbazone	0.08 + 0.01	Bayer CropScience Research Triangle Park, NC cropscience.bayer.com
6	Outlook	6.0 E	dimethenamid -P	0.84	BASF Research Triangle Park, NC basf.com
6	Aatrex	4 L	atrazine	1.12	Syngenta Greensboro, NC syngenta.com
7	Outlook	6.0 E	dimethenamid -P	0.84	BASF Research Triangle Park, NC basf.com

Table 2.2 (continued). Herbicide active ingredients and rates used.

7	Aatrex	4 L	atrazine	1.68	Syngenta Greensboro, NC syngenta.com
8	Verdict	5.57 EC	dimethenamid -P + saflufenacil	0.56 + 0.07	BASF Research Triangle Park, NC basf.com
9	Outlook	6.0 E	dimethenamid -P	0.84	BASF Research Triangle Park, NC basf.com
9	Capreno	3.45 SC	tembotrione + thiencarbazone	0.08 + 0.01	Bayer CropScience Research Triangle Park, NC cropscience.bayer.com
10	Laudis	3.5 SC	tembotrione	0.09	Bayer CropScience Research Triangle Park, NC cropscience.bayer.com
11	Capreno	3.45 SC	tembotrione + thiencarbazone	0.08 + 0.01	Bayer CropScience Research Triangle Park, NC cropscience.bayer.com

Abbreviations: L, Liquid; ME, Micro-encapsulated; SC, Soluble concentrate; E/EC, emulsifiable concentrate

Table 2.3. Environmental conditions 14 days before herbicide application, between herbicide application and interseeding, 35 days after interseeding, and 10 year average values in Lexington, KY.

	Before herbicide application (14 days)				Between herbicide application and interseeding			After interseeding (35 days)		
	Precip (mm)	Temp (C)		# of days	Precip (mm)	Temp (C)		Precip (mm)	Temp (C)	
		Max	Min			Max	Min		Max	Min
2016	71	21	10	28	116	30	18	126	31	20
10 year average	51	24	13		90	29	18	177	30	19
2017	39	23	13	36	137	25	14	205	29	19
10 year average	76	21	10		137	25	14	149	30	19

Table 2.4. Effect of pre-emergent herbicide treatments on initial density of interseeded annual ryegrass and red clover measured 3 weeks after interseeding in 2016 and 2 weeks after interseeding in 2017 in Lexington, KY.

Treatment	Rate	Annual ryegrass		Red clover	
		2016[a]	2017[a]	2016[a]	2017[a]
	kg ai ha⁻¹	# m⁻²			
Control	-	137	94	187	172
acetochlor + atrazine	1.39 + 1.12	272	92	212	169
acetochlor + atrazine	2.28 + 1.12	103	72	153	168
acetochlor + atrazine	2.28 + 1.12	107	129	138	144
acetochlor + tembotrione + thiencarbazone	1.96 + 0.08 + 0.01	234	82	188	163
dimethenamid -P + atrazine	0.84 + 1.12	97*	96	181	159
dimethenamid -P + atrazine	0.84 + 1.68	131	108	157	175
dimethenamid -P + saflufenacil	0.56 + 0.07	196	79	196	151
dimethenamid -P + tembotrione + thiencarbazone	0.84 + 0.08 + 0.01	158	119	191	174
tembotrione	0.09	213	95	207	154
tembotrione + thiencarbazone	0.08 + 0.01	267	108	207	162
SEM (±)[b]		13	5	6	4

[a] Treatment means followed by (*) are significantly lower than the no herbicide control at alpha=0.05.
[b] Standard error of the mean based on untransformed data.

Table 2.5. Comparisons of pre-emergent herbicide treatments effect on initial annual ryegrass density 3 weeks after interseeding in 2016 in Lexington, KY.

Treatment	Initial density	vs	Treatment	Initial density	P value
[treatment number] (kg ai ha^{-1})	# m^{-2}		[treatment number] (kg ai ha^{-1})	# m^{-2}	
[2] acetochlor (1.39) + atrazine (1.12)	272		[3] acetochlor (2.28) + atrazine (1.12)	103	0.0141
[2] acetochlor (1.39) + atrazine (1.12)	272		[4] acetochlor (2.28) + atrazine (1.12)	107	<0.0001
[3] acetochlor (2.28) + atrazine (1.12)	103		[4] acetochlor (2.28) + atrazine (1.12)	107	0.7526
[2] acetochlor (1.39) + atrazine (1.12)	272		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234	0.2910
[3] acetochlor (2.28) + atrazine (1.12)	103		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234	0.0357
[4] acetochlor (2.28) + atrazine (1.12)	107		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234	<0.0001
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234		[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158	0.1671
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234		[10] tembotrione (0.09)	213	0.3994
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	234		[11] tembotrione (0.08) + thiencarbazone (0.01)	267	0.3025
[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158		[10] tembotrione (0.09)	213	0.6844
[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158		[11] tembotrione (0.08) + thiencarbazone (0.01)	267	0.0799
[10] tembotrione (0.09)	213		[11] tembotrione (0.08) + thiencarbazone (0.01)	267	0.2209
[2] acetochlor (1.39) + atrazine (1.12)	272		[6] dimethenamid -P (0.84) + atrazine (1.12)	97	<0.0001

Table 2.5 (continued). Comparisons of pre-emergent herbicide treatments effect on initial annual ryegrass density three weeks after interseeding in 2016 in Lexington, KY.

[3] acetochlor (2.28) + atrazine (1.12)	103	[6] dimethenamid -P (0.84) + atrazine (1.12)	97	0.9208
[4] acetochlor (2.28) + atrazine (1.12)	107	[6] dimethenamid -P (0.84) + atrazine (1.12)	97	0.5669
[6] dimethenamid -P (0.84) + atrazine (1.12)	97	[7] dimethenamid -P (0.84) + atrazine (1.68)	131	0.0763
[6] dimethenamid -P (0.84) + atrazine (1.12)	97	[8] dimethenamid -P (0.56) + saflufenacil (0.07)	196	0.0001
[6] dimethenamid -P (0.84) + atrazine (1.12)	97	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158	0.5033
[7] dimethenamid -P (0.84) + atrazine (1.68)	131	[8] dimethenamid -P (0.56) + saflufenacil (0.07)	196	0.0150
[7] dimethenamid -P (0.84) + atrazine (1.68)	131	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158	0.9905
[8] dimethenamid -P (0.56) + saflufenacil (0.07)	196	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	158	0.3354
[4] acetochlor (2.28) + atrazine (1.12)	107	[7] dimethenamid -P (0.84) + atrazine (1.68)	131	0.2194
[2] acetochlor (1.39) + atrazine (1.12)	272	[7] dimethenamid -P (0.84) + atrazine (1.68)	131	<.0001
[3] acetochlor (2.28) + atrazine (1.12)	103	[7] dimethenamid -P (0.84) + atrazine (1.68)	131	0.4358

Table 2.6. Comparisons of pre-emergent herbicide treatments effect on annual ryegrass biomass in the spring prior to termination 2018 in Lexington, KY.

Treatment	Biomass	vs	Treatment	Biomass	P value
[treatment number] (kg ai ha^{-1})	g m^{-2}		[treatment number] (kg ai ha^{-1})	g m^{-2}	
[2] acetochlor (1.39) + atrazine (1.12)	14		[3] acetochlor (2.28) + atrazine (1.12)	5	0.2205
[2] acetochlor (1.39) + atrazine (1.12)	14		[4] acetochlor (2.28) + atrazine (1.12)	13	0.9462
[3] acetochlor (2.28) + atrazine (1.12)	5		[4] acetochlor (2.28) + atrazine (1.12)	13	0.2460
[2] acetochlor (1.39) + atrazine (1.12)	14		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6	0.2408
[3] acetochlor (2.28) + atrazine (1.12)	5		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6	0.9568
[4] acetochlor (2.28) + atrazine (1.12)	13		[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6	0.2680
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6		[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13	0.2676
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6		[10] tembotrione (0.09)	21	0.0258
[5] acetochlor (1.96) + tembotrione (0.08) + thiencarbazone (0.01)	6		[11] tembotrione (0.08) + thiencarbazone (0.01)	28	0.0019
[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13		[10] tembotrione (0.09)	21	0.2335
[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13		[11] tembotrione (0.08) + thiencarbazone (0.01)	28	0.0303
[10] tembotrione (0.09)	21		[11] tembotrione (0.08) + thiencarbazone (0.01)	28	0.2987
[2] acetochlor (1.39) + atrazine (1.12)	14		[6] dimethenamid -P (0.84) + atrazine (1.12)	10	0.5843
[3] acetochlor (2.28) + atrazine (1.12)	5		[6] dimethenamid -P (0.84) + atrazine (1.12)	10	0.4904
[4] acetochlor (2.28) + atrazine (1.12)	13		[6] dimethenamid -P (0.84) + atrazine (1.12)	10	0.6311

Table 2.6 (continued). Comparisons of pre-emergent herbicide treatments effect on annual ryegrass biomass in the spring prior to termination 2018 in Lexington, KY.

[6] dimethenamid -P (0.84) + atrazine (1.12)	10	[7] dimethenamid -P (0.84) + atrazine (1.68)	25	0.0332	
[6] dimethenamid -P (0.84) + atrazine (1.12)	10	[8] dimethenamid -P (0.56) + saflufenacil (0.07)	9	0.8658	
[6] dimethenamid -P (0.84) + atrazine (1.12)	10	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13	0.6306	
[7] dimethenamid -P (0.84) + atrazine (1.68)	25	[8] dimethenamid -P (0.56) + saflufenacil (0.07)	9	0.0227	
[7] dimethenamid -P (0.84) + atrazine (1.68)	25	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13	0.0910	
[8] dimethenamid -P (0.56) + saflufenacil (0.07)	9	[9] dimethenamid -P (0.84) + tembotrione (0.08) + thiencarbazone (0.01)	13	0.5166	
[4] acetochlor (2.28) + atrazine (1.12)	13	[7] dimethenamid -P (0.84) + atrazine (1.68)	25	0.0908	
[2] acetochlor (1.39) + atrazine (1.12)	14	[7] dimethenamid -P (0.84) + atrazine (1.68)	25	0.1035	
[3] acetochlor (2.28) + atrazine (1.12)	5	[7] dimethenamid -P (0.84) + atrazine (1.68)	25	0.0064	

Chapter 3: Species selection for interseeding in Kentucky

3.1. Introduction

One way cover crops can be established is by interseeding which is when the cover crops are planted between the rows of a main crop while that main crop is growing. Interseeding could be done in several ways such as aerially broadcast or using a drill. Interseeding timing varies too from planting the cover crops with the main crop to planting just before senescence. Since traditional cover crops are planted after harvesting the main crop, the soil is left bare and susceptible to weed emergence until the cover crop establishes and the biomass covers the ground. In a corn – winter annual cover crop – full season soybean rotation, one desired trait of interseeded cover crops is that they can readily produce biomass as the corn (*Zea mays* L.) senesces. With additional biomass by corn harvest, weeds will have more competition for light which has been shown to reduce weed emergence (Teasdale 1996). Another issue with planting cover crops after harvesting the main crop is that conditions for cover crop establishment may not be ideal. Higher precipitation than average and cold temperatures after harvesting the main crop hindered cereal rye (*Secale cereale* L.), seeded after harvest, from establishing and highlighted the benefits of interseeding (Hively and Cox 2001).

Any cover crop that would be interseeded into the corn in the year prior to soybeans (*Glycine max* (L.) Merr.) should be a grass species to reduce nitrogen leaching concerns and help reduce disease pressure. An ideal interseeded grass species would be a winter annual to survive through the fall, winter, and into the following spring, but still have enough heat and drought tolerance to survive the hot, often dry summers in Kentucky. Once the corn canopy closes, the interseeded cover crop will have limited

sunlight, therefore any interseeded cover crop should also have the ability to survive in shaded conditions. Lastly, high corn yields are desired so it is important that the cover crops do not compete with the corn while it is growing. Using a grass that has summer dormancy so it is not actively growing during the summer will help limit the competition between the cover crop and corn so yields are not reduced.

Using the qualities given above, six grass entries were chosen to be tested with interseeding in Kentucky. Five of the six entries are fescue species. Fescues are cool season perennial grasses that are often used as forages or turf in Kentucky. Many species have been bred to survive both summers and winters in Kentucky. Meadow fescue (*Festuca pratensis* Huds.) is a fescue with a broader leaf that originated in northern Europe and the mountainous regions in southern Europe (Casler et al. 2008). Meadow fescue is expected to do well when interseeded because of its drought tolerance (Staniak 2016). Festulolium (*x Festulolium* Asch. & Graebn.) is a cross between a ryegrass species (*Lolium* spp.) and a fescue species. By crossing those two species, it is thought that festulolium will have the growth potential of a ryegrass and the stress tolerance of a fescue. North African tall fescue (*Festuca arundinacea* Schreb.) is tall fescue that is native to the mountainous regions of north Africa (Casler et al. 2008). It was chosen due to its drought tolerance (Tim Phillips, personal communication). There are two tall fescue pre-cultivars (KYFA 0601 and KYFA 1304), experimental populations from a breeding program at the University of Kentucky, chosen since tall fescue is well adapted to hot, dry, and moderately shaded conditions (Munshaw 2015). While these entries are not commercially available, they are expected to do well with interseeding since they have been bred specifically to grow in Kentucky. Timothy (*Phleum pratense* L.) is the only

entry that is not a fescue species. It is a perennial bunch grass that is native to Europe and Asia (Lacefield et al. 2002). It is expected to do well due to its good seedling vigor (Tim Phillips, personal communication).

The objective of this study was to determine which grass entries that are adapted to Kentucky would be best to interseed in corn. All the entries chosen have qualities such as heat, drought, and shade tolerance that we expect will make them good for interseeding.

3.2. Material and methods

This experiment was conducted at the Kentucky Agricultural Experiment Station Spindletop Research Farm near Lexington, KY (38°8′7.4″ N, 84°29′57.6″ W) and the University of Kentucky Research and Education Center in Princeton, KY (37°5′52.5″ N, 87°51′39.9″ W). At the Princeton location the soil type was Crider silt loam (Fine-silty, mixed, active, mesic Typic Paleudalfs) and the Lexington location had Bluegrass-Maury silt loam soil (Fine/Fine-silty, mixed, active, mesic Typic Paleudalfs). The experimental layout was a randomized complete block design. The plots were 3 m wide and 15 m long.

3.2.1. Plot establishment.

3.2.1.1. Lexington: In both years, the fields for this experiment were cropped to cereal rye with fallow over fall and winter prior to establishment of this experiment. Dates of major field events are in Table 3.1. An initial burndown herbicide application to control existing vegetation was completed on 22 March 2016 and 29 March 2017 using 0.84 kg ae ha^{-1} of glyphosate with 1% v:v ammonium sulfate. Fertility was applied based on soil sampling and the University of Kentucky's recommendations and 155 kg ha^{-1} of nitrogen (as urea) and 58 kg ha^{-1} of potassium (as potassium chloride) were applied on 29

April 2016. On 25 April 2017, 168 kg N ha^{-1} (as urea) was applied with no additional potassium needed. On 7 May 2016, soil was worked with a disk followed by a soil finisher on 20 May 2016. Both tillage implements were used on 18 April 2017. In 2017, drip irrigation was used in each plot. A total of 1.8 cm was applied during the growing season. Dates and amounts are in Table 3.1.

3.2.1.2. Princeton: The field previously had soybeans before the establishment of this experiment in 2016. The field used in 2017 previously had wheat (*Triticum aestivum* L.). The dates of major field events are shown in Table 3.1. The initial burndown was done on 29 March 2016 with 0.84 kg ae ha^{-1} of glyphosate and 20 March 2017 with 0.84 kg ae ha^{-1} of glyphosate plus 0.56 kg ai ha^{-1} of dicamba. Fertility was applied based on soil sampling and the University of Kentucky's recommendations, 441.5 kg ha^{-1} of 33-0-0-12 and 262.4 kg ha^{-1} diammonium phosphate (18-46-0) were broadcast on 10 April 2016 and on 12 April 2017, 112 kg ha^{-1} of nitrogen (as urea) was applied. While the wheat was growing in the field used in 2017-18, 101 kg ha^{-1} of nitrogen (as UAN) was applied. In 2017, drip irrigation was used in each plot. A total of 7.2 cm was applied during the growing season. Dates and amounts are in Table 3.1.

3.2.2. Corn establishment. Each plot contained four rows of corn spaced 76 cm apart. In Lexington, Stine 'R9740VT3pro' was planted with a six-row planter (MaxEmerge 1755, John Deere) on 24 May 2016 at approximately 74,000 seeds ha^{-1} and Stine '9714G' was planted with a four-row planter (MaxEmerge 1750, John Deere) on 3 May 2017 at approximately 68,000 seeds ha^{-1}. In Princeton, Stine 'R9740VT3pro' was planted with a four-row planter (MaxEmerge2 7200, John Deere) on 18 April 2016 at

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approximately 74,000 seeds ha^{-1} and Stine '9714G' was planted with a six-row planter (MaxEmerge XP 1780, John Deere) on 14 April 2017 at approximately 70,000 seeds ha^{-1}.

3.2.3. Cover crop planting. The cover crop treatments were interseeded using a two-row interseeder unit (Interseeder Technologies) This unit plants three cover crop rows 19 cm apart between the corn rows. The entries used are shown in Table 3.2. In Lexington, interseeding was done on 26-27 June 2016 and 12 June 2017 when the corn was at approximately stage V6. In Princeton, interseeding occurred on 24 May 2016 and 23 May 2017 when the corn was at approximately stage V5.

3.2.4. Weed and pest management.

3.2.4.1. Lexington: Post emergent application of glyphosate (0.84 kg ae ha-1 with 1% v:v ammonium sulfate) was applied to control emerged weeds prior to interseeding on 9 June 2016 and glyphosate was applied immediately after interseeding on 12 June 2017.

3.2.4.2. Princeton: Immediately following interseeding in 2016, glyphosate (1.26 kg ae ha^{-1}) was applied in 140 L ha^{-1} with 0.55% AMS v/v using a high clearance sprayer. In 2017, glyphosate (0.84 kg ae ha^{-1}) was applied immediately after interseeding with the same equipment. On 10 May 2017 a post emergent application of glyphosate (1.26 kg ae ha^{-1}) plus 0.05 kg ai ha^{-1}of halosulfuron plus 0.38% nonionic surfactant v/v was applied. Pests were scouted for throughout the season and on 12 July 2016 Lambda-cyhalothrin (Warrior®, Syngenta) was applied at a rate of 0.05 kg ai ha^{-1} to control Japanese beetles.

3.2.5. Data collection.

3.2.5.1. Corn. In Lexington only, corn height was measured on 21 June, 11 July, and 1 August 2016. Height was measured from the ground to the top of the tallest newly emerged leaf. Once tasseling commenced, height was measured from the ground to the base of the tassel. Developmental stage was measured by counting the number of fully exposed collars on 21 June. Height and stage were not measured in Princeton. The inner two corn rows of each plot were harvested with a plot combine at both locations. Yield was corrected to 15.5% moisture prior to analysis.

3.2.5.2. Cover crop. Cover crop density was measured periodically each year and dates are shown in Table 3.1. Cover crop density was measured by counting the number of cover crop plants within two $0.25m^2$ quadrats per plot. Two rows of the cover crop were included in each quadrat. In spring 2017, due to low density at cover crop termination data were collected from between two corn rows (76 cm wide) and 6 m long. Cover crop biomass was collected at the dates given in Table 3.1. At corn harvest in 2016 and 2017, cover crop biomass was collected from two $0.25m^2$ quadrats per plot. Before cover crop termination in 2017 and 2018, biomass was collected from the area between two corn rows (76 cm wide) and 6 m long. The biomass was dried at 60C until consistent weight was achieved and then weighed to obtain dry weight.

3.2.5.3. Weed density and biomass. Weeds were identified to species and counted at corn harvest and biomass was collected from two $0.25m^2$ quadrats per plot in 2016. In 2017, weed biomass was collected at corn harvest from one $0.25m^2$ quadrat per plot; weed density was not measured. The biomass was dried at 60C until consistent weight was achieved and then weighed to obtain dry weight.

3.2.6. Data analysis. Prior to analysis all data were checked for normality and homogeneous variances. When assumptions were not met transformations were used. In several cases, common transformations did not allow the data to meet the assumptions. In those cases (cover crop density at corn harvest in Lexington and Princeton in 2016 and prior to termination in 2017 in Lexington, cover crop biomass prior to termination in 2017 in Lexington and 2018 in Princeton, and weed biomass at corn harvest in Lexington 2016) Friedman's Rank test was used. Post hoc analysis of nonparametric data was done by using Tukey's HSD on the mean ranks (Pereira et al. 2015). All data that met the assumptions were subject to ANOVA using PROC GLIMMIX in SAS v 9.4 (SAS Institute 2012). When ANOVA indicated significant treatment differences ($P < 0.05$), means were compared using Tukey's HSD. Years were analyzed separately due to differences in management. Treatment was considered fixed and block was considered random.

3.3. Results

3.3.1. Lexington

3.3.1.1. Corn: In both years there were no differences in corn yield due to the different cover crop treatments ($P > 0.05$). Yield was also similar following the no cover control treatment and all interseeded cover crops. Corn yield averaged 181 and 192 bu acre^{-1} in 2016 and 2017, respectively. Other indicators of competition were measured in 2016 (i.e. early season corn height and stage) and no differences were detected (data not shown).

3.3.1.2. Cover crop:

2016: The effect of treatment on initial interseeded cover crop density 2 weeks after interseeding (WAI) was significant ($P < 0.01$; Table 3.3). The initial density of timothy was the lowest followed by festulolium and KYFA 1304 (Figure 3.1A). Meadow fescue and KYFA 0601 had the highest initial density in 2016. By early August the density of all entries had declined, though differences between entries were detected ($P < 0.01$; Table 3.3). KYFA 0601 density declined 56% and had the highest August density (Figure 3.1A). On the other hand, timothy declined 96% and had only 1 remaining plant m^{-2} in early August. Density continued to decline and by corn harvest, while there were still treatment differences ($P < 0.01$), most treatments did not have many remaining cover crop plants (Table 3.4). KYFA 0601 had the most surviving cover crop plants at corn harvest with 11.5 plants m^{-2}. All other treatments were not significantly different from zero. The following spring, density did differ based on treatment ($P < 0.001$), however all treatments had very low density (Table 3.4). KYFA 0601 continued to have to highest density but only had an average of 1 plant m^{-2} remaining. Spring cover crop biomass was not influenced by treatment ($P > 0.05$) which would be expected with the low densities that were observed. Average biomass was < 1 g m^{-2} in the spring (data not shown).

2017: Initial density 2 WAI in 2017 was also affected by interseeded cover crop treatment ($P < 0.05$; Table 3.3). The range in initial densities in 2017 was not as large as in 2016 (Figure 3.1B). Similar to 2016, meadow fescue had the highest initial density with 173 plants m^{-2} though, unlike 2016, KYFA 0601 had the lowest with 94 plants m^{-2} (Figure 3.1B). Early August density did not differ between treatments ($P > 0.05$; Figure 3.1B). All treatments saw a decline in density over the summer but not as extreme as in

2016. KYFA 1304 density declined 69% from 2 WAI to early August and North African tall fescue had only a 39% decrease from 2 WAI to early August. Even though irrigation was used, density of all cover crops continued to decline (data not shown). Cover crop biomass at corn harvest was not influenced by treatment (P > 0.05). On average there was 2.7 g m^{-2} at corn harvest. The following spring prior to termination there were differences due to treatment (P < 0.05; Table 3.3). Timothy had the most biomass and KYFA 0601 and festulolium had the least (Table 3.6).

3.3.1.3. Weeds: Weed density and biomass at corn harvest in 2016 did not differ due to interseeded cover crop treatment (P > 0.05). On average there were 12 weeds m^{-2} weighing 2 g m^{-2} at corn harvest in 2016. In 2017, weed density was not measured but weed biomass at corn harvest did not differ between cover crop treatments (P > 0.05). The average weed biomass was 65 g m^{-2} at corn harvest in 2017.

3.3.2. Princeton

3.3.2.1. Corn: In both years there were no differences in corn yield due to the different cover crop treatments (P > 0.05). In Princeton, there was not a no cover control treatment so it cannot be determined if having interseeded cover crops decreased corn yields compared to having no cover. Yield averaged 194 and 163 bu acre^{-1} in 2016 and 2017, respectively.

3.3.2.2. Cover crop:

2016: Initial cover crop density 2 WAI was not affected by cover crop treatment (P > 0.05; Table 3.3). Densities ranged from 280-343 plants m^{-2} (Figure 3.2A). By early August, density of all entries had decreased by about 70% and treatments were significantly different (P < 0.01; Figure 3.2A). KYFA 1304 had the most surviving cover

crops with 107 m^{-2} and timothy had the fewest with only 3 plants m^{-2} (Figure 3.2A). The stand continued to decline through corn harvest but treatments were still significantly different (P < 0.01; Table 3.3). KYFA 0601 had the highest density at corn harvest and festulolium and timothy had no surviving plants (Table 3.5). The following spring prior to termination, density and biomass differed among entries (P < 0.05; Table 3.3). KYFA 1304 had the highest density and biomass and timothy had the lowest (Table 3.5).

2017: The initial density 2 WAI was affected by cover crop treatment (P < 0.05; Table 3.3). North African tall fescue had the highest initial density and timothy had the lowest (Figure 3.2B). All treatments had declined 67%-91% by early August. Density was affected by treatment in early August (P < 0.05; Table 3.3). North African tall fescue continued to have the highest density and timothy the lowest (Figure 3.2B). Though irrigation was used, the density in all treatments had declined by 56-91% before the irrigation was installed. Cover crop biomass at corn harvest differed between treatments (P < 0.05; Table 3.3). KYFA 0601 had the most biomass and had more than festulolium and timothy (Table 3.5). The following spring, cover crop biomass also differed between cover crop treatments, but the conservative means comparison test used (Tukey's HSD) did not detect significant differences between treatments (Table 3.3). The average cover crop biomass was 7 g m^{-2} in the spring prior to termination including timothy and festulolium which had no remaining plants.

3.3.2.3. Weeds: Weed density and biomass were not influenced by cover crop treatment at corn harvest in 2016 (P > 0.05). On average there were 51 weeds m^{-2} weighing 33 g m^{-2}. In 2017, weed density was not measured due to high grass weed

pressure but weed biomass did not differ due to interseeded cover crop treatment (P >

0.05). The average weed biomass at corn harvest in 2017 was 182 g m^{-2}.

3.4. Discussion

In this study, corn yield was not reduced when cover crops were interseeded at

V5-V7. Similar results have been found in other studies that interseeded grass species

such as cereal rye, winter wheat, oat (*Avena sativa* L.), annual ryegrass (*Lolium*

multiflorum L.), and perennial ryegrass (*Lolium perenne* L.) (Belfry and Van Eerd 2016;

Uchino et al. 2012; Baributsa et al. 2008; Scott et al. 1987). In these studies, different

species were used and establishment and survival were better than in this study. If the

species used in this study are not more competitive than the species used in the other

studies, there would be little risk to corn yield even in years with good summer survival

of the interseeded cover crop. However, since there was not adequate summer survival of

the interseeded cover in this study it should be repeated to ensure there will be no

reduction in corn yields.

The survival of interseeded cover crops was inconsistent across the years and

locations. While all site-years saw a decline in interseeded cover crop stand through the

summer, the magnitude of the decline differed among site-years. In Lexington, the

decline in 2016 was sharper than 2017 whereas in Princeton the decline was more

extreme in 2017. In both locations a shorter corn hybrid was used in 2017. This may have

allowed more light to infiltrate down to the cover crops. If light was limiting their

growth, this may have allowed them to survive the summer better. Other studies have

also reported early senescence of interseeded cover crops and attribute it to lack of solar

infiltration (Belfry and Van Eerd 2016). In Lexington, the average temperature between

June-August in 2016 was 1.4C higher than the 30-year average and in 2017 it was 0.5C lower than the 30-year average (data not shown). These slight differences in average temperature are probably not enough to impact the cover crop. The highest maximum air temperature from June-August did not differ greatly between the two years; the maximum air temperature was 35C in 2016 and 34.4C in 2017. While temperatures at the soil surface will differ from the recorded air temperature, the relative difference will be similar suggesting there was not a large difference in temperatures in the two years. Insufficient rainfall was probably not an issue. In both years, there was about two cm more precipitation between June-August than the 30-year average and in 2017 drip irrigation was used ensuring adequate moisture was available for the cover crops. The main difference in Lexington between the two years was the shorter corn hybrid. More light infiltration may have helped the interseeded cover crops survive in Lexington in 2017 better than in 2016.

In Princeton, the temperature trend was similar to Lexington in that the 2016 June-August average temperature was higher than the 30 year average and 2017 was lower. The highest maximum air temperature in Princeton was 35.5C in 2016 compared to 34.4C in 2017. The subtle temperature differences may not cause enough stress to result in the differences in survival of the interseeded cover crop observed. However the average rainfall in 2016 in Princeton was almost double the 30 year average and in 2017 it was 26% less than the 30 year average. While irrigation was used in 2017 in Princeton, the density had declined sharply before the irrigation was installed. Inadequate moisture in Princeton in 2017 may have contributed to the cover crop decline.

In general, the performance of the interseeded cover crops can be summarized this way: two tall fescue pre-cultivars > meadow fescue > festulolium > North African tall fescue > timothy. The two tall fescue entries usually had some of the highest initial densities and early August densities. They were able to survive the summer better than most of the other entries. Tall fescues are well adapted to hot, dry, and moderately shaded conditions (Munshaw 2015). Tall fescue under heat stress for 28 days had an increase in stomatal conductance, electrolyte leakage, and dark respiration rate but other factors such as relative water content and rate of photosynthesis were not affected compared to plants grown under optimal heat conditions; drought stress posed a bigger challenge for these plants than temperature stress (Yu et al. 2012). Tall fescue has better heat and drought tolerance than perennial ryegrass (*Lolium perenne* L.) (Jiang and Huang 2001). The authors suggest the long roots of tall fescue compared to perennial ryegrass are part of why it grew better in these conditions. Both studies however report the combination of the two stressors are more problematic than either one alone (Yu et al. 2012; Jiang and Huang 2001). While tall fescue is known to have long roots, which help it in drought conditions, one study found root biomass was reduced when tall fescue was grown in shaded conditions (Wherley et al. 2005). Once the corn canopy closes interseeded tall fescue will be growing in partially shaded conditions. The results from this study however, would suggest that even if root growth is limited by shade, tall fescue was still better suited to summer conditions than the other cool season grasses studied.

Despite good summer survival, KYFA 0601, one of the tall fescue pre-cultivars examined, had among the lowest spring biomass prior to termination in Lexington 2018. This may not be due to tall fescue doing poorly, given that it still had more biomass than

other site-years, but the increased summer survival of the other entries. It may also be due to the lower initial establishment of tall fescue in Lexington 2017 compared to Lexington 2016. In 2016 there was 3 cm more rainfall in the two weeks prior to interseeding as well as higher average temperatures than in 2017. These cooler and drier conditions in 2017 may have contributed to the lower initial establishment. While average temperatures 14 days after interseeding were similar between the years, the higher temperatures before interseeding in 2016 may have warmed the soil. It has been shown that tall fescue germination rate is linearly correlated with temperature from 5-27.5C (Sharifiamina et al. 2016). The warmer temperatures in 2016 could be related with faster germination than in 2017. Since tall fescue did not have high initial establishment, the spring density and therefore biomass was lower than other entries that had higher establishment.

Meadow fescue also had good initial establishment and summer survival compared to the other entries. Meadow fescue has been reported to have more root biomass than either festulolium, tall fescue, or perennial ryegrass (Barnes et al. 2014). Increased root biomass would help meadow fescue reach water in drought conditions. Another study reported that meadow fescue biomass was reduced 20-30% when grown under drought conditions (40% of field capacity) compared to well watered conditions (70% field capacity) which was the second smallest of the species the studied (Staniak 2016). Meadow fescue may be able to tolerate drought conditions longer before stands or biomass decline. The same study reported meadow fescue biomass yielded 91% of the well watered control after 10 days without water (Staniak 2016). In this study the ability of meadow fescue to maintain biomass production under drought conditions may have allowed it to survive the summer better than other entries.

Festulolium in general had intermediate initial establishment compared to the other entries. It also did comparatively well surviving through early August (Figure 3.1 and 3.2). This may be due to festulolium's drought tolerance which comes from the fescue part of the cross. One study found about a 14% decrease in one festulolium genotype that had been grown in drought conditions for 14 weeks. They also report that after re-watering the plants for two weeks that same genotype had the same regrowth as the control that had been growing in rainfed conditions (Perlikowski et al. 2014). Another study found the drought tolerance of festulolium was the same as meadow fescue and greater than tall fescue but tall fescue had higher yields when grown in optimal water conditions (Fariaszewska et al. 2017). In this study though, by corn harvest in both years in Princeton, festulolium had low density and biomass compared to the other entries. The spring measurements were inconsistent for festulolium. In Lexington 2018, when the other entries did well, festulolium had comparatively low biomass. Conversely in Princeton 2017, festulolium had one of the highest spring biomass amounts. It is unclear why there was high biomass in some years and low biomass in others.

North African tall fescue had inconsistent results as well. In Princeton in both years, North African tall fescue had good establishment, summer survival, and biomass production compared to the other entries. However in Lexington 2017, there was low initial establishment. Besides low initial establishment, another reason North African tall fescue might have had low survival and biomass is poor root growth. One study found that North African tall fescue grown in 12-60% of full sunlight, had less root biomass and more leaf biomass than plants grown in full sun (Robson and Jewiss 1968b). Shorter roots may prevent North African tall fescue from accessing water deeper in the soil. The

results from this study suggest that North African tall fescue needs to have good establishment and adequate water in order to survive the summer.

Timothy in general did the worst compared to the other entries. It consistently had lower initial and early August densities but in one year, it had produced the most biomass by the following spring. Shade conditions were probably not a limiting factor as timothy has been shown to produce similar amounts of biomass whether grown in full sunlight or 50% shade (Lin et al. 1999). Moisture however may be responsible for the lower summer survival. While timothy has been shown to tolerate drought conditions as well as some other species, its recovery after drought was worse (Okamoto et al. 2011). In Lexington 2018, timothy had the most spring biomass despite having low initial density. It has been reported that the LT_{50}, the temperature at which 50% of the plants survive, of timothy grown in field conditions is around -20C (Andrews and Gudleifsson 1983). North African tall fescue and tall fescue had lower LT_{50} values, -16 and -13C, respectively. These plants were grown in a greenhouse and only exposed to the temperatures treatments for 6 hours so these values may not translate to field conditions where daily fluxuations may cause repeated stress (Robson and Jewiss 1968a). The lowest minimum temperature during the winter in 2018 was -19C. Since timothy has more cold tolerance than some of the other species it is possible this allowed it to better survive the winter and have more biomass the following spring.

All of these entries were chosen because they had one or more attributes that were thought to help them survive contrasting environmental conditions during the summer and winter, while creating biomass. The results from this study suggest that no one attribute alone is enough. The most successful entries, the two tall fescue pre-cultivars,

had multiple qualities that helped them to be more successful than the others. However even having many attributes did not ensure success in every site-year. More research is needed to determine why some entries did better in certain site-years and if those conditions can be replicated to ensure success every year.

Even when the cover crops survived the summer, there was no additional weed suppression compared to lower surviving species or the control. Several studies have found reductions in weed biomass (Uchino et al. 2009; Mohammadi 2010; Fakhari et al. 2015; Uchino et al. 2012) as well as weed density (Uchino et al. 2015) when cover crops were interseeded. One study found a negative correlation between hairy vetch (*Vicia villosa* Roth.) dry weight and weed dry weight (Mohammadi 2010). In this study weed suppression may have been minimal due to low cover crop biomass. However weed biomass was not measured in spring 2018 when cover crop biomass was the highest seen during this study. But given that at corn harvest the previous year there were no differences in weed biomass between the no cover and the interseeded treatments, any additional weed suppression that occurred through the spring would also be provided by post-harvest planted cover crops.

Table 3.1. Dates of major field events and data collection in species selection experiment.

Event	Lexington		Princeton	
	2016-17	2017-18	2016-17	2017-18
Initial burndown	22 March	29 March	29 March	20 March
Fertilized	29 April	25 April	10 April	12 April
Corn planted	24 May	3 May	18 April	14 April
Interseeded	26 June 27 June	12 June	24 May	23 May
Weed management	9 June	12 June	24 May	10 May 23 May
Insect management	N/A	N/A	12 July	N/A
Irrigated (cm applied)	N/A	24 July (1.8)	N/A	25 July (2.2) 26 July (2.4) 11 August (1.3) 24 August (1.3)
Corn harvested	4 October	6 October	22 September	20 September
Data collected				
Cover crop density	11 July 1 August 15 August 4 October 25 November 20 April	26 June 13 July 24 July 8 August*	6 June 9 August 21 September 26 October 12 April	7 June 21 June 12 July 25 July 10 August*
Cover crop biomass	20 April 2017	21 September 22 April 2018	21 September 12 April 2017	15 September 12 April 2018
Weed density	4 October	N/A	21 September 2 March 2017	N/A
Weed biomass	4 October	21 September	21 September	15 September

* Cover crop density was not collected in spring 2018 in Lexington due to tillering of plants which precluded an accurate measurement. In Princeton it was not measured due to low density.

Table 3.2. Entries examined in species selection experiment. Some entries (i.e. Festulolium var. 'FLOP') were not examined in the second year of the experiment due to poor performance in the first year, so alternative entries were selected.

Treatment	Lexington 2016-17	Lexington 2017-18	Princeton 2016-17	Princeton 2017-18
Festulolium (*x Festulolium* Asch. & Graebn.) variety 'FLOP'	X		X	
Festulolium variety 'Kenfest'		X		X
Meadow fescue (*Festuca pratensis* Huds.) pre-cultivar KYFP 1301 MF	X	X	X	X
North African tall fescue (*Festuca arundinaceus* Schreb.) variety 598863		X	X	X
Tall fescue pre-cultivar KYFA0601	X	X	X	X
Tall fescue pre-cultivar KYFA1304	X	X	X	X
Timothy (*Phleum pretense* L.) variety 'Clair'	X		X	
Timothy pre-cultivar KYPP0901		X		X

Table 3.3. Effect of interseeded cover crop treatment on cover crop density (2 weeks after interseeding (WAI) and in early August) and cover crop biomass the following spring in Lexington and Princeton, KY, in 2016-17 and 2017-18. Effect of treatment on density at corn harvest and the following spring is also shown for 2016-17; effect of treatment on cover crop biomass at corn harvest is shown for 2017-18. NS= not significant; *= significant at the 0.05 level; **= significant at the 0.01 level; ***= significant at the 0.001 level.

	Lexington		Princeton	
	2016-17	2017-18	2016-17	2017-18
Initial density [a]	**[e]	*[e]	NS	*
August density [b]	**[e]	NS[e]	**	*
Density at harvest [c]	**	NA	**	NA
Biomass at harvest [c]	NA	NS	NA	*
Density the following spring [d]	***	NA	*	NA
Biomass the following spring [d]	NS	*[e]	*	*

[a] 2 weeks after interseeding (WAI)
[b] 5 WAI in Lexington 2016, 8 WAI in Lexington 2017, and 11 WAI in Princeton
[c] Harvest dates are given in Table 3.1; harvest was 14 WAI in Lexington 2016, 16.5 WAI in Lexington 2017, and 17 WAI in Princeton
[d] Refer to Table 3.1 for sampling dates
[e] The no cover control was not included in the analysis.

Table 3.4. Interseeded cover crop density at corn harvest in 2016 and cover crop density prior to termination in 2017 in Lexington, KY.

	2016-2017	
Treatment	Density at corn harvest*	Density at termination*
	# m^{-2}	# m^{-2}
KYFA 0601	11.5^a	1.0^a
KYFA 1304	5^{ab}	0.4^a
Festulolium	2.5^{ab}	0.5^a
Meadow Fescue	0.5^{ab}	0.2^{ab}
Timothy	0^b	0^b
No cover	0^b	0^b
SEM (±)	1.2	0.1

* Within a column, treatment means followed by different letters are significantly different at alpha= 0.05.

Table 3.5. Interseeded cover crop density at corn harvest in 2016, density and biomass in the spring prior to termination in 2017, and interseeded cover crop biomass at corn harvest in 2017 in Princeton, KY.

| Treatment | 2016-2017 | | | 2017-2018 |
	Density at corn harvest*	Density at termination*	Biomass at termination*	Biomass at corn harvest *
	# m^{-2}	# m^{-2}	g m^{-2}	g m^{-2}
KYFA 0601	39^a	6.5^{ab}	21.3^{ab}	1.6^a
KYFA 1304	23.5^{ab}	7.1^a	26.9^{ab}	1.2^{ab}
Meadow Fescue	17.5^{ab}	5.4^{ab}	17.8^{ab}	0.3^{ab}
N. Afr. Tall Fescue	12^{ab}	4.5^{ab}	17.7^{ab}	1.0^{ab}
Festulolium	0^b	3.9^{ab}	29.0^a	0.2^b
Timothy	0^b	0.32^b	1.9^b	0.3^b
SEM (±)	4.5	0.69	2.9	0.16

* Within a column, treatment means followed by different letters are significantly different at alpha= 0.05.

Table 3.6. Interseeded cover crop biomass prior to spring termination in 2018 in Lexington, KY.

Treatment	Biomass at termination*
	g m^{-2}
Timothy	319^a
Meadow Fescue	231^{ab}
N. Afr. Tall Fescue	188^{ab}
KYFA 1304	184^{ab}
KYFA 0601	161^b
Festulolium	149^b
SEM (±)	17

*Treatment means followed by different letters are significantly different at alpha= 0.05.

Figure 3.1. Average cover crop density in (a) 2016 and (b) 2017 two weeks after interseeding (WAI) and in early August (5 WAI in 2016 and 8 WAI in 2017) in Lexington, KY. Within each date, bars with different letters are significantly different at alpha= 0.05. Error bars indicate standard error of the mean for each treatment. North African tall fescue was not included in 2016.
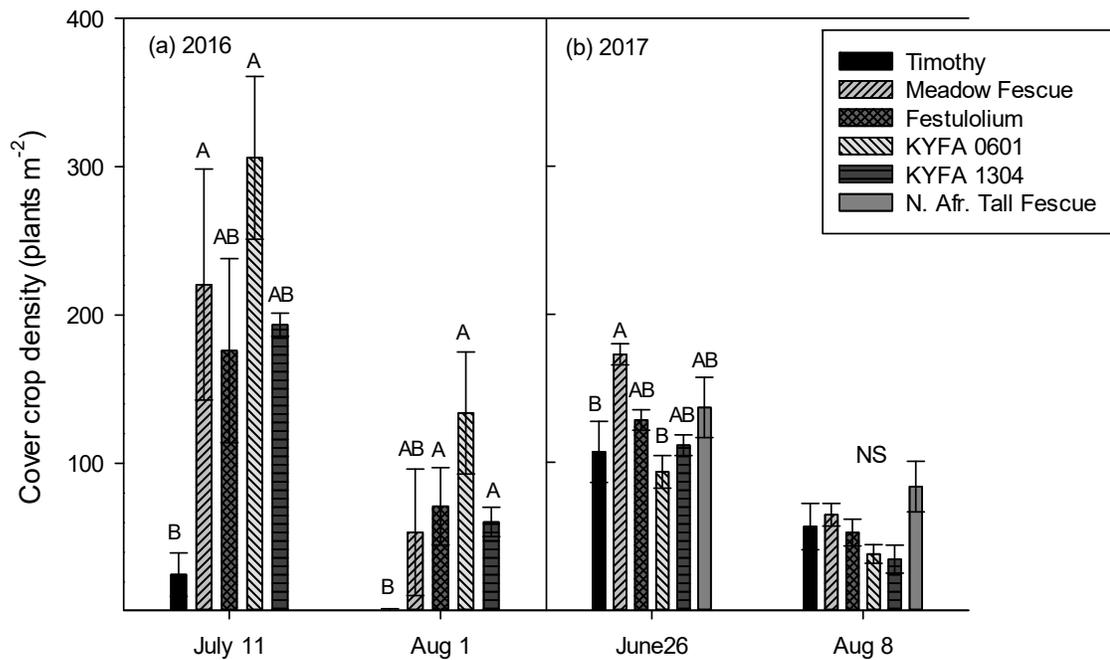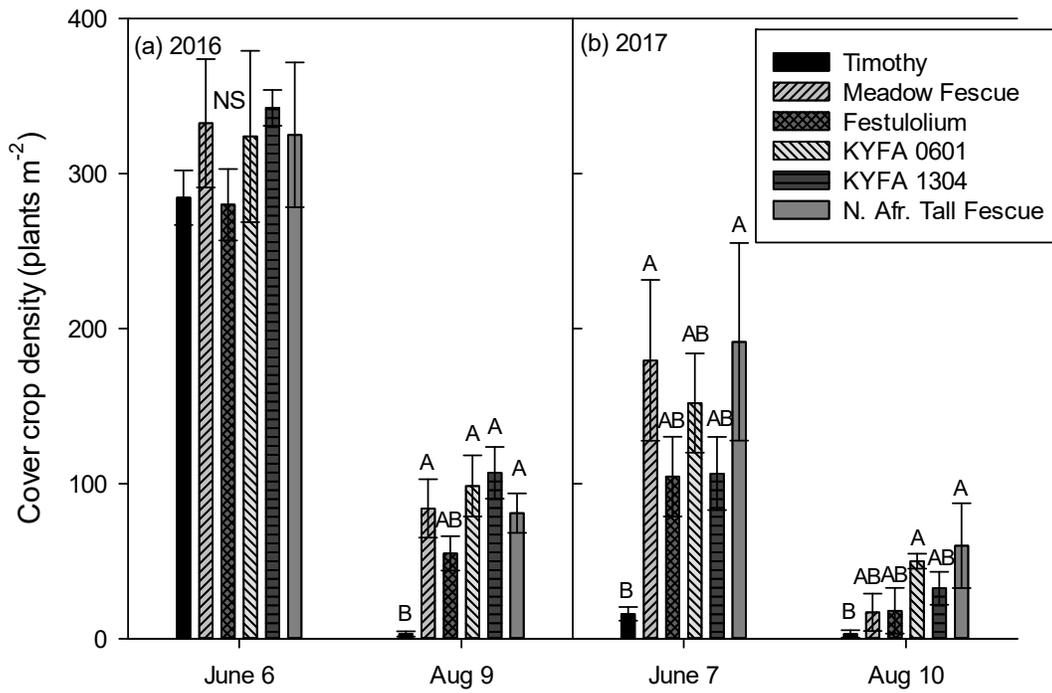
Figure 3.2. Average cover crop density in (a) 2016 and (b) 2017 two weeks after interseeding (WAI) and in early August (11 WAI) in Princeton, KY. Within each date, bars with different letters are significantly different at alpha= 0.05. Error bars indicate standard error of the mean for each treatment.

Chapter 4: Interseeded cover crops vs post-harvest planted cover crops

4.1. Introduction

A traditional cover crop is planted after harvesting the main crop. This leaves the soil bare and susceptible to weed emergence until the cover crop establishes and the biomass covers the ground. An alternative is to interseed the cover crop into the main crop while it is big enough to handle competition but before the canopy closes. In corn (*Zea mays* L.), this would correspond to the critical weed free period, ending at V5-V7. This would allow the cover crops to establish before the corn canopy closes and start producing biomass as the corn senesces. Interseeding can be done in several ways such as aerially broadcast or using a drill. Interseeding timing varies too from planting the cover crops with the main crop to planting just before senescence. A balance between planting when light will reach the ground so the seeds can germinate and minimizing competition needs to be found. Given that the interseeded cover crops will be alive under the main crop in low light conditions, they need to be shade tolerant. They should also be able to survive in the hot, dry summer conditions found in Kentucky while still winter hardy so they can survive during the summer and rapidly produce biomass after corn harvest.

Some studies have shown how interseeding can allow cover crops to produce more biomass and ground coverage compared to cover crops planted after cash crop harvest in the fall (Hively and Cox 2001; Scott et al. 1987). In one study interseeded cover crops including annual ryegrass (*Lolium multiflorum* L.), creeping red fescue (*Festuca rubra* L.), and several legume species had more ground cover and biomass than the post-harvest planted cereal rye (*Secale cereale* L.) in the following spring (Hively and Cox 2001). During one year of that study there was more precipitation than average and

cold temperatures during the fall which hindered the cereal rye seeded post-harvest from establishing and highlighted the benefits of interseeding (Hively and Cox 2001). Another study found that ground cover was generally higher when cover crops were interseeded when the corn was 0.15- 0.30 m compared to cover crops seeded after cash crop harvest (Scott et al. 1987). Other studies have documented the relationship between cover crop biomass and weed biomass (Mohammadi 2010). That study reports a negative correlation between cover crop dry weight and weed dry weight (Mohammadi 2010). If interseeded cover crops will have more biomass than post-harvest planted cover crops, we would expect them to suppress more weeds.

One challenge with interseeding cover crops in a conventional corn-soybean (*Glycine max* (L.) Merr.) rotation is the use of herbicides. It is common to apply an herbicide with soil residual activity before or at planting of the main crop. This means herbicides will be in the soil when the cover crops are interseeded and may reduce the establishment or growth of the cover crops. Some studies have found reductions in stand and biomass when certain herbicides were applied prior to planting (Tharp and Kells 2000; Wallace et al. 2017; Cornelius and Bradley 2017a). Wallace et al. (2017) found reductions in interseeded annual ryegrass fall biomass when using s-metolachlor + mesotrione + atrazine compared to an untreated control. Italian ryegrass (*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot), a close relative of annual ryegrass, stand and biomass 28 days after planting was reduced compared to an untreated control when glyphosate + s-metolachlor + mesotrione + atrazine was applied to the previous corn crop (Cornelius and Bradley 2017a). These studies suggest that there may be risk to interseeded cover crops establishment when s-metolachlor + mesotrione + atrazine is applied; this

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combination of active ingredients is commonly applied in corn production. The use of post-emergent herbicides is also limited where interseeded cover crops are used. Once the interseeded cover crops have emerged, herbicide applications that would kill the cover crops cannot be made. This means that if a field is weedier than desired, herbicides cannot be used to regain control.

Another consideration is competition between the interseeded cover crop and the corn. Since the interseeded cover crop will be establishing and growing while the corn is growing there is the possibility they will compete for nutrients and water. Some studies have found reductions in the yields of the main crop when both grass and legume species were interseeded compared to no cover controls (Abdin et al. 1998; Fakhari et al. 2015; Scott et al. 1987). In this study the corn will be monitored for potential signs of stress (i.e. shorter or less green in color) as well as monitoring soil moisture to see if there could be competition for water between the corn and interseeded cover crop.

The objectives of this study were to determine (i) if interseeded annual ryegrass and interseeded orchardgrass (*Dactylis glomerata* L.) would suppress weeds similar to post-harvest planted cereal rye and (ii) how a commonly used pre-emergent herbicide (containing s-metolachlor, mesotrione, and atrazine as active ingredients) would impact interseeded cover crop establishment and survival. We expected interseeded orchardgrass and annual ryegrass to suppress weeds once the corn starts senescing and through the winter better than post-harvest planted cover crops given that they will already be established by corn harvest. We also expected a full rate of this herbicide to reduce interseeded cover crop density compared to a lower or zero rate.

4.2. Materials and methods

4.2.1. Plot establishment. This experiment was conducted at the University of Kentucky Research and Education Center in Princeton, Kentucky (37°5′52.5″ N, 87°51′39.9″ W). The soil at this location was Crider silt loam (Fine-silty, mixed, active, mesic Typic Paleudalfs). Before establishing this experiment in 2016, the field was cropped to soybeans with fallow over the fall and winter. The field used in 2017 had been cropped to wheat (*Triticum aestivum* L.). The field was not tilled for the duration of the experiment. Cover crop species and herbicide rates were assigned to plots as a split plot randomized complete block in both years with cover crop species as the main plot factor and herbicide rate as the subplot factor. The main plots were 4.6 meters wide and 27.4 meters long. The subplots were 4.6 meters wide and 9.1 meters long. The cover crop treatments were no cover, interseeded orchardgrass (variety 'Benchmark Plus'), interseeded annual ryegrass (variety 'Marshall'), and post-harvest planted cereal rye (variety 'Aroostook'). A soil residual herbicide (trade name Lexar®, Syngenta) containing three active ingredients (s-metolachlor: mesotrione: atrazine) was applied at three different rates: 0, 3.5, and 5.8 L ha^{-1} (corresponding to 0:0:0, 0.73:0.09:0.73, 1.21:0.16:1.21 kg ha^{-1} of s-metolachlor: mesotrione: atrazine, respectively) at corn planting.

Dates of major field events are shown in Table 4.1. An initial burndown herbicide application to control winter annual weeds was done on 29 March 2016 with 0.84 kg ae ha^{-1} of glyphosate and 20 March 2017 with 0.84 kg ae ha^{-1} of glyphosate plus 0.56 kg ai ha^{-1} of dicamba. Lime was applied on 13 April 2016; no additional lime was needed on

the 2017 field. While the wheat was growing previously, 101 kg ha^{-1} of nitrogen (as UAN) was applied. Fertility was applied based on soil sampling and the University of Kentucky's recommendations, 441.5 kg ha^{-1} of 33-0-0-12 and 262.4 kg ha^{-1} diammonium phosphate (18-46-0) were broadcast on 10 April 2016 and on 12 April 2017 112 kg ha^{-1} of nitrogen (as urea) was applied but no additional nutrients were needed.

4.2.2. Corn planting and herbicide treatment application. Each plot contained six corn rows spaced 76 cm apart; Stine 'R9740VT3pro' was planted at a rate of approximately 74,000 seeds ha^{-1} using a six row no-till planter (MaxEmerge XP 1780, John Deere) on 19 April 2016 and Stine '9714G' was seeded at a rate of approximately 70,400 seeds ha^{-1} on 14 April 2017 using the same planter. The soil residual herbicide treatments were applied at corn planting with a backpack sprayer calibrated to apply 140 L ha^{-1} using DG8004 nozzles (TeeJet Technologies).

4.2.3. Cover crop planting. The orchardgrass and annual ryegrass were interseeded at 28 kg ha^{-1} using a two-row interseeder unit (Interseeder Technologies) on 23 May 2016 and 23 May 2017. The corn was at V5 when interseeding occurred. This unit is a high clearance drill that plants three cover crop rows 19 cm apart between the corn rows. Two passes were made per plot to insure three rows of cover crop were seeded in the data collection rows. After corn harvest the cereal rye was planted at 118 kg ha^{-1} with a no till drill (Lilliston).

4.2.4. Weed and pest management. On 10 May 2017 glyphosate (1.26 kg ae ha^{-1}) plus 0.05 kg ai ha^{-1} of halosulfuron plus 0.38% nonionic surfactant v/v was applied to the corn to control emerged weeds including yellow nutsedge (*Cyperus esculentus* L.). Immediately following interseeding in 2016, glyphosate (1.26 kg ae ha^{-1}) was applied in

75

140 L ha^{-1} with 0.55% AMS v/v using a high clearance sprayer. In 2017, glyphosate (0.84 kg ae ha^{-1}) was applied immediately after interseeding with the same equipment. Pests were scouted for throughout the season and on 12 July 2016 Lambda-cyhalothrin was applied at a rate of 0.05 kg ai ha^{-1} to control Japanese beetles. No additional pest control was warranted in 2017.

4.2.5. Irrigation, soil moisture and temperature, etc. monitoring. In 2017, drip irrigation was used in half of each main plot. A total of 7.26 cm was applied during the growing season. Dates and amounts are in Table 4.1. In 2017, two dataloggers (Onset, HOBO UA-002-08) were placed in one subplot per main plot to record temperature and light intensity 2.54 cm above the soil surface.

4.2.6. Data collection.

4.2.6.1. Cover crop. Cover crop density was measured periodically each year and dates are shown in Table 4.2. The number of cover crop plants was counted within two 0.25m^2 quadrats per subplot with two rows of the cover crop included in each quadrat. Cover crop density was measured again in the spring before cover crop termination. Due to low density at termination in 2017, density was measured between two rows of corn stubble (area 76 cm wide) and a length of 3 m in each subplot. All cover crop density counts were standardized to number per m^2 prior to analysis. Interseeded cover crop biomass was collected from two 0.25m^2 quadrats per subplot at corn harvest in both years. In fall 2017, biomass was collected only from the subplots that had orchardgrass due to low survival of annual ryegrass. Biomass was collected again before cover crop termination. In spring 2017, biomass was collected between two corn rows (area 76 cm wide) and 3 m long in each subplot. In spring 2018, cover crop biomass was collected

from two 0.25m^2 quadrats per subplot in the orchardgrass and cereal rye treatments. Annual ryegrass had few surviving plants so cover crop biomass was not collected in those plots. All biomass was dried at 60C until a consistent weight was achieved and then weighed to obtain dry weight.

4.2.6.2. Weeds. Weeds were identified to species from 0.25m^2 areas per subplot on the dates given in Table 4.2. At all times except for spring 2017, density was measured from two quadrats per subplot; in spring 2017 only one quadrat per subplot was measured. In spring 2018, weed density was not measured in the annual ryegrass plots due to minimal survival of the annual ryegrass and general lack of weeds in the plots. Weed biomass was collected at corn harvest from two 0.25m^2 quadrats per subplot in 2016 and again at cover crop termination from one quadrat per subplot in spring 2017. In fall 2017, weed biomass was only collected from one quadrat per subplot in the plots that had orchardgrass. Due to low survival of annual ryegrass, weed biomass was not collected from those plots. In spring 2018, weed biomass was not measured due to low weed density. All biomass was dried at 60C until a consistent weight was achieved and then weighed to obtain dry weight.

4.2.6.3. Soil Moisture. Soil moisture was obtained by taking soil cores in the corn row and between corn rows from each main plot every other week starting two weeks after interseeding. Samples were collected to 20 cm depth on 24 May 2016, 6 June 2016, and 23 May 2017. On the remaining sample dates in both years, cores were separated into 0-10 cm and 10-20 cm. Most samples consisted of eight soil cores but in extreme dry conditions fewer samples were taken. Wet weight of the soil was determined and then the

soil was dried at 60C until consistent weight was achieved and then weighted to obtain

dry weight. Percent gravimetric soil moisture was calculated as follows:

% gravimetric soil moisture = (wet soil weight – dry soil weight) / dry soil weight * 100

4.2.6.4. Corn. Corn height was measured in each subplot every two weeks

starting two weeks after interseeding until one month after the corn reached R1. Height

was measured from the ground to the top of the tallest newly emerged leaf. Once

tasseling commenced, height was measured from the ground to the base of the tassel.

Developmental stage was measured in each subplot by counting the number of fully

exposed collars and was assessed every two weeks starting two weeks after interseeding

until tassels could be felt. The chlorophyll content was measured from seven plants per

subplot using a SPAD meter (Minolta) in 2016 only. In 2016, the inner four corn rows of

each subplot were harvested with a plot combine. In 2017, two rows were harvested from

the irrigated and unirrigated sections of each subplot. In both years, yield was corrected

to 15.5% moisture prior to analysis.

4.2.7. Data analysis. Prior to analysis all data were checked for normality and

homogeneous variances. When assumptions were not met, either a log or square root

transformation was used. Once assumptions were met, data were subject to ANOVA

using PROC GLIMMIX in SAS v 9.4 (SAS Institute 2012). When ANOVA indicated

significant treatment differences ($P < 0.05$), means were compared using Tukey's HSD.

Years were analyzed separately due to differences in management (i.e. irrigation) and

significant year * treatment interactions. Cover crop treatment and herbicide rate were

considered as a fixed factor, while block was considered a random factor. For 2017 data

irrigation was considered as a fixed factor. Repeated measures analysis was conducted with time as a fixed factor for cover crop density over the summer in both years.

4.3. Results

4.3.1. Cover crop:

2016: Initial interseeded cover crop density, measured 2 weeks after interseeding (WAI), did not differ based on cover crop treatment or herbicide treatment ($P > 0.05$; Figure 4.1). While initial densities were about 450 and 350 plants m^{-2} for annual ryegrass and orchardgrass respectively, by 11 WAI both species had around 40 plants m^{-2} (Figure 4.1). Densities continued to decline with annual ryegrass declining more than orchardgrass (Figure 4.1, see means separations with lowercase letters). By corn harvest in 2016, there was about 13 annual ryegrass plants m^{-2} and 28 orchardgrass plants m^{-2}. The following spring prior to termination that number had further declined to <1 annual ryegrass plant and <5 orchardgrass plants m^{-2} which were significantly different (data not shown).

The spring prior to termination, post-harvest planted cereal rye had significantly more biomass than either interseeded treatment ($P < 0.001$; Figure 4.2a). Between the interseeded treatments, the orchardgrass had more biomass than the annual ryegrass which would be expected given the higher density of orchardgrass.

2017: Unlike 2016, the initial density of the interseeded annual ryegrass and interseeded orchardgrass differed ($P < 0.05$) with the orchardgrass having higher density than annual ryegrass (Figure 4.3). From 2 WAI to 9 WAI, the densities of both orchardgrass and annual ryegrass declined significantly (Figure 4.3). The annual ryegrass density declined by about 85% while the orchardgrass declined only 36%. Using the

unirrigated half of each subplot at 11 WAI, orchardgrass significantly declined compared to 9 WAI while annual ryegrass did not significantly decline (Figure 4.3).

Using both the irrigated and unirrigated densities, early August (11 WAI) density was influenced by cover crop treatment, herbicide rate, and irrigation ($P < 0.05$; Table 4.3). The only significant differences within a cover crop species were in the no herbicide subplots in annual ryegrass where the irrigated half had lower density than the unirrigated and within the high herbicide rate subplots in orchardgrass where the irrigated had lower density than the unirrigated (data not shown). Given the high weed pressure across the field, using irrigation may have benefited the weeds more than the interseeded cover crop. This may be why the density is lower within the irrigated halves. It is unclear, however why only these two cover crop-herbicide rate combinations had significant differences due to the irrigation.

Interseeded orchardgrass biomass at corn harvest was not influenced by herbicide rate or irrigation ($P > 0.05$; Table 4.3). By corn harvest there were no surviving annual ryegrass plants therefore those plots could not be sampled from this point forward. Cover crop biomass prior to spring termination was influenced only by cover crop species ($P < 0.05$; Table 4.3). The post-harvest planted cereal rye had significantly more biomass than the interseeded orchardgrass (Figure 4.2b).

4.3.2. Weeds:

2016: Weed density was measured 2 WAI and then again at corn harvest. Weed density did not differ due to cover crop treatment or herbicide rate at either time ($P > 0.05$; Table 4.4). Spring weed density (e.g. common ragweed (*Ambrosia artemisiifolia* L.), giant ragweed (*Ambrosia trifida* L.), marestail (*Conyza canadensis* (L.) Cronquist),

common lambsquarters (*Chenopodium album* L.), morningglory species (*Ipomoea spp.*), and grasses) was influenced by the interaction of cover crop treatment and herbicide rate ($P < 0.05$; Table 4.4). There were differences due to herbicide rate within annual ryegrass and orchardgrass and there were differences due to cover crop species within the low rate of herbicide (Table 4.7 and 4.8). The trends within the interseeded cover crop species were opposite. Weed density in annual ryegrass plots was highest where there was no herbicide applied the previous spring; in the orchardgrass subplots with no herbicide applied the previous spring, weed density was the lowest (Table 4.7). We might expect the high rate of the herbicide to lower the interseeded cover crop establishment which might allow more weeds to emerge over time. We would also expect the stand of the interseeded cover crops to be better following no herbicide the previous year; this more vigorous stand might suppress weeds more. However since there were no differences between the cover crop densities due to herbicide rate these trends are not well explained by differences in cover crop suppression. Within the low rate of the herbicide, the no cover plots had higher weed density than the cereal rye seeded after corn harvest (Table 4.8). By this point in the rotation, we would not expect the pre-emergent herbicide to be impacting the weeds. Therefore these differences are probably due solely to cover crop biomass. Given that the post-harvest seeded cereal rye had the most biomass it would be expected those plots would have fewer weeds.

Cover crop species affected spring weed biomass in 2017 ($P < 0.001$; Table 4.4). The post-harvest planted cereal rye had lower weed biomass than either interseeded treatment or the no cover control (Figure 4.4). The two interseeded treatments did not reduce the weed biomass compared to no cover. This result may be due to low summer

survival of the interseeded cover crop. Since there was little survival, the expected weed suppression benefits were not observed.

2017: In 2017, neither cover crop main plot treatment nor the herbicide subplot treatment influenced the weed density at 2 WAI, at corn harvest, or the following spring ($P > 0.05$; Table 4.4). The field used in this year was heavily dominated by summer annual grasses. Since these grasses are summer annuals, they were able to outgrow the interseeded winter annuals and overtake the field. Due to the seedbank being dominated by summer annual grasses, prior to termination weed density of early emerging summer annuals was minimal with an average of 8 weeds m^{-2}.

4.3.3. Corn: There were no differences in corn yield between interseeded cover crop or herbicide rate ($P > 0.05$) treatments in either year (Table 4.5). In 2017, there was a significant interaction between herbicide rate and irrigation on corn yield. However, Tukey's HSD test did not detect significant differences between the treatments. In 2016, the average corn yield was 174 bu acre^{-1} and in 2017 the average yield was 115 bu acre^{-1}. In 2017, though irrigation was used in half of each subplot, there was no difference in yield between irrigated and unirrigated corn (Table 4.5).

Other indicators of competition between the corn and cover crop were measured. In 2016 early season corn height (2 WAI) was only influenced by the subplot factor, with the no herbicide subplots having shorter corn than the plots with the high rate of herbicide (data not shown). These differences could be due to higher weed density in the no herbicide plots although early season weed density was not affected by this treatment. It could also be related to the cover crop density if the high rate of the herbicide reduced the cover crop density compared to the no herbicide plots. If cover crop density was

reduced by the herbicide then there would be less competition with the weeds possibly resulting in higher weed density. However, cover crop initial stand was also not affected by herbicide rate. In 2017, there were no differences in early season corn height due to any treatment factor. Corn stage was not affected by treatment two weeks after interseeding in 2016. SPAD values did differ based on treatment 4 WAI ($P < 0.05$; Table 4.5). The no cover treatment had higher SPAD values than the corn where annual ryegrass had been interseeded (data not shown). The corn where orchardgrass was interseeded was similar to both the no cover and the annual ryegrass (data not shown). Early season differences had minimized by 6 WAI as there were no differences in SPAD values at that date ($P > 0.05$).

4.3.4 Soil conditions: Gravimetric soil moisture did not differ between any main plot or subplot treatments in 2016 (data not shown). In 2017 there were differences in moisture based on location (data not shown). Seven WAI, soil moisture was approximately 10% higher between the rows of corn than in the rows of corn. After irrigation started, the in-row area had approximately 11% more moisture than between the row. There were no differences in soil moisture due to treatment. The differences based on location in 2017 may be due to the high weed pressure that was observed between the rows of corn. The weeds may have benefited from the added irrigation and therefore reduced soil moisture between the corn rows. Given the high weed pressure in 2017, adding irrigation may have allowed the weeds to further grow between the rows which would allow them to uptake more moisture therefore reducing the soil moisture.

In 2016, the average air temperature from June-August was about 0.5C higher than the 30-year average and in 2017 it was about 1C lower than the 30-year average.

Data loggers used in 2017 show there were on average 51 hours between 8 June and 14 July that temperatures were above 40C and 6 hours above 54C, 2.54 cm above the soil surface in the plots where annual ryegrass was seeded (Table 4.6). The average maximum temperature reported by the dataloggers during this period was 58C (Table 4.6).

4.4. Discussion

Under the conditions observed during these two years, corn yield was not affected by the interseeded cover crops, which led us to conclude that there was minimal competition between the cover crop and corn. This supports the results of several other studies where no difference in the main crop yield was observed when cover crops were interseeded (Baributsa et al. 2008; Belfry and Van Eerd 2016; Scott et al. 1987; Uchino et al. 2012). In 2016 both species had low survival and in 2017 annual ryegrass had little if any survival by corn harvest. This low survival may have diminished any competition between the cover crop and corn. These results may differ in years with substantial cover crop growth and survival, or extremely dry conditions, since there may be increased competition and therefore reduced corn yields.

There were no reductions in interseeded cover crop stand when the herbicide used was applied at low or high rates compared to no herbicide. The few effects of the herbicide and herbicide rate were not consistent across cover crop treatments and were probably influenced by other environmental factors. Initial densities were not reduced by the low or high herbicide rate. However, 2016 was wetter than average and 2017 was warmer than average— in 2016 there was 3.1 cm more precipitation between herbicide application and interseeding than the 10 year average and in 2017 while there was less precipitation than the 10 year average, the temperatures between application and

interseeding were 1.4C higher. Both increased moisture and temperature will speed up the degradation of herbicides (Curran 2001). Thus, while results of this study lead us to conclude that this herbicide can likely be used prior to interseeding annual ryegrass and orchardgrass in western Kentucky, under different environmental conditions, the impact of the herbicide may be more damaging than seen in these two years.

In this study the post-harvest planted cereal rye had more biomass at termination than either interseeded treatment. The post-harvest seeded cereal rye plots also had lower weed biomass in 2016. This supports one study that found a negative correlation between cover crop dry weight and weed dry weight (Mohammadi 2010). It should be noted that while there was good establishment of the post-harvest cover crop in the two years of this study, not all years will be conducive to cover crop establishment after the main crop harvest. That was reported in one study where there was more precipitation than average and cold temperatures which hindered the cereal rye seeded after harvesting the main crop from establishing (Hively and Cox 2001). While the post-harvest seeded cereal rye in this study had good establishment, growth, and therefore suppressed weeds, care should be taken to plant in a timely manner.

The interseeded orchardgrass in general did better than the interseeded annual ryegrass. The initial density of the orchardgrass was higher than the annual ryegrass in one year (Figure 4.3) and in both years the orchardgrass survived the summer better than the annual ryegrass (Figure 4.1 and 4.3). Both species have similar optimal temperatures although the results from this study would suggest orchardgrass is more heat tolerant that annual ryegrass. Orchardgrass top growth has been shown to decrease only 30% as temperatures reach 28C though growth was reduced by 64% as temperatures reached 35C

(Baker and Jung 1968). Annual ryegrass starts showing severe signs of stress as temperatures reach 38C but at temperatures lower than that heat stress was not observed (Richardson 2004). The temperature values from the dataloggers given in Table 4.6 are well above the values where signs of stress were reported for annual ryegrass. Given air temperatures in 2016 were higher than 2017, we would expect the temperatures above the soil surface prior to corn canopy closure to also be higher in 2016. This means there was likely more than 51 hours in 2016 in which the temperature where the cover crops were growing were above 40C and the maximum temperature might have been higher. The higher temperatures may have contributed to the sharp decline in density observed in 2016. Given that the annual ryegrass did not survive the summer in 2017, these temperatures are likely too high for annual ryegrass but orchardgrass appears to have more tolerance to these conditions.

Another reason annual ryegrass did not survive the summer could be due to shading from the corn. While a shorter corn hybrid was used in 2017 than in 2016 and both years saw significant decline in annual ryegrass density. It is possible even with a shorter corn hybrid there was still too little light infiltration. It has been reported that orchardgrass had no significant reduction in dry matter weight when it was grown in 50% compared to full sun (Lin et al. 1999). Annual ryegrass however produced 47% less biomass under 50% shade compared to in full sun (Soares 2016). The increasing shade imposed on the intersedeed cover crops as the corn canopy closes may have contributed to the reduction in annual ryegrass density. However other studies have noted adequate survival of interseeded annual ryegrass in shaded conditions (Wallace et al. 2017; Curran 2018).

Insufficient rainfall may have played a part as well. The average rainfall in 2016 was almost double the 30 year average but in 2017 it was 26% less than the 30 year average. While irrigation was used in 2017 in Princeton, the density of annual ryegrass in particular had declined sharply before the irrigation was installed. One study reported that after 7 days without water, orchardgrass root length increased whereas the roots of perennial ryegrass (*Lolium perenne* L.), a close relative of annual ryegrass, decreased 16% compared to well watered plants (Molyneux and Davies 1983). If orchardgrass roots are still able to grow during water stress conditions we would expect they can reach water further down in the soil and survive longer. The results from this study may suggest orchardgrass has some mechanism to surviving when water is limited whereas annual ryegrass may not.

The interseeded cover crop did not suppress the weeds more than the no cover control. In 2016-2017 this is likely due to the low survival of the cover crop resulting in low cover crop biomass. One study found a negative correlation between hairy vetch (*Vicia villosa* Roth.) dry weight and weed dry weight (Mohammadi 2010). Since we had little cover crop biomass we would not expect much weed suppression. In 2017-18 however there was moderate survival of the orchardgrass but that did not result in fewer weeds. Again, that may be the result of the seedbank in that field being dominated by summer annual grasses which were better able to compete with the interseeded cover crops during the summer. The following spring there were few weeds in the field regardless of if cover crops were present of not.

Overall, the results of this study suggest interseeded cover crops will not result in more weed suppression than cereal rye planted post-harvest. This is largely due to the

lack of summer survival of the interseeded cover crops. If it can be determined why annual ryegrass and orchardgrass do not consistently survive the summer in western Kentucky and those conditions can be mitigated, better results may be possible.

Table 4.1. Dates of major field events.

Event	2016-17	2017-18
Initial burndown	29 March	20 March
Fertilized	10 April	12 April
Corn planted	19 April	14 April
Pre-emergent herbicide application in subplots	19 April	14 April
Orchardgrass and annual ryegrass interseeded	23 May	23 May
Irrigated (cm applied)	N/A	25 July (2.2) 26 July (2.4) 11 August (1.3) 24 August (1.3)
Corn harvested	22 September	20 September
Cereal rye planted	27 September	5 October
Spring cover crop sampling prior to termination	12 April	12 April

Table 4.2. Dates of data collection.

Data collected	2016-17	2017-18
Cover crop density [a]	6 June	6 June
	9 August	21 June
	30 August	12 July
	21 September	25 July
	26 October	10 August
	12 April	
Cover crop biomass	21 September	15 September
	12 April	12 April
Corn height	6 June	7 June
	20 June	21 June
	5 July	12 July
	18 July	25 July
Corn stage	6 June	7 June
	20 June	21 June
SPAD reading	20 June	N/A
	5 July	
	18 July	
Weed density [b]	6 June	6 June
	21 September	12 April
	26 October	
	2 March	
	12 April	
Weed biomass [c]	21 September	15 September
	12 April	
Soil moisture	24 May	23 May
	6 June	7 June
	20 June	21 June
	5 July	12 July
	18 July	25 July
	9 August	10 August

[a] Cover crop density was not measured in spring 2018 due to tillering of the cover crops which precluded an accurate count

[b] Due to low winter annual weed density in 2017-18, density was not measured as frequently.

[c] Due to low winter annual weed biomass in spring 2018, it was not collected.

Table 4.3. The effect of cover crop treatment (CC) and herbicide rate (HR) on cover crop biomass and density prior to termination in Princeton, KY, in 2016 and 2017. Also shown is the effect of time on cover crop density through the summer (2, 11, 14, 17 weeks after interseeding (WAI) in 2016; 2, 9, 11 WAI in 2017). The effect of irrigation (IR) on cover crop density 11 WAI, cover crop biomass at corn harvest and the following spring in 2017-18 is also shown. For summer density in 2017 only unirrigated density measurements were used and density 11 WAI includes both irrigated and unirrigated. NS=not significant; *= significant at the 0.05 level; **= significant at the 0.01 level; ***= significant at the 0.001 level.

| | Summer density | | Density 11 WAI | Harvest Biomass | Spring biomass | | Spring density |
	2016	2017[a]	2017[a]	2017[b]	2016-17	2017-18	2016-17
CC	NS	*	*	N/A	***	*	*
HR	NS	NS	NS	NS	NS	NS	NS
CC X HR	NS	NS	NS	N/A	NS	NS	NS
IR	-	-	**	NS	-	NS	-
CC X IR	-	-	NS	N/A	-	NS	-
HR X IR	-	-	NS	NS	-	NS	-
CC X HR X IR	-	-	*	N/A	-	NS	-
Time	***	***	-	-	-	-	-
CC X time	**	***	-	-	-	-	-
HR X time	NS	NS	-	-	-	-	-
CC X HR X time	NS	NS	-	-	-	-	-

[a] For the summer density measurement only the unirrigated density at 11 WAI was used; for density 11 WAI both unirrigated and irrigated densities were used.

[b] By harvest 2017 there was no surviving annual ryegrass therefore it was not included in the analysis for harvest biomass or spring biomass.

Table 4.4. The effect of cover crop treatment (CC) and herbicide rate (HR) on weed density 2 weeks after interseeding (WAI), at corn harvest (2016 only), and the following spring in Princeton, KY. The effect on weed biomass at corn harvest (2017 only) and the following spring (2016 only) in 2016 is also shown. Due to much higher weed pressure in 2017, weed biomass was collected instead of weed density. NS=not significant; *= significant at the 0.05 level; ***= significant at the 0.001 level.

	Weed density 2 WAI		Harvest		Spring weed density		Spring weed biomass	
	2016	2017	2016 weed density	2017 weed biomass[a]	2016-17	2017-18[a]	2016-17	2017-18[b]
CC	NS	NS	NS	NS	NS	NS	***	N/A
HR	NS	NS	NS	NS	NS	NS	NS	N/A
CC X HR	NS	NS	NS	NS	*	NS	NS	N/A

[a] By harvest 2017 there was no surviving annual ryegrass therefore weed biomass and density were not measured in those treatments at harvest or the following spring.
[b] Weed biomass was minimal in spring 2018 and therefore was not collected.

Table 4.5. The effect of cover crop treatment (CC) and herbicide rate (HR) on early season corn height (Ht) 2 weeks after interseeding (WAI) and corn yield in Princeton, KY, in 2016 and 2017. The effect on early season corn stage (St) 2 WAI and SPAD 4 WAI are also shown for 2016. Also shown is the effect of irrigation (IR) on corn yield is also shown for 2017; irrigation was not used in 2016. NS=not significant; *= significant at the 0.05 level.

	2016				2017	
	Ht	St	SPAD	Yield	Ht	Yield
CC	NS	NS	*	NS	NS	NS
HR	*	NS	NS	NS	NS	NS
CC X HR	NS	NS	NS	NS	NS	NS
IR	-	-	-	-	-	NS
CC X IR	-	-	-	-	-	NS
HR X IR	-	-	-	-	-	*
CC X HR X IR	-	-	-	-	-	NS

Table. 4.6. Average maximum temperature and average number of hours with temperatures over 40, 43, 46, 49, 52, 54, and 57 C, 2.54 cm above the soil surface under the corn canopy from 8 June to 14 July 2017 in Princeton, KY. Only data from plots where annual ryegrass was seeded are shown.

	Average number of hours over selected temperature						
Max temp (C)	40C	43C	46C	49C	52C	54C	57C
58	51	29	21	16	11	6	1

Table 4.7. The effect of herbicide rate on weed density at cover crop termination when annual ryegrass and orchardgrass were interseeded in 2017 in Princeton, KY. Weed density did not differ due to any treatment factor and the average density was 8 weeds m^{-2} in spring 2018.

Herbicide rate	Interseeded annual ryegrass*	Interseeded orchardgrass*
	Weed density (plants m^{-2})	
No herbicide	104[a]	27[b]
Low herbicide	52[b]	67[ab]
High herbicide	27[b]	88[a]
SEM (±)	16	16

* Within a column, treatment means followed by different letters are significantly different at alpha= 0.05.

Table 4.8. The effect of cover crop treatment on weed density at cover crop termination when a low herbicide rate was applied at corn planting in 2017 in Princeton, KY. Weed density did not differ due to any treatment factor and the average density was 8 weeds m^{-2} in spring 2018.

Cover crop treatment	Weed density*
	plants m^{-2}
No cover	100[a]
Interseeded annual ryegrass	61[ab]
Interseeded orchardgrass	61[ab]
Post-harvest cereal rye	27[b]
SEM (±)	26

* Treatment means followed by different letters are significantly different at alpha=0.05.
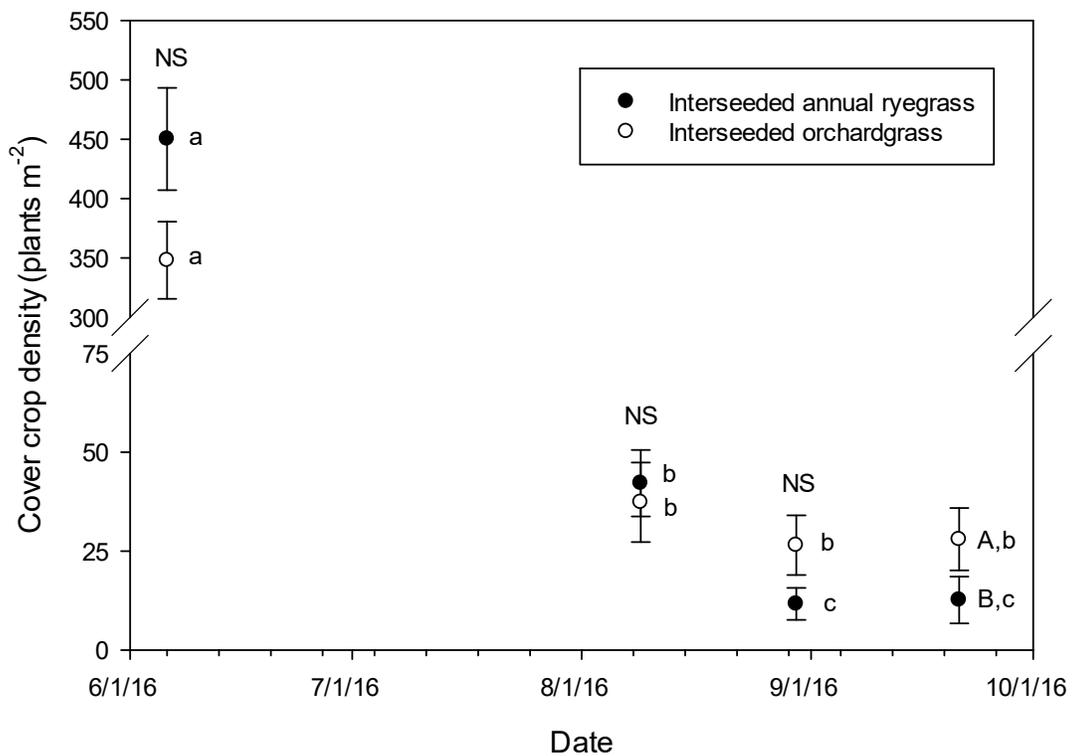

Figure 4.1. Interseeded annual ryegrass and interseeded orchardgrass density in the summer of 2016 (2, 11, 14, and 17 weeks after interseeding) in Princeton, KY. Within a date (i.e. across species), treatment means with different capital letters are significantly different at alpha= 0.05. Across all dates, treatment means within each species with different lower-case letters are significantly different at alpha= 0.05.
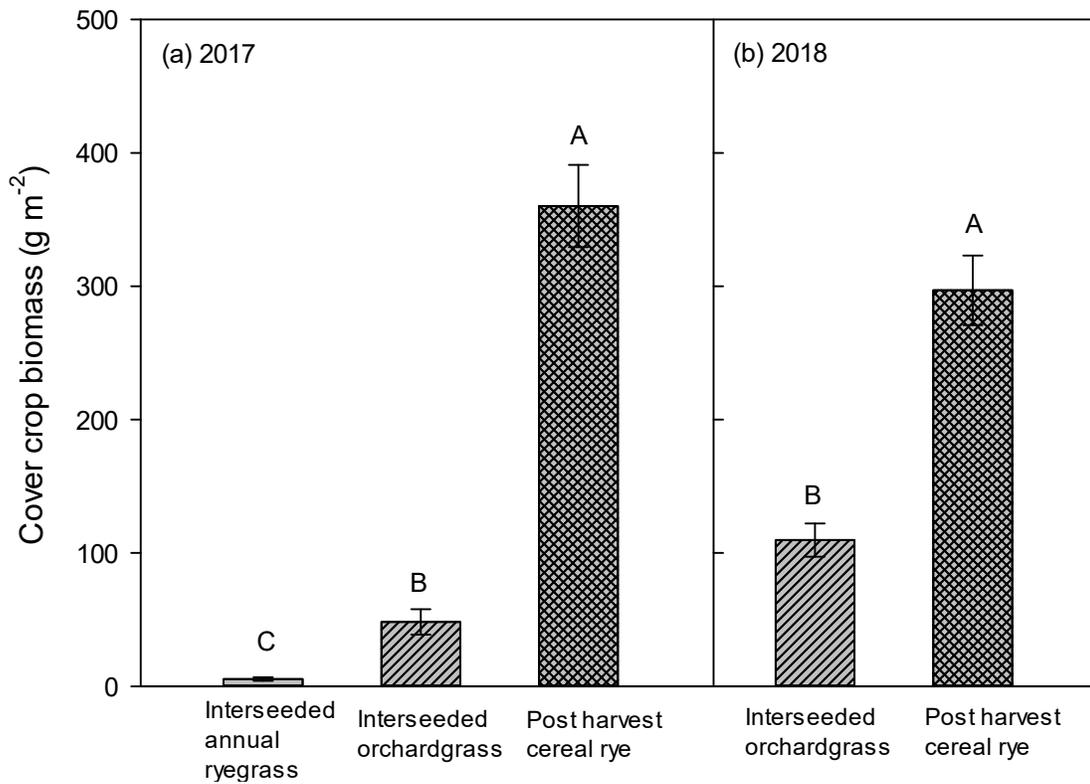
Figure 4.2. Cover crop biomass before spring termination in (a) 2017 and (b) 2018 in Princeton, KY. Within each year, bars with different letters are significantly different at alpha= 0.05. In 2018, due to minimal survival of the interseeded annual ryegrass, biomass was not collected.
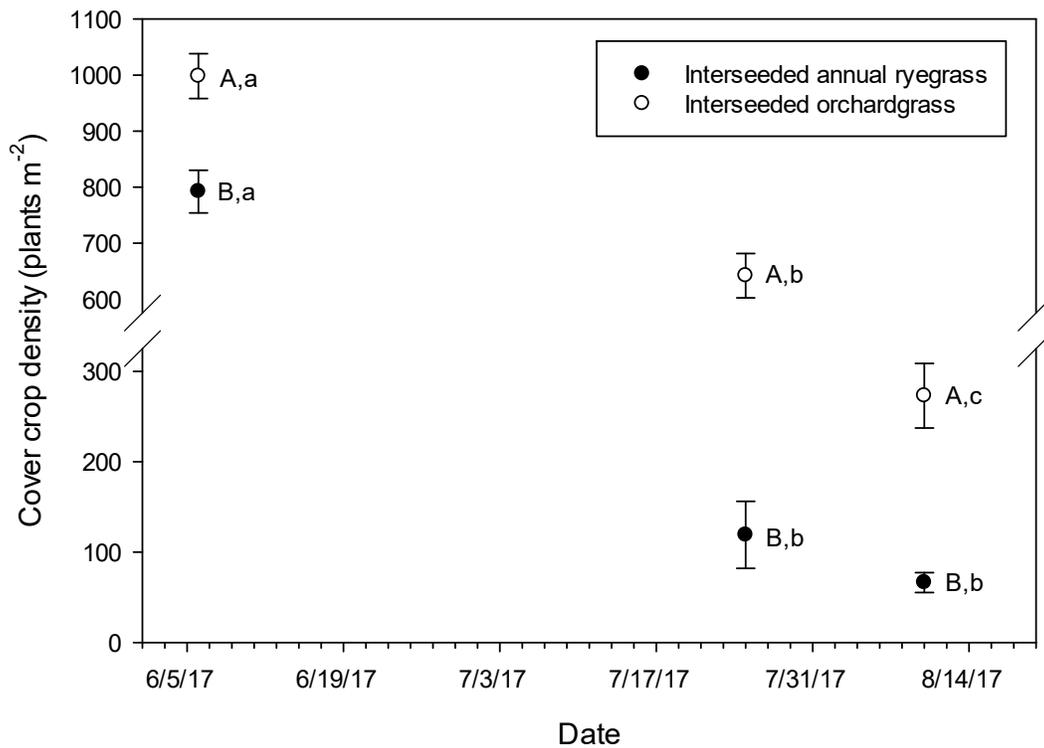
Figure 4.3. Interseeded annual ryegrass and interseeded orchardgrass density 2, 9, and 11 weeks after interseeding (WAI) in Princeton, KY, in 2017. At 11 WAI only unirrigated densities are shown. Within a date (i.e. across species), treatment means with different capital letters are significantly different at alpha= 0.05. Across all dates, treatment means within each species with different lower-case letters are significantly different at alpha= 0.05.
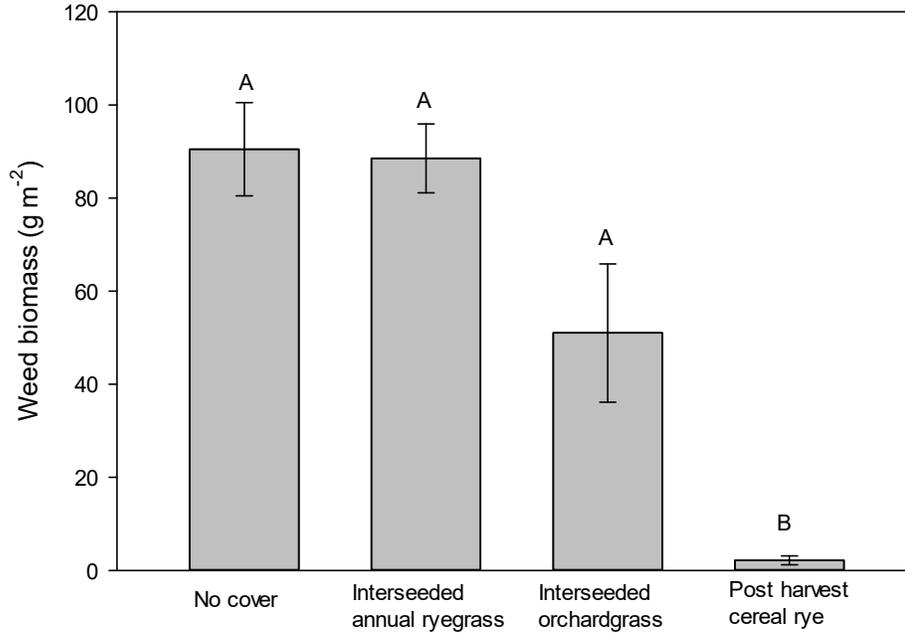
Figure 4.4. Effect of cover crop on weed biomass before spring termination in Princeton, KY, in 2017. Bars with different letters are significantly different at alpha= 0.05. Weed biomass was not collected and weed density did not differ due to any treatment factor in 2018. The average weed density was 8 weeds m^{-2} in spring 2018.

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