ABSTRACT

HENRIQUEZ INOA, SHANNON KOSS. Cover Crop Development and Utilization. (Under the direction of Dr. S. Chris Reberg-Horton.)

Two studies were performed from fall 2017 to fall 2019 focused on two major agronomic benefits of utilizing covers crop; aid in early season weed control and legume-derived nitrogen. The first study was aimed to improve weed suppressive capabilities of cereal rye. Our breeding efforts started with a bulked population from a greenhouse allelopathic screening of USDA National Small Grain Library accessions. Individuals were started in a greenhouse then transplanted in the field in order to screen for weed suppressive abilities as well as agronomic performance. Individuals were selected for weed suppression based on weed emergence and weed injury around the base of the plant. These selections allowed us to plant half-sib families the following year, and evaluate on an individual and family level for weed suppression. The second year of breeding yielded 105 individuals that showed high allelopathic activity.

The second study focused on utilizing fall-planted crimson clover as a partial or full nitrogen replacement for cotton as well as the potential for crimson clover to self-seed. Five cover crop termination dates were investigated; no crimson clover, two weeks before cotton planting (2WBP), at planting, two weeks after cotton planting (2WAP), and no termination. Two sidedress nitrogen treatments were applied around match head square flowering stage. Biomass, SPAD reading, and yield were collected. There were no yield differences among cover crop treatments; only sidedress rate affected yield. Clover biomass was maximized when terminated at planting while the 2WBP, 2WAP, and no termination were not significantly different. The later termination 2WAP and no termination had the best reseeded clover biomass the following fall.

These results indicate that planting a crimson clover cover crop prior to cotton will not negatively affect yield. Late or no termination of crimson clover will provide enough viable seed for the following year's cover crop, which can aid in reducing cover crop seed costs for growers.

Cover Crop Development and Utilization

by
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DEDICATION

I would like to dedicate my thesis to my family. To my parents, who raised me and taught me strong work ethic, solid moral compass, and humble desires for life. To my twin brother, who has always pushed me to do better for myself and to be unchanging and distinct in my values and perspective. Lastly to my husband, who has encouraged and supported me to be the most confident in myself and my abilities; and most importantly, not take life to seriously.

BIOGRAPHY

Shannon Henriquez Inoa was born and raised in Raleigh, NC. She graduated high school in 2011 and started her Associate Degree at Wake Tech Community College. After 2 years at wake Tech, Shannon transferred to NC State as sophomore. She graduated with a Crop and Soil Science with a concentration in Agronomic Science undergraduate degree in fall 2016. After graduation Shannon became a research technician with Organic Cropping Systems lab at NC State, which led to the opportunity to obtain a Master’s Degree in Crop Science. While working on her Master’s degree Shannon started as research technician with the new Alternative Crops lab under Dr. David Suchoff.

ACKNOWLEDGMENTS

The author would like to extend serious graduate to her advisor, Dr. Chris Reberg-Horton, for his wisdom and distinct passion for young professionals to work, learn, and succeed. Gratitude is also extended to her other committee members, Dr. Paul Murray and Dr. Ramon Leon for guidance and input throughout her master's career. Within Dr. Reberg-Horton's program, the author would like to give thanks to Sarah Seehaver and Rachel Vann for insight, energy, and setting a great example of impactful woman in agriculture. She would also like to thank Alyssa Woodard, Marcela Chavez, and Elder Lopez for their efforts towards Shannon's degree and the friendship that came along with our work. It should be acknowledged that numerous others within the Crop and Soil Department aided in Shannon's degree and their efforts are appreciated.

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Chapter II: Crimson Clover Cover Crop Utilization in Cotton Production

CHAPTER I: Breeding Highly Allelopathic Cereal Rye (Secale *cereal*)

ABSTRACT

Weed management through the use of cover crops is of great interest among growers. The use of cover crops as a weed management strategy can help mitigate the proliferation of herbicide-resistant weeds in conventional systems and help organic growers alleviate dependency on tillage as their main weed control method. Utilizing allelopathic species can augment the weed suppression potential of cover crops. Previous research was conducted screening cereal rye (*Secale cereale* L.) germplasm from the USDA National Small Grains Library for allelopathic activity. Individuals possessing high allelopathic activity were crossed with 'Wrens Abruzzi' cereal rye, a cultivar adapted and used commonly in the Southeast. These hybrids were randomly intermated in isolation over two generations. The random mated progeny were the subject of the 2017-18 field screening in North Carolina to quantify allelopathic-mediated weed suppression. Based on allelopathic ratings and field observations, the highest 150 allelopathic rye individuals were harvested and created the families that made up the following year's half-sib nursery. The 2018-19 half-sib nursery consisted of five single plants per family replicated three times. Based on similar allelopathic evaluations of the initial field screening, 104 single plant selections were harvested within 25 families. The 2018-19 selections constructed the 2019-20 headrow nursery. Over the two years of selections, the allelopathic rye population was reduced from the original 3,000 individuals to half-sib 25 families. This work represents critical foundation for creating a cereal rye cultivar that is acclimated to the Southeast climate with high allelopathic activity to aid early season weed control for summer cash crops.

INTRODUCTION

The evolution of herbicide-resistant weeds limit the effectiveness of chemical weed control. Cover crop utilization is one avenue for mitigating this problem. A cover crop is a monoculture or polyculture of species planted during fallow field periods, which generate benefits such as reduced soil erosion, decreased nitrogen leaching, weed suppression, added soil organic matter, and improved general soil health (NRCS, 2009). Improving the weed suppression ability of cover crops can aid in reducing the reliance on herbicides and manage resistant weed populations (Schulz et al., 2013). Cereal rye (*Secale cereale L.*) is the most common cover crop species used by growers in the United States due to its wide adaptability and cold tolerance (SARE, 2013-16).

Cover crop residues aid in weed suppression in three ways: (1) physical obstruction to weed growth (Teasdale and Mohler, 2000); (2) physical barrier limiting light penetration to the soil surface, where many weed species require light for germination; and (3) production and release of allelopathic compounds near the soil surface that inhibit weed germination and seedling growth (Barnes et al., 1983; Weston, 1996). Within tobacco (Nicotiana *tabacum* L.), soybean (Glycine *max*), and sunflower (Helianthus *annuus*) systems, cereal rye cover crop residue can reduce common lambsquarter (Chenopodium *album* L.), redroot pigweed (Amaranthus *retroflexus* L.), and common ragweed (Ambrosia *artemisiifolia* L.) populations by 65%, 74%, 80%, respectively (Shiling et al., 1985). Similarly, in pea (Pisum *sativum* L.) production under cereal rye residue, barnyard grass (Echinochloa *crus-galli* L.) and redroot pigweed biomass were reduced by 74% and 55%, respectively, without affecting pea yield (Barnes & Putnam, 1983). Growers and plant breeders can take advantage of this natural phenomenon to aid in weed control.

Shilling et al. (1986) found 17 phytotoxic compounds in ‘Abruzzi’ cereal rye, a common cultivar in the southeastern United States. The most phytotoxic compounds found in cereal rye were 2,4-Dihydroxy-1,4(2H)-benzoxazine-3-1 (DIBOA) and 2,3-benzoxazolinone (BOA) (Barnes & Putnam, 1987; Shilling et al., 1985). DIBOA and BOA belong to the benzoxazinoid (BX) chemical group. Of this chemical family, DIBOA is the more active (Niemeyer, 2009; Barnes et al., 1986; Shilling et al., 1985; Burgos and Talbert, 2000). BOA is the breakdown product of DIBOA (Barnes & Putnam 1987; Burgos & Talbert, 2000). Suggesting that cereal rye allelopathic activity has broad sprectrum weed control potential, dicots are more sensitive to BOA, while monocots are more sensitive to DIBOA (Nair et al., 1990; Barnes & Putnam, 1987).

Reiss et al. (2017) found that cereal rye root tissue concentrations of DIBOA were 3 times higher than triticale (x Triticosecale) and 48 times higher than wheat (Triticum *aestivum*). The authors further demonstrated that cereal rye shoot tissue had even more dramatic DIBOA concentrations than triticale and wheat, by factors of 90 and 937, respectively. There was also a strong relationship between early vigor (NDVI and LAI) and rye canopy height to benzoxazinoids (BX) concentration (Reiss et al., 2017), suggesting that both allelopathic activity and competitive ability are major traits for weed suppression potential. The term plant interference is used to describe the combined effect of competition and allelopathy (Weston & Duke, 2003). In field settings, these two factors are difficult to differentiate and may not be useful to separate because both contribute to weed suppression.

There are tenfold differences in allelopathic compound production among commercially available rye cultivars (Burgos & Talbert, 1999; Reberg-Horton et al., 2005; Rice 2005). The differences among rye cultivars support the pursuit of breeding for a highly allelopathic cereal rye cultivars. Correlations between DIBOA, BOA, and total hydroxamic acids, all members of the BX chemical group, are $R^2 > 0.8$. The high correlations indicate that differentiating between allelopathic compounds would be unnecessary from a breeding perspective (Rice et al., 2005). Heritability estimates of DIBOA production in cereal rye were comparable to corn yield, indicating that allelopathic compound production is a quantitative trial likely controlled multiple genes (Brooks et al., 2012, Hallauer et al. 2010). In addition to allelopathic compounds concentrations, maturity is also an important physiological trait that impacts allelopathy in cereal rye. The maximum concentration of allelopathic compounds occurs at the boot stage or the transition from vegetative to reproductive growth (La Hovary, 2011). In order to maximize weed suppression, this period of growth should occur close to cash crop planting.

The most common cultivars of rye were developed decades ago (Wrens Abruzzi in 1970 and Wheeler in 1972) (Morey, 1970; USDA Agricultural Marketing Service). The differences in allelopathic potential and broad genetic control of allelochemical production within the cultivated rye gene pool confirm the need for a cereal rye breeding program geared towards enhancing allelopathic activity. The development of highly allelopathic cereal rye cover crops has the potential to aid in early season weed control in a cash crops both physically and chemically and assist in controlling herbicide resistant weeds. To date there is no cereal rye breeding program centered on allelopathic potential for cover crop use. The goal of our research was to establish a cereal rye breeding program concentrating on allelopathic activity and high agronomic performance for southeastern U.S. growers. The initial cereal rye population was based on greenhouse bioassay screening of National Small Grains Library cereal rye accessions (Reberg-Horton, 2002). Screening allelopathic activity under field settings was the basis of rye breeding advancement because of the relevant implications to grower use.

METHODS AND MATERIALS

Developing Initial Population

Reberg-Horton (2002) evaluated 268 cereal rye accessions from the USDA National Small Grain Library, plus ten commercially available cultivars, for allelopathic activity. The initial allelopathic cereal rye population was developed based on results of tissue extract bioassays collected from the rye entries grown in a field setting. Each extract was added to a petri dish lined with filter paper and weed seeds of either redroot pigweed or goosegrass (Eleusine *indica* L.), then incubated in a germination chamber.

Radicle length was chosen as the indicator for weed growth inhibition. Based on Burgos and Talbert (2000) findings, root elongation is a better indicator of allelopathic activity because of more inhibitory effects on root tissue and less variation in radicle lengths. An allelopathic index was created based on weed seed root length and standard deviation for each rye accession. Based on the replicated checks, an estimate of standard error for radicle length of goosegrass and pigweed was established.

All accessions that were both late maturating and highly allelopathic were selected for the initial rye population. The number of accessions with both traits was small, thus accessions with either late maturity or with high allelopathic activity were added to the population to ensure ample allelopathic genetics. Based on these criteria, 15 accessions were selected to advance in the breeding population along with two publicly available cultivars. ‘Wrens Abruzzi ‘, a southeastern adapted cultivar, was chosen because of its allelopathic activity, among the highest of all rye entries tested. The ‘Wheeler’ cultivar was also chosen because of its late maturity and allelopathic activity, though less than ‘Wrens Abruzzi’. These 17 genotypes were allowed to cross-pollinate in isolation over two generations; ant their progeny was bulked for future breeding efforts, ending the 2001 study.

Nursery Preparation

The bulked allelopathic population from the 2001 cereal rye screening was the F_2 parent population to start the cereal rye breeding program of 2017. The population was planted in the greenhouse in the summer of 2017. This allowed a second generation of crossing in isolation while screening for viable seed because of the long storage time. Seed was harvested from 150 randomly selected plants then bulked, which produced the advanced population that was to be screened in a field setting for allelopathic activity in the fall of 2017.

In late October, 7,000 seeds from the third generation were planted into individual containers that were started in the greenhouse to ensure uniform germination. Four weeks after greenhouse planting, 6,000 individuals were randomly chosen to be transplanted in three field locations: Central Crops Research Station in Clayton, NC, Upper Coastal Plain Research Station in Rocky Mount, NC, and the Piedmont Research Station in Salisbury, NC. Each location was selected to represent the major soil types in the southeast United States. Nutrients were incorporated into the fields weeks before planting based on individual field soil recommendations from the NCDA&CS soil test reports. Each station established horticultural beds based on station spacing before rye transplanting. Split application of nitrogen was applied using 30% liquid urea-ammonium totaling 100.9 kg N h applied with a backpack sprayer. In the fall of 2017, 33.6 kg N h was applied two weeks after transplanting and the remaining nitrogen applied once plants broke winter dormancy in March of 2018.

Plants were hand transplanted into raised beds, spaced 0.91 m apart to ensure the evaluation of weed populations surrounding each plant. Each rye plant was a unique genotype, therefore, nurseries were not replicated. A reference genotype was randomized throughout each field, ‘Wrens Abruzzi’, which were used to compare the initial population against commercially available cereal rye. To establish what the natural weed population was in each field, the second reference plot was a fallow plot with nothing planted.

During the initial field screening, an indicator species was planted around cereal rye to quantify the allelopathic activity among individuals. Redroot pigweed was used as the indicator species because of the pervasive issues with Amaranthus weed species, including herbicide resistance in Palmer Amaranth. In early May 2017, we applied a pigweed sand mixture to a 20 cm x 45cm rectangle that was mechanically disturbed with a hoe on the south side of each plant and the mixture applied. The target rate of pigweed seed was 333m^2. This application strategy was designed to simulate pigweed emergence early in the cash crop season. Only half of the individuals per location received this treatment because of time and labor constraints.

Data Collection

Physical differences among individuals were dramatic, therefore, phenotypic ratings were taken twice during the vegetative growth period in early and late April at all three locations. Evaulated traits included growth pattern, leaf shape, and biomass production. Growth pattern was categorized as either prostrate, semi-prostrate, or upright growth. Once the prostate individuals reached reproductive growth, the growth pattern became upright resulting in plants with a wider crown. Leaf shape also differed among genotypes and ratings consisted of a dichotomy of wide versus narrow leafed individuals. Lastly, a visual rating scale from one to nine was used to capture biomass differences throughout the growing season.

An allelopathic rating was taken in mid-May within the disturbed area of the weed seed application. The pigweed application did not yield constant emergence, but carpetweed (Mollugo *verticillata* L.) was pervasive across the field. Therefore, carpetweed was used as the indicator species. The allelopathic rating consisted of four observations. First, a dichotomy rating of yes or no, indicating allelopathic activity present or absence around a cereal rye individual. Second, a weed density rating from one to nine was taken based on control plots with no rye plant (i.e. full coverage of indicator species). Third, a weed injury rating was taken on a scale of one to nine. Lastly, a weed injury gradient rating was taken on a scale of one to three: One indicating no gradient of weed injury, and up to three indicating a distinct gradient of increasing weed health moving away from the base of the cereal rye plant. These ratings informed our preliminary selections with a final visual decision made at harvest.

Half-sib Families

Cereal rye is an outcrossing species, thus the progeny of an individual plant is related by only one parent, making them half-siblings (half-sib). Based on seed quantity, 150 families of the previously selected plants advanced to the 2018-19 nurseries. In late October 2018, 100 seeds from each selection and 'Wrens Abruzzi' were planted in a similar greenhouse fashion to the previous year. Half-sib families were transplanted with the same reference plots used from the previous year: 'Wrens Abruzzi' and fallow plots.

Field preparation mirrored the previous year with the absence of bed formation for better weed population evaluation around individuals. Plants were transplanted in mid-November with the same split application of nitrogen as fall 2017.In a completely randomized disgn, each entry was replicated three times per location with five plants of each family making up a plot. A total of 15 individuals from each family were represented in each field.

Because of the failure of the previous year's weed seed application, the application strategy was altered. Pigweed was still used as the target weed but we added lettuce (Lactua *sativa*) into the mixture because of its shallow seeding depth and early emergence at a similar rate to pigweed. Timing and method of weed seed application was changed to a frost seeding technique using a Gandy drop spreader and eliminating some of the dormancy issues of the pigweed we saw the previous year.

Phenotypic data were collected based on the previous year's rating structure of growth pattern, leaf shape, and biomass production. Data was only collected once during vegetative growth in mid-April. Despite the better weed seed application method, once more the pigweed emergence was not consistent enough to be used as the indicator species. Only, one location had sufficient natural weed pressure to confidently rate allelopathic activity. The same allelopathic ratings were taken as the 2017-18 growing season with one exception: The weed injury gradient was not observed like in the previous year, instead, we saw a distinct weed-free ring present around the base of the plant or no ring at all. This observation was captured with a categorical yes or no rating.

As in the previous year, the allelopathic ratings informed the initial selections with the final decision of harvest based on field observations. However, there was a major discrepancy when comparing our prepared selections to observations in the field at the time of harvest. Some Individuals that should have been selected based on the allelopathic ratings did not have pronounced weed-free rings compared to those individuals around it or within the same family. Because of this inconsistency, selections were made based on the field observations on the day of harvest.

RESULTS AND DISCUSSION

Nursery Development

The primary objective of 2017-18 field screening was to identify and harvest the most allelopathic rye individuals. We took advantage of the diverse genetic background of the initial rye population by also creating two other populations: disease resistant and high agronomic performance. The progeny of these selections became the half-sib families for the following year's nurseries. The number of individuals selected within each population and the subsequent selection pressure is displayed in Table 1.

During the second year, the disease-resistant and agronomic nurseries were planted in a head-row fashion. There was no disease pressure present during the second year at the disease-resistant nursery location, as a result selections could not be made for this trait. The agronomic nursery was allowed to grow until the 'Wrens Abruzzi' rye cultivar flowered. At this point, the genotypes that flowered before or at the time of Wrens Abruzzi were killed along with the 'Wrens Abruzzi' plots. Only those genotypes that were late flowering were allowed to intermate. Final selections of the late flowering agronomic nursery were based on biomass accumulation and stand uniformity (Table 1).

Year 1 Population Improvements

Two of the three locations developed enough weed pressure to allow for allelopathic scoring and selection. Allelopathic activity of rye individuals was scored based on the surrounding weed density as a percent weed density of the control plot. Also, the intensity of weed injury symptoms and the degree of injury gradient around the base of the plant. The total number of plants selected for high allelopathic activity was 174 individuals across two locations. Scoring results are displayed as histograms to compare the entire scored population to the selected individuals in year 1, Figure 1a-3b.

The weed density scores of the entire scored population followed a nearly normal distribution (Figure 1a), where the check cultivar, 'Wrens Abruzzi', is highlighted. Our selection efforts are aimed towards individuals on the right tail of the curve where the weed density is significantly reduced compared to the control plot. The histogram of selected plants (Figure 1b) has a similar distribution but with a lower percentage of plants with lower emergence inhibition. The weed injury score of both the entire population and the selected individuals are understandably right-skewed, where most rye individuals did not cause significant weed injury (Figures 2a and 2b). Within the selected plants, there is a proportion of individuals that caused weed injury. Within the entire scored population, there is a low proportion of the population that has a significant weed injury gradient (Figure 3a) compared to the selected distribution (Figure

3b). Overall, the selected individuals had a reduced proportion of individuals with no emergence inhibition, a higher proportion of plants that caused weed injury, and a clear weed injury gradient. There were still some individuals in the selected population that were not rated as highly allelopathic; these individuals were selected based on agronomic characteristics such as flowering time or plant structure. These results are consistent with our breeding efforts towards a highly allelopathic rye nursery.

Year 2 Population Improvements

Even with a weed seed application, only one location of year two developed enough weed pressure to evaluate allelopathic activity. The 2018-19 season did not produce weed injury circles exhibiting gradients like the previous year. For this reason, only weed emergence inhibition and weed injury ratings were collected for the 2018-19 allelopathic nursery.

In year two, 104 individuals were selected for their high allelopathic activity. The entire allelopathic nursery presented a right skewed distribution for both weed emergence inhibition and weed injury (Figures 2a and 2c), reinforcing the notion that visual allelopathic activity is an infrequent phenotype. The allelopathic plants had a higher proportion of inhibition and injury. Year two had strong selection pressure for allelopathic individuals based on the frequency of both weed emergence inhibition (Figure 8) and weed injury (Figure 10) compared to the entire population (Figure 7 and 9). Weed injury scores of allelopathic individuals show high variation remaining within the population. In both cases, ‘Wrens Abruzzi’ scored below the most allelopathic individuals, suggesting there is the capacity to breed for more allelopathic rye than what is currently available.

Within the selected plants, the majority had high emergence inhibition except a notable proportion, 14 plants, had less than 20% inhibition (Figure 8). This could be due to the weed injury effect of those individuals as the reasoning for selection. Of the selected plants, 37% caused severe weed injury (Figure 10). The plants that were selected that scored under 20% injury could have been selected for their emergence inhibition. It is important to note that both the weed emergence inhibition and weed injury scores aided in selection. Based on the presence of a weed free ring around the plant and/or weed injury, the final decision was made in the field.

At the end of the first year of evaluating allelopathic activity, a total of 174 individuals were selected across two locations. Of those selected, the majority (130 individuals) exhibited prostrate or semi-prostrate growth patterns**.** In the second year, 105 plants were selected for allelopathy, of which 94 plants grew in a prostrate or semi-prostrate pattern (Figure 11). The wider crown of these individuals could have made them more competitive for light, nutrients, and space, contributing to the reduced weed density score. This reasoning supports the notion that plant competition plays a role in weed suppression. The term plant interference is used to describe the combined effect of competition and allelopathy (Weston & Duke, 2003). The differentiation between competition and allelopathic activity are not easily separated especially under field conditions, but both play a role in weed suppression. Since our selections were based on weed symptomology, we cannot definitively state that our breeding effects are based solely on allelopathic effects. Nonetheless, our goal was to develop a cereal rye cover crop that aids in weed suppression and competitive ability is a part of that development. The selections with an upright growth pattern could be the most allelopathic because of the lack of competitiveness compared to the other growth patterns.

Under artificial conditions factors such as soil type, microbial populations, and plant interference cannot be observed, resulting in conclusions that do not represent real-world circumstances. Brooks et al. (2012) investigated the heritability of allelopathic compounds in cereal rye under field conditions. They concluded that the cereal rye tissue DIBOA content was not sufficient to represent the amount of weed suppression observed in the field. This finding reinforces that notion of plant inference, where allelopathic compounds alone do not account for all of the weed suppressive qualities of cereal rye. Additionally, the weed suppression found in field-grown cereal rye was significantly higher than that found in plants grown under artificial environments (Mwaja, 1995). Similar results were found in field-grown rice (Khanh et al., 2009). These studies give support the notion of additive environmental effects on weed suppression potential. The lack of congruency between artificial and field environments leaves doubt in the translation of experimental results to grower conditions and utility. Our breeding efforts were centered on field evaluation of rye allelopathy, because of this lack of congruency.

Table. 1 Cereal rye selection nurseries, selection pressures, and total plants selected in 2017 and 2018

		Allelopathic Nursery		Rust Resistant Nursery		Agronomic Nursery	
Year	Location	Total Plants Selected	Selection Pressure	Total Plants Selected	Selection Pressure	Total Plants Selected	Selection Pressure
1	Clayton	106	5.4%	27	1.35%	87	4.35%
1	Rocky Mount	68	3.4%	No Disease Pressure		84	4.2%
1	Salisbury	-	-			84	4.2%
2	Kinston	104	4.5%			-	-
2	Clayton	-	-			26	6.5%

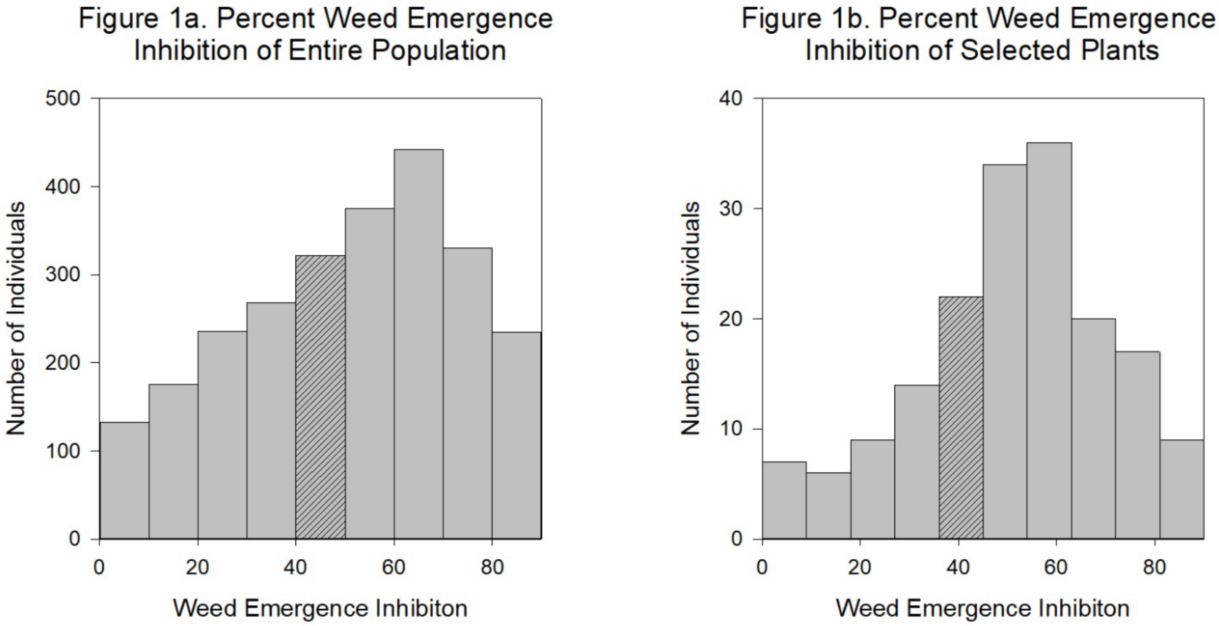


Figure 1a. Distrubution of weed emergance inhibition of the entire population. Figure 1b. Distrubtion of weed emergnace inhibtion of selected individuals. Shaded area indicates ‘Wrens Abruzzi’

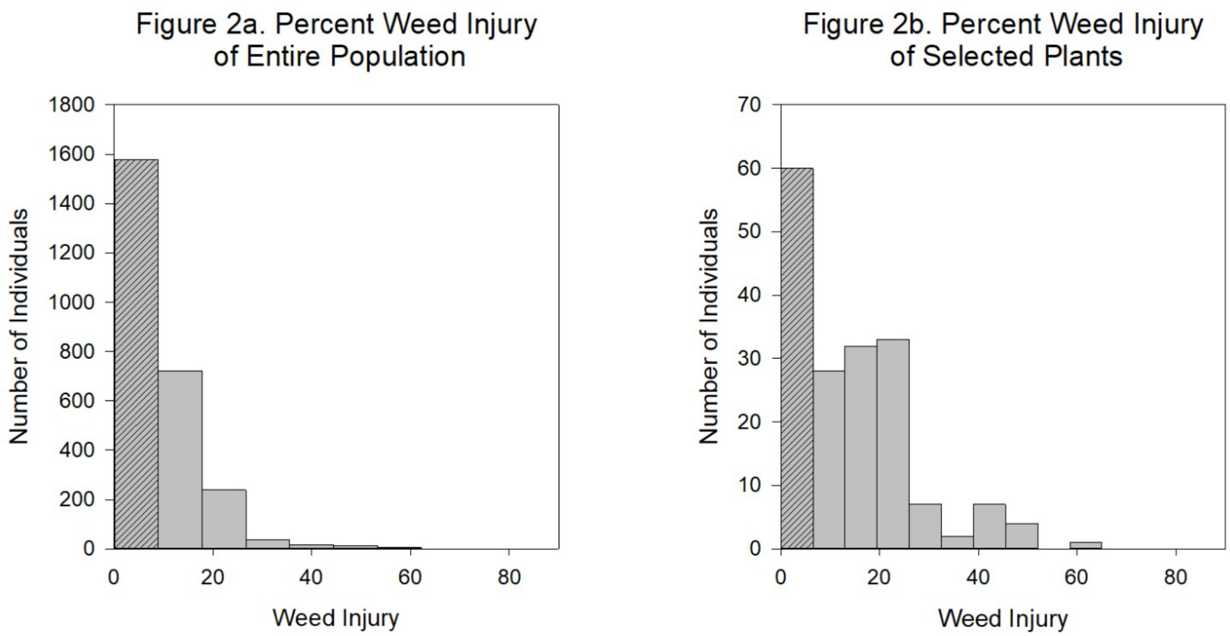


Figure 2a. Distribution of weed injury of the entire population. Figure 2b. Distribution of weed injury of selected individuals. Shaded area indicates 'Wrens Abruzzi'.

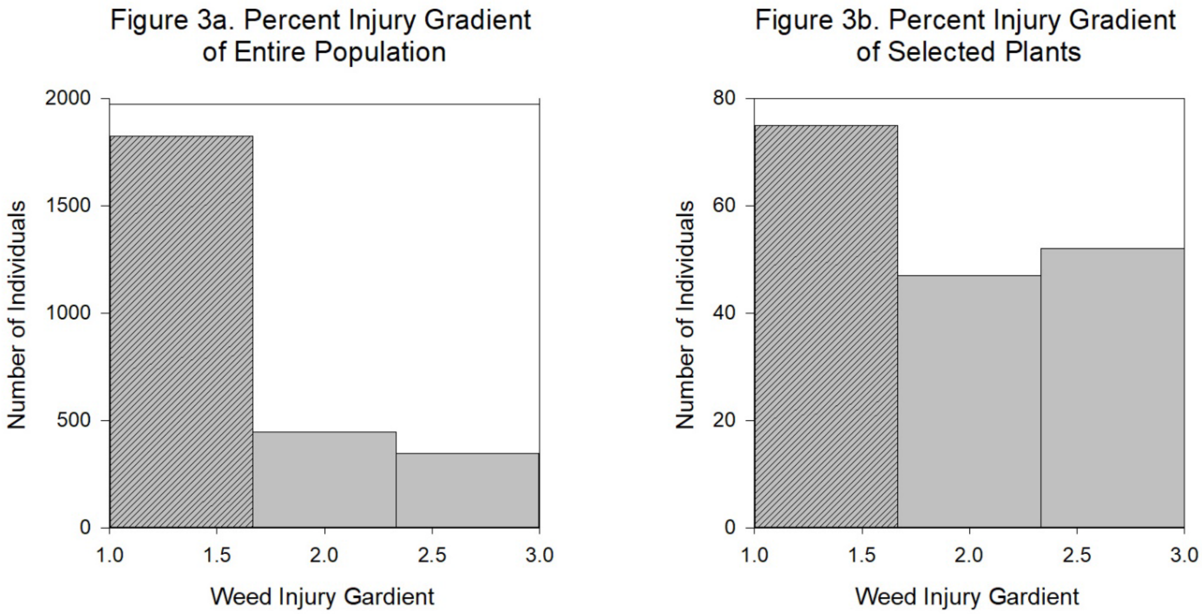
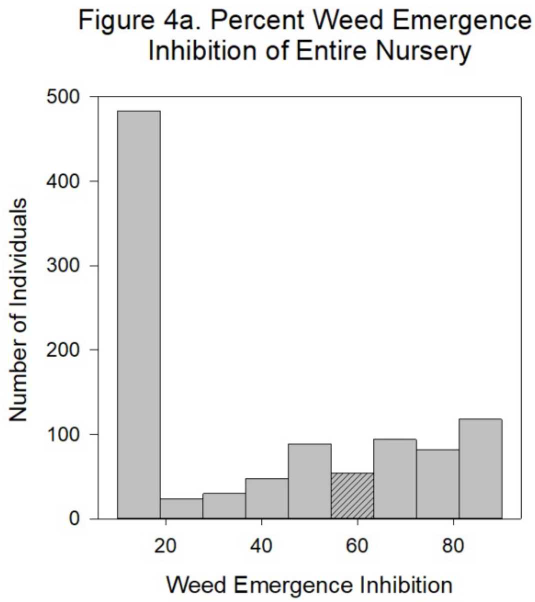


Figure 3a. Distribution of weed injury gradient for entire population. Figure 3b. Distribution of weed injury gradient of selected individuals. Shaded area indicates 'Wrens Abruzzi'.



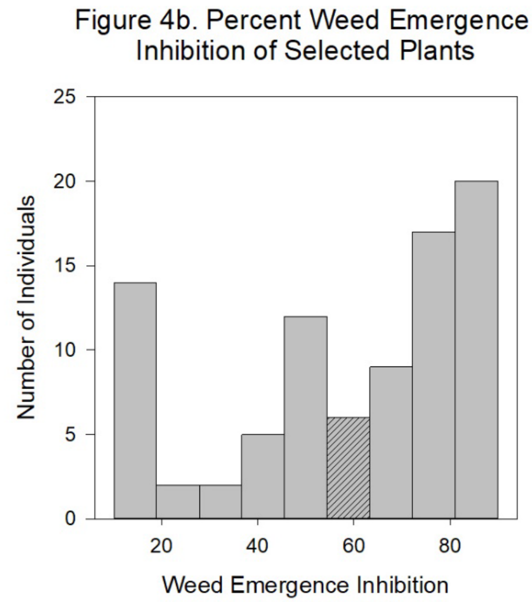


Figure 4a. Distribution of weed emergence inhibition of entire population. Figure 4b. Distribution of weed emergence inhibition of selected individuals. Shaded area indicates 'Wrens Abruzzi'.

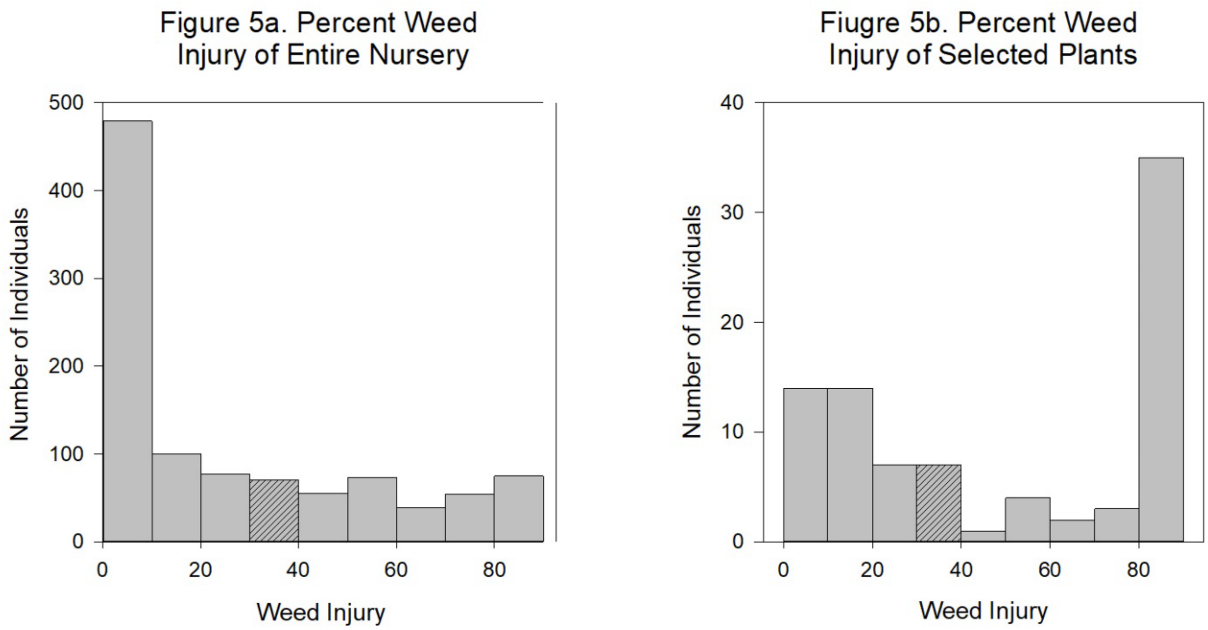


Figure 5a. Distribution of weed injury of entire nursery. Figure 5b. Distribution of weed injury of selected individuals. Shaded area indicates 'Wrens Abruzzi'.

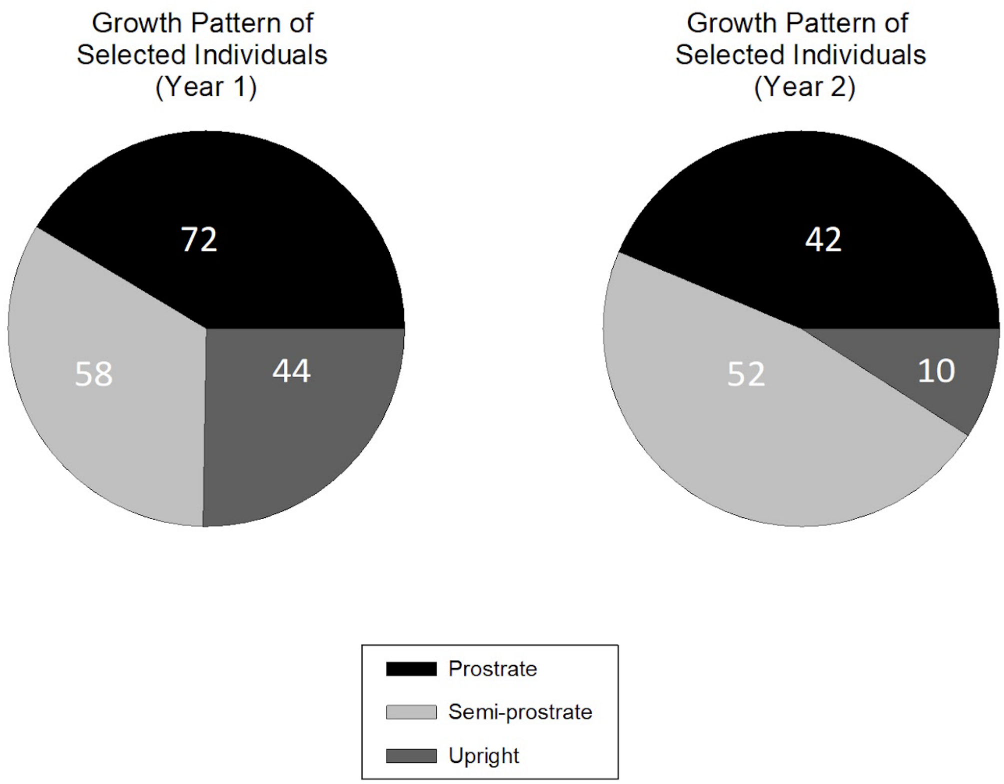


Figure 6. Growth patterns distribution of selected cereal rye individuals in Year 1 and Year 2.

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Chapter II: Crimson Clover (Trifolium *incarnatum*) Cover Crop Utilization in Cotton (Gossypium *hirsutum*) Production

ABSTRACT

Legume cover crop use in row crop production has gained popularity in North Carolina over the past several years because of their associated soil and agronomic. To date, there is no research quantifying the nitrogen contribution from the legume to the subsequent cash crop. In addition, there are no recommendations for when or if to kill the cover crop prior to cash crop planting. This study is focused on the nitrogen contribution from a legume cover crop to the subsequent cotton cash crop. Crimson clover was selected as the cover crop of interest because of the high biomass production and potential for self-reseeding. We planted crimson clover in late fall and allowed to grow until early spring. Different cover crop termination times were investigated: before, during, and after planting the cotton crop. To quantify the nitrogen contribution of the legume, crimson clover biomass was collected and different side dress nitrogen applications (30 and 60lb N/acre) were applied including no side dress control. A randomized split-plot design was employed and regional practices were used in the agronomic management of cotton. We found that cover crop termination at cotton planting produced the most clover biomass. The late and no termination treatments produced the most reseeded biomass the following year. There were no significant differences among yields of the cover crop treatments. Side dress nitrogen positively affected yield. Our results suggest that crimson clover can be used in cotton production systems to partially replace nitrogen inputs, and that the reseeding capability of this cover crop may help reduce farmer input costs related to sequential cover cropping.

INTRODUCTION

Cover crop utilization across the US has increased by 50% from 2012 to 2017 (US Census). Cover crop incorporation onto farmers' operations can be difficult from a logistical standpoint at the field level, while reluctance is also based on the lack of consistent yield and economic benefits across time and locations (Roesch-McNally et al. 2018; Lewis et al. 2018).

Soil health benefits are the primary reason growers first implement a cover crop (Toler et al. 2019; SARE 2020). Cover crop benefits include decreased soil erosion and sediment runoff, reduced nutrient leaching and runoff, improved soil structure, and nitrogen contributions, if legume species are used (Kasper and Singer, 2011; Hancock et al. 2019).

Nitrogen (N) supply is a frequent yield limiting factor after water availability (Zhou and Yin, 2018). In cotton production, insufficient nitrogen can lead to reduced yield and quality, while excessive nitrogen can lead to undesirable late season growth, delayed cotton maturity, reduced yield and quality (Zhou and Yin, 2018). There is interest in legume derived N sources to supplement or even replace inorganic fertilizer sources. Both crimson clover and hairy vetch have the potential to supply season-long N requirements for cotton (Foote et al. 2014; Tonitto et al. 2006).

Syncing cover crop decomposition and nitrogen mineralization with cash crop needs is the major barrier to replacing synthetic fertilizers. Winter legumes such as hairy vetch, winter pea, or crimson clover can accumulate enough nitrogen to provide total season long nitrogen needs for summer cash crops (Bauer 1993; Foote et al. 2014; Zablotowicz et al. 2011). Biomass production, flowering, and termination timings are factors that affect nutrient synchronization (Foote et al., 2014; Schomberg and Endale, 2004). Cover crop N content follows dry matter accumulation (Ranells and Wagger, 1996). Cover crop C:N ratio is a reliable indicator of decomposition and mineralization rate of plant material. Hairy vetch and winter pea have lower C:N ratios compared to crimson clover, in turn decomposition is faster (Wagger, 1989; NRCS 2011).

The legume cover crop species that can be best utilized in cotton production is crimson clover (Ranells and Wagger, 1992). The time frame of crimson clover achieving maximum biomass and N content coincides with the cotton planting period (Boquet and Breitenbeck, 2000). Maximum N assimilation in cotton occurs during bloom and early boll set growth stages. Crimson clover decomposition and mineralization is slower than hairy vetch or peas because of the higher C:N ratio. The delay in mineralization of crimson clover could sync better with cotton's N requirements compared to other winter legumes.

Crimson clover also has the reseeding potential due to seed dormancy. Seed can go through several wetting events, especially during high temperatures, without initiating germination (Knight and Hollowell, 1974). This characteristic allows for planted crimson clover to set mature seed that will germinate the following year. Reseeded crimson clover can eliminate the need for annual clover planting, which can decrease the production cost of incorporating cover crops into row crop systems (Hancock et al., 2019).

The objective of this research was to evaluate the reseeding potential of crimson clover in cotton production and, to assess the cover crop N contribution to the following cotton cash crop.

METHODS AND MATERIALS

This study was conducted over two years, at the three locations: Rocky Mount, Goldsboro, and Salisbury, North Carolina. A split-plot experimental design was used: the main-plot consisted of five different crimson clover termination timings: no cover crop, termination two weeks before planting (2WBP), termination at cotton planting (@P), termination two weeks after planting (2WAP), and no termination. The sub-plot treatments included different sidedress N rates: 0, 34, or 67 kg/h. All subplots received 20 pounds of N at planting. Station equipment was used to plant crimson clover and cotton, conduct field management, and cotton harvest.

Crimson clover cover crop was sowed in the fall prior to cotton planting. In the fall of 2017 and 2018, crimson clover, cultivar 'AU Robin', was planted at a rate of 20 lbs /A at a depth of 0.5 in. In the winter of 2018 and 2019, the no cover crop main plots were terminated using glyphosate plus dicamba or glyphosate alone, dependent on weather conditions. The second week of May in 2018 and the first week of May in 2019 were the target planting dates for cotton. Clover termination treatments; 2WBP, @P, and 2WAP occurred according to cotton planting date. Crimson clover biomass was collected using a $0.5m^2$ quadrat on the day of termination. Herbicides used to terminate clover coincided with herbicide-tolerant trait cultivars used at each location. Cotton stand counts were collected early in the growing season while soil moisture and temperature measurements were collected throughout the season. Sidedress nitrogen treatments, using 30% UAN, were applied approximately at match-head square growth stage or when cotton had seven to eight nodes. SPAD measurements were collected from the most nearly expanded leaf occurring at flowering, 2 weeks, and 4 weeks after flowering. Cotton defoliation occurred in October, but harvest dates differed across locations because of weather conditions. Reseeded crimson clover biomass was collected the following spring.

RESULTS AND DISCUSSION

Cover Crop Biomass

The crimson clover biomass peaked in early to mid-May achieving 9269 kg/h of dry matter. Clover biomass at cotton planting was higher than other termination timings (Figure 1). These results illustrate that crimson clover development and maturation incorporates well into the timeline of cotton production. Ranells and Wagger (1996) reported that maximum N content in crimson clover occurred at late bloom/ early seed set stage which is approximately early to mid-May. Clover termination at planting allowed for maximum nitrogen accumulation in the crimson clover. Clover biomass decreased after planting possibly due to the seed set and natural senescence of the clover.

Reseeded crimson clover biomass in the second year was strongly affected by cover crop termination timing in year one. Self-seeded crimson clover biomass was significantly lower in the two earlier termination timings: 2WBP and @P (Figure 1). The lower biomass production was due to the previous clover not being able to set sufficient viable seed before termination. The later termination timings, 2WAP and no termination, had significantly higher reseeded biomass values because the clover was allowed to produce more viable seed before termination. Touchton et al. (1982) found similar results; self-seeded clover produced sufficient biomass the following year.

Crimson clover termination at planting provided the most cover crop biomass for the subsequent crop but did not produce enough viable seed production to reseed itself for the following year. Termination of crimson clover at cotton planting will allow clover biomass to be maximized, but adequate seed development for the following year's cover crop will not be optimized. Therefore, there is a tradeoff between maximizing cover crop biomass for the following cash crop and producing viable seed for the subsequent year.

Cotton stand count was significantly affected by crimson clover presence regardless of termination time (Table 2). Bauer et al. (1993) also found stand differences between cover crop treatments with no impact on yield. The amount of cover crop biomass at planting and planter type are two factors that influence cotton stand count.

SPAD

Sampling date significantly affect SPAD. As the growing season progressed, SPAD readings increased. The first time point had the lowest SPAD reading (Figure 2). The third sampling date had the highest SPAD readings (Figure 2). Below average rainfall occurred before and during sampling date 2 which may have affected nitrogen uptake of new growth influencing the lack of separation of SPAD readings between date 1 and 2. Sidedress N treatments also significantly affected SPAD readings. SPAD readings across sampling dates increased as sidedress N rate increased (Table 4).

Termination timing significantly affected SPAD readings across locations. No cover crop treatment had the highest SPAD mean across sampling dates (Table 5). 2WAP and no termination treatments were the second highest SPAD readings, 2WAP treatment having a slightly higher value than no termination. The lowest SPAD values were observed in the 2WBP and at planting termination timings (Table 5). The significant separation between cover crop treatment SPAD readings may be due to termination timing of the clover with respect to the decomposition and nitrogen release of the clover residue. Wilson and Hargrave (1986) found that the N content decreased dramatically during the first few weeks of crimson clover residue in the field. The lowest SPAD readings, 2WBP and at planting, may be due to the residue N from those treatments being lost prior to cotton utilization. The later terminations, 2WAP and no termination, had higher SPAD values, consistent with the idea that delayed clover termination better synchronizes N release with cotton uptake.

Yield

Only sidedress N rate significantly affected cotton seed yield. Yield results followed a similar linear pattern to SPAD readings of sidedress N rates (Figure 3). The lowest yield was from no sidedress N application. The highest yield, 1285 lbs./A seed cotton, was produced by the highest sidedress nitrogen rate, 60lbs. N/A.

Conclusion

This study sought to investigate the use of crimson clover as a partial- or full-nitrogen replacement for cotton as well as the ability of reseed to reduce cover crop seed inputs the following year. There was a tradeoff between maximizing clover biomass and reseeding potential. Clover termination at planting will produce the most clover biomass but reduce reseeding potential. While later termination of clover will result in lower clover biomass but improve reseeding for the following year, which could aid in operational costs to the grower. Though there were differences between cover crop treatments in the SPAD readings during the season, end of season cotton yields were only significantly affected by sidedress nitrogen rates. Previous research has shown that legume cover crops can meet cash crop N requirements

(Mcclanahan, 2019; Balkcom et al., 2019; Foote et al., 2014). The lack of yield differences between crimson clover and bare ground plots may be due to N immobilization that occur with crimson clover having a higher C: N ratio compared to hairy vetch or winter pea. A crimson clover cover crop prior to a cotton cash crop will not increase or hinder cotton yield but can provide a self-seeding cover crop. The self-seeding advantage of crimson clover can aid in reducing input cost for incorporating cover crops into field rotations.

Table 2. Analysis of variance of yield influenced by crimson clover termination timing and sidedress treatment

	Yield		
Source of Variation	DF	F Value	P>F
Environment (E)	3	19.22	<.0001**
Kill Treatment (K)	4	0.93	0.4829
Sidedress Treatment (S)	2	3.23	0.0414*
E X K	12	5.64	<.0001**
E X S	6	1.60	0.1489
E X K X S	24	0.76	0.7783

Figure 1. Crimson Clover biomass based on termination time and reseeded clover biomass the following year.

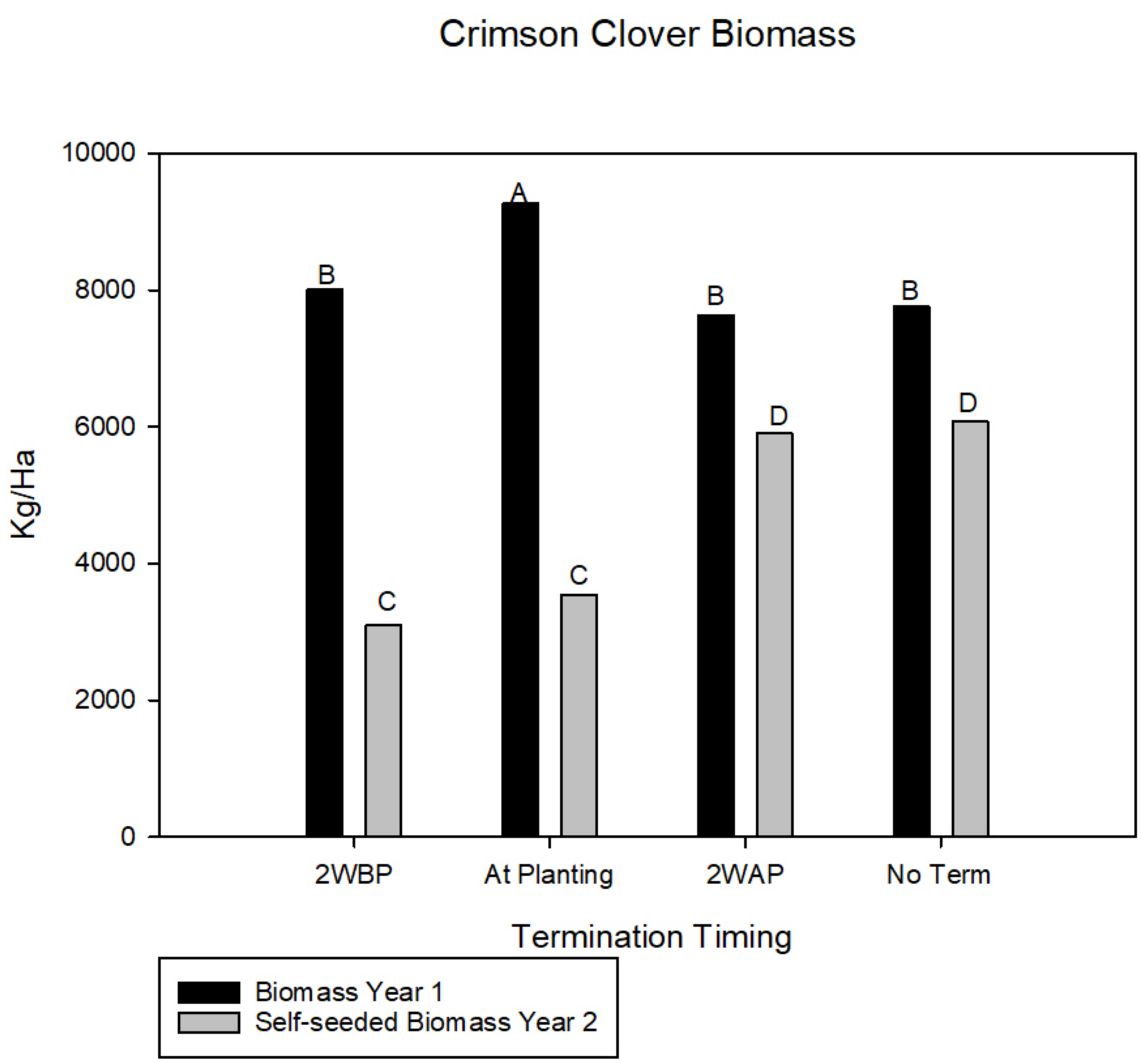


Table 3. Cotton stand count means under different crimson clover termination timings. Crimson clover biomass did effect cotton stand count but there is no difference between termination timings.

Termination Timing	Average Stand Count
No Cover Crop	10.06 A
2 Weeks before Planting	6.03 B
At Planting	6.8 B
2 Weeks after Planting	6.03 B
No Termination	6.08 B

Figure 2. SPAD reading values at three sampling times during the growing season. SPAD readings increased as the season progressed.

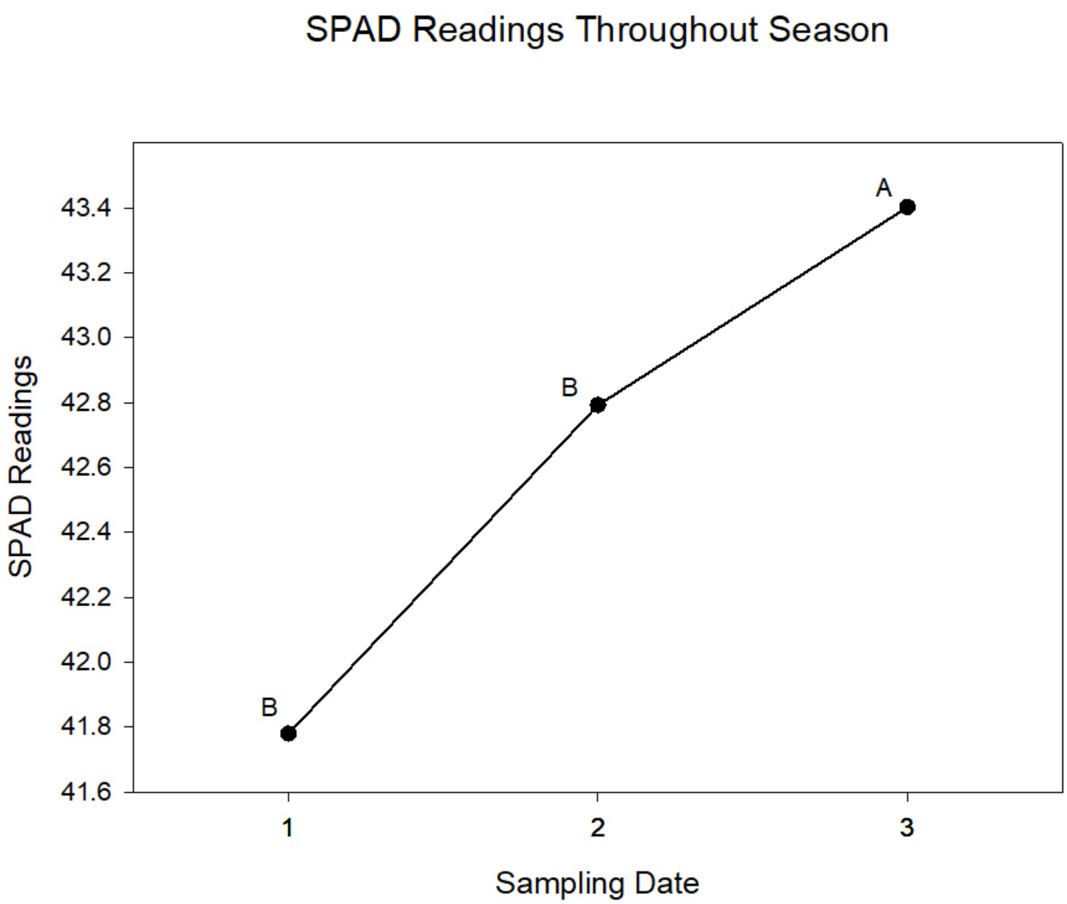


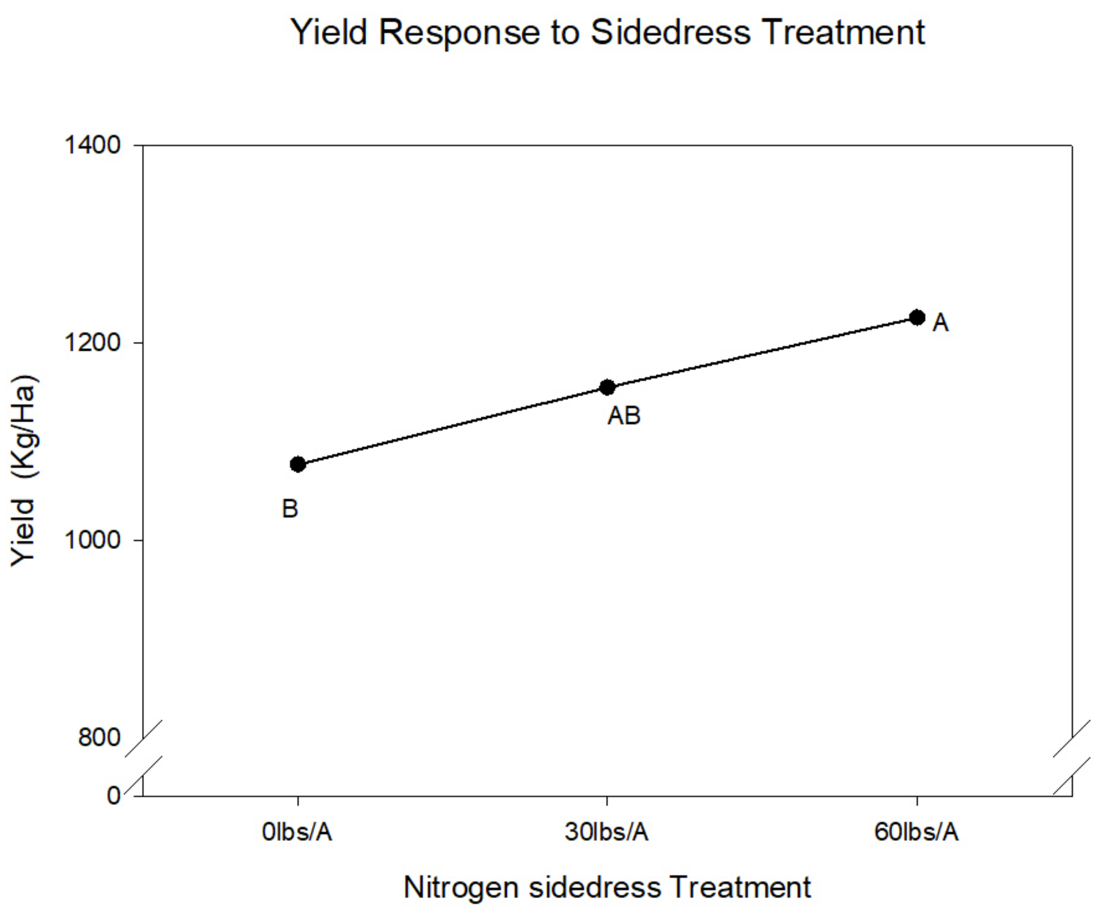
Table 4. SPAD readings different nitrogen sidedress treatments. The highest nitrogen sidedress rate corresponded to the highest SPAD reading. The no sidedress nitrogen treatment had the lowest SPAD readings.

Sidedress Treatments	SPAD Readings
0 lbs./A	40.55 ^{C}
30lbs./A	41.45 ^{B}
60lbs./A	42.05 ^{A}

Table 5. SPAD reading differences between different cover crop termination timings. No cover crop treatment had the highest SPAD readings followed by 2WAP and No Termination treatments. The earlier termination timings had the lowest SPAD reading, 2WBP and at planting.

Termination Timing	SPAD Readings
No Cover Crop	43.41 ^{A}
2 Weeks before Planting	42.31 ^{B}
At Planting	42.48 ^{B}
2 Weeks after Planting	42.92 ^{AB}
No Termination	42.52 ^{AB}

Figure 3. Yield response from sidedress treatments. Cover crop treatments did not significantly affect yield only sidedress nitrogen rate. The yield response of nitrogen rates is linear.



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