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AGRONOMY AND SOILS

Defoliant Effects on Cover Crop Germination, Cover Crop Growth, and Subsequent Cotton (*Gossypium hirsutum*) Development

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ABSTRACT

The price of nitrogen (N) fertilizer has increased to the point where it may be cost effective to grow winter legume cover crops as a sole source of nitrogen for a subsequent cotton crop in North Carolina. Establishing these cover crops is critical to the success of this strategy. In order to optimize legume cover crop establishment, cotton producers may have to overseed legumes into cotton that has or will be sprayed with cotton harvest aids, which may interfere with legume germination and growth. A greenhouse experiment was conducted to determine the effects of commonly used cotton harvest aids on legume germination and growth. This was followed by a field study to determine the optimum time to overseed legume cover crops in cotton, to determine the effects of cotton defoliants on legume establishment in the field, and to determine the effects of cover crop species and overseeding timing on cotton growth and yield in a field in which N was not depleted. Cotton defoliants containing thidiazuron plus diuron reduced greenhouse legume germination and growth more than any other cotton harvest aid tested; however, field studies indicate that cover crop germination and cover crop dry weight are not affected by thidiazuron plus diuron. Crimson clover (*Trifolium incarnatum* L.) and Austrian winter pea (*Pisum sativum* L.) positively affected cotton yield equally. However, timing of cover crop overseeding played an important role in cover crop germination, accumulated biomass, and lint yield. We observed that overseeding legumes 14 days prior to defoliation resulted in the highest cover crop dry weight and cotton yield.

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The average cost of nitrogen (N) has remained relatively constant at $ 0.48 kg^{-1} N^{-1} (30% solution) from 1974 to 2000; since then, the cost has rapidly increased. The latest five- year (2006 to 2011) average cost is $1.20 kg^{-1} N^{-1} from urea ammonium nitrate (UAN) (United States Department of Agriculture-Economic Research Service (USDA-ERS), 2012). This rising cost of N has reinvigorated research involving legume cover crops as an alternative source of N. Rain-fed cotton may be a good crop to incorporate legume cover crops into because it is planted in early to mid-May in the southeastern United States (U.S.) (Edmisten, 2012a); it is herbicide tolerant enabling post-emergent termination of cover crops, and it requires less than 100 kg N ha^{-1} to obtain high yields (Crozier et al., 2012). Recent studies involving summer annual crops and legume cover crops indicates that May cotton plantings may be synchronized with high spring cover crop biomass production and timely N release and mineralization of legumes such as crimson clover (*Trifolium incarnatum* L.), Austrian winter pea (*Pisum sativum* L.), and hairy vetch (*Vicia villosa* Roth) (Cook et al., 2010; Norsworthy et al., 2010; Parr et al., 2011).

Growers in North Carolina face unique challenges associated with cotton when they incorporate legume cover crops into cotton production systems. In order to achieve high germination and growth prior to winter, legume cover crops should be planted from early-September to early-October (Crozier et al., 2011). The presence of un-harvested cotton and the use of chemical harvest aids further complicates legume planting. Also, growers are reluctant to divert limited time and resources away from cotton harvest, which may continue into November, to plant cover crops. Growers are more likely to plant an income-producing crop such as wheat, even if cotton was harvested in early September and fields were prepared for planting. Therefore, in order to plant cover crops during a busy time of the year, growers must adopt some non-traditional practices that require little investment in time, such as aircraft overseeding, or overseeding with their high clearance sprayer. Overseeding legumes into standing cotton presents another problem due to potential cotton harvest aid effects on legume germination, growth, and development.

Harvest aids function as defoliants, boll openers, and desiccants, and are typically applied to cotton approximately 14 days prior to harvest to assure timely harvest. Benefits of proper defoliation include: 1) removal a major source of trash and staining; 2) more efficient picker operation; 3) quicker crop drying; 4) reduced boll rot; 5) reduced plant lodging; and 6) promotion of earliness and yield (Edmisten, 2012b). Harvest aids can be classified as either herbicidal or hormonal and work in a variety of ways to promote abscission, leaf drop, and boll opening. They are often used in mixtures to obtain optimal results (Edmisten, 2012b). Ultimately, most of these chemicals work by stimulating ethylene synthesis in the cotton plants, either by injuring the plant or by direct exposure to ethylene-producing compounds, by disrupting auxin transport, or by acting as a cytokinin to promote ethylene synthesis (Burton et al., 2008; Edmisten, 2012b; Radhakrishnan et al., 2009). Response by legume seeds when exposed to harvest aid chemicals is relatively unknown or not documented.

Some of the most commonly used harvest aids used in North Carolina contain thidiazuron (TDZ). Manufacturers' labels of cotton harvest aids containing TDZ caution users to avoid planting legumes within two months of application (Anonymous, 2005). Thidiazuron is a growth regulator that mimics cytokinin-like activity and may lead to a wide variety of responses in plants depending on the species (Radhakrishnan et al., 2009). Thidiazuron can cause leaf abscission in some Malvacea species including cotton, release dormancy in lateral buds of apple (Wang et al., 1987), encourage shoot regeneration in faba bean (*Vicia faba* L.) cotyledonary nodal explants ((Khalafalla and Hattori, 2000), and promote shoot and root formation on soybean [*Glycine max* (L.) Merr.] explants in vitro regeneration studies (Radhakrishnan et al., 2009). Other commonly used harvest aids contain ethephon, which is absorbed by plant tissue and broken down into ethylene to act as a plant growth regulator. Ethylene has been shown to promote seed germination, alleviate thermo-inhibition, and may even be involved in breaking seed dormancy (Linkies and Leubner-Metzger, 2012). Cyclanilide is sometimes used in combination with ethephon as a synergist to accelerate defoliation and boll opening. Little is understood about how this material physiologically functions; however, its mode of action has been attributed to disruption of polar auxin transport possibly by interaction with indole-3-actic acid (IAA) transport proteins (Burton et al., 2008).

Several harvest aid materials in the protoporphyrinogen oxidase (PPO) inhibitor or photosystem II (PSII) electron flow disruptor class of herbicides may directly prevent seed germination and photosynthesis of developing seedlings, respectively, in some plant species. Acifluoren, a similar PPO inhibiting herbicide to cotton defoliants, is commonly used as a soybean herbicide and was found to reduce lentil (*Lens cullinaris* Medikus) germination in greenhouse studies (Wright et al., 1995). Diuron, a PSII herbicide, is commonly used in combination with thidiazuron as a cotton defoliant and should not be applied to alfalfa (*Medicago sativa* L.) stands established less than one year indicating potential injury to developing legume seedlings (Anonymous, 2009). Cotton harvest aids have the potential to affect legume germination and development in many unpredictable ways, especially when they are used in combination with each other.

In order to refine the practice of overseeding legumes in cotton, an experiment was conducted (greenhouse experiment followed by a field experiment) to determine the effects of harvest aids on legume germination and legume growth, and to determine the proper time to overseed. The greenhouse portion was conducted to compare harvest aid materials representing several modes of action to determine a "worst case" combination of two legume species and harvest aid material. The field portion of this study incorporated the previously selected legume species and the most injurious harvest aid material found in the greenhouse study to test several legume overseeding dates. Legume germination, legume biomass, cotton morphological response and cotton yield were documented.

MATERIALS AND METHODS

A greenhouse experiment was conducted at the North Carolina State University Method Road Greenhouse Complex in Raleigh, North Carolina in 2009 followed by a field experiment on a Goldsboro fine sandy loam soil (fine-loamy, siliceous, thermic, Aquic Paleudults) near Rocky Mount in 2010 and on a Norfolk loamy sand soil (fine-loamy, siliceous, thermic, typic) in Clayton in 2011. The greenhouse experiment consisted of a split plot design including three cover crops and eight cotton defoliation treatments to screen for deleterious defoliant effects on legume germination and seedling growth. After determining defoliant effects on each cover crop in the greenhouse, the defoliant representing the worst case scenario was selected to be used on the two most sensitive legume crop species in subsequent field experiments. Field experiments consisted of a factorial arrangement of treatments within a randomized complete block design and included two cover crops, three cover crop planting dates, and two cotton defoliation treatments.

Greenhouse Experiments. Eight defoliation treatments, with different modes of action, were applied to three legume cover crops. Three cover crop species (Austrian winter pea, crimson clover, and hairy vetch) were selected to represent a wide variety of seed sizes expressing a great deal of germination variability, when overseeded in cotton. One hundred seeds of each species (300 total) were planted in a 46 cm by 66 cm sterilized tray containing sterilized Norfolk loamy sand soil (6 cm depth) which served as the experimental unit. Seeds were equally spaced in nine rows and randomly planted within trays. Each tray was then sprayed with one of the following defoliants using a Teejet® XR11002 even flat tip at 4.8 km h^{-1}calibrated to 94 l ha^{-1}: 1) thidiazuron (Dropp® SC, Bayer CropScience, Research Triangle Park, NC) at 0.22 kg a.i. ha^{-1}; 2) carfentrazone-ethyl (Aim® EC, FMC Corporation, Philadelphia, PA) at 0.28 kg a.i. ha^{-1}; 3) thidiazuron plus diuron (Ginstar® EC, Bayer CropScience, Research Triangle Park, NC) at 0.088 kg a.i. ha^{-1} thidiazuron plus 0.044 kg a.i. ha^{-1} diuron; 4) urea sulfate plus ethephon (FirstPick™, Nufarm Americas Inc., Burr Ridge, IL) at 1.28 kg a.i.ha^{-1}; 5) ethephon phosphonic acid (Prep™, Bayer CropScience, Research Triangle Park, NC) at 2.23 kg a.i.ha^{-1}; 6) phosphorotrithioate (Def® 6, Bayer CropScience, Research Triangle Park, NC) at 1.68 kg a.i. ha^{-1}; 7) ethephon phosphonic acid plus cyclanilide (Finish® 6 Pro, Bayer CropScience, Research Triangle Park, NC) at 1.68 kg a.i. ha^{-1} ethephon plus 0.11 kg a.i. ha^{-1} cyclanilide, and 8) no defoliant (NTC). Seed trays were then covered with sterilized sand (0.5 cm depth), lightly watered, and randomly placed on a bench under scheduled irrigation to promote germination. Germination data and shoot biomass was collected by cutting seedlings at the soil line with a razor blade 14 to 19 days after planting (DAP), they were then forced-air dried at 90°C for 48 hours and weighed using a digital scale, except in run #1 where no shoot weights were taken. Germination percent was determined by counting live plants. Treatments were replicated four times and the experiment was repeated three times, planting dates were 1 May 2009, 29 May 2009, and 25 June 2009.

The experimental design was a split-plot with defoliant serving as the whole plot unit and cover crop species serving as the sub-plot unit. Germination rating and shoot biomass data were subjected to analysis of variance (ANOVA) using the general linear model in SAS (Version 9.2, SAS Institute, Cary, NC) for a three (runs) by three (legume species) by eight (defoliant) treatment structure. Means of significant main effects and interactions were separated by Fisher's Protected LSD at $p \leq 0.05$.

Field Experiment. Results from the greenhouse experiment indicated that hairy vetch was least affected by harvest aids; therefore, vetch was omitted from field experiments. Crimson clover and Austrian winter pea were overseeded into standing cotton on three dates; 14 days prior to cotton defoliation, immediately prior to defoliation, and immediately prior to cotton harvest. Legumes were inoculated with commercial innoculants, Nitragin C (Nitragin Inc. Milwaukee, WI) for Austrian winter pea and Nitragin R (Nitragin Inc., Milwaukee, WI) for crimson clover, and spread over four cotton rows using a hand spreader calibrated to deliver 28 kg ha^{-1} actual live seed in 3.6 m by 9 m plots. Legume overseeding in Rocky Mount took place on 16 September 2009, 30 September 2009, and 19 October 2009. Legume overseeding took place in Clayton on 17 September 2010, 12 October 2010, and 26 October 2010. Cotton defoliation treatments consisted of no defoliant or thidiazuron plus diuron at 0.088 kg a.i. ha^{-1} and 0.044 kg a.i. ha^{-1}, respectively, applied with a CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1}. All cotton stalks were shredded within four days of cotton harvest. Legume germination was determined in early-March by randomly selecting two locations within the center two rows of each plot and counting live plants inside 0.25 m^2 quadrants. Cover crop biomass was obtained immediately prior to termination by removing all shoot biomass within two 0.25 m^2 quadrants randomly placed within the center two rows of each plot. Shoot biomass was placed in cloth sacks, dried at 75°C for 72 hours, and weighed. Cover crops were terminated 11 days prior to planting in Rocky Mount (27 April 2010) and nine days prior to cotton planting in Clayton (2 May 2011), using paraquat (Gramoxone Inteon®, Syngenta Crop Protection, Greensboro, NC) at 1.12 kg a.i. ha^{-1} plus diuron (Direx® 4L, Makhteshim Agan of North America Inc., Raleigh, NC) at 0.90 kg a.i. ha^{-1} plus a non-ionic surfactant (Induce®, Helena Chemical Co., Collierville, TN) at 0.25 % V/V using a backpack sprayer calibrated to 140 L ha^{-1}.

Cotton was established using a strip tillage tool (KMC 2-36, Kelley Manufacturing Company, Tifton, GA) at 91 cm row width on 7 May 2010 and 11 May 2011. The cotton cultivar 'WRF485' (PhytoGen, Dow AgroSciences, Indianapolis, IN) was planted at 1.5 cm depth after strip tillage at 14.8 seeds m-row^{-1} on the same day. After planting, the experiment received a broadcast treatment of lambda-cyhalothrin insecticide (Karate®, Syngenta Crop Protection, Greensboro, NC) at 0.022 kg a.i. ha^{-1} to prevent cutworm (*Noctuidae* spp.) damage. No nitrogen was applied to cotton during the growing season; otherwise, cotton was grown in accordance with North Carolina Cooperative Extension recommendations (Bacheler, 2012; Crozier et al., 2012; Edmisten, 2012a; Edmisten, 2012b; York, 2012).

Cotton height, total nodes, number of total bolls, and sympodial bolls were collected from six randomly selected plants from the middle two rows of each plot prior to harvest. The center two rows of each plot were machine harvested with a two-row spindle picker modified for small-plot research. A one kg sample of seed cotton was collected from each plot during harvest to determine lint percentage using a 10-saw laboratory gin (Continental Gin Co., Birmingham, AL). Cotton was harvested on 9 September 2010 in Rocky Mount and 5 October 2011 in Clayton.

The experimental design was a randomized complete block design (RCBD) consisting of a factorial arrangement of two (defoliant) by two (legume species) by three (legume planting date) main plot factors. Cover crop germination, cover crop biomass, cotton yield, plant height, total nodes, sympodial bolls, and total bolls data were subjected to ANOVA using the general linear model in SAS for a two (sites) by two (legume species) by two (defoliants) by three (overseed dates) treatment structure. Treatment means for significant main effects and interactions were determined using Fisher's Protected LSD at $p \leq 0.05$.

RESULTS AND DISCUSSION

Greenhouse Experiments. Cover crop germination was affected by defoliation treatment, but not consistently across run, cover crop, or defoliation treatment due to the presence of run by legume species by defoliant treatment interaction (Table 1). This interaction was attributed to soil moisture, cloud cover, and air temperature variability. The greenhouse irrigation system was automatically controlled but the controller had to be manually adjusted to minimize over-watering during cloudy periods. Environmental conditions conducive to cover crop germination improved with run as the experimenter gained experience with the irrigation system. Hairy vetch germination ranged from 73 to 93 %; however, it was not significantly affected by any defoliation treatment regardless of run. Crimson clover and Austrian winter pea germination were reduced by TDZ plus diuron only in run 3 compared to the untreated check. However, crimson clover germination was reduced by ethephon phosphonic acid, TDZ, and TDZ plus diuron in run 2 compared to the untreated check. Although not significant, crimson clover germination tended to be lower when carfentrazone, ethephon plus cyclanilide, and urea sulfate plus ethephon defoliation treatments were applied in runs 2 and 3, compared to the non-treated check. These results indicate that most defoliants have the potential to reduce crimson clover germination, and response to defoliation treatments are species dependent.

Cover crop dry weights were negatively affected by several defoliation treatments in runs 2 and 3 (Table 2). Thidiazuron plus diuron reduced dry weights more than any other defoliant treatment across all cover crops and greenhouse runs compared to the non-treated control. The TDZ plus diuron treatment reduced crimson clover, hairy vetch, and Austrian winter pea dry weight by a minimum of 50% in both runs compared to the non-treated control. Since no defoliation treatment had any effect on vetch germination, it was not selected for subsequent field testing in order to minimize the size of the field experiment. Thidiazuron plus diuron exhibited the greatest potential to reduce cover crop germination and biomass; therefore, it was chosen as the defoliation treatment for subsequent field experiments.

Table 1. Legume germination (%) in greenhouse by defoliant treatment.

Defoliation treatment	Run 1			Run 2			Run 3		
	Clover	Pea	Vetch	Clover	Pea	Vetch	Clover	Pea	Vetch
	% germination								
NTC[z]	81.8	90.7	84.5	81.5 a	93.0	91.0	81.5 ab	94.3 a	87.0
Carfentrazone	71.3	69.8	79.0	73.8 ab	93.5	93.3	79.0 ab	94.8 a	88.0
Phosphorotrithioate	85.0	84.5	82.0	70.5 abc	88.3	86.5	83.0 a	89.5 ab	86.0
Ethephon plus Cyclanilide	82.8	80.5	85.5	69.5 abc	92.8	85.0	69.0 b	92.8 a	87.3
Urea Sulfate/Ethephon	87.8	83.5	90.0	75.5 ab	90.5	87.8	73.5 ab	93.0 a	87.0
Ethephon Phosphonic Acid	68.9	81.5	77.3	67.0 bc	89.5	87.8	70.8 ab	94.3 a	87.0
Thidiazuron	78.8	88.0	73.2	65.5 bc	89.0	84.5	71.0 ab	93.5 a	87.3
Thidiazuron plus Diuron	70.5	77.3	82.0	58.5 c	86.8	91.5	47.8 c	84.3 b	85.3
P > F	0.1079	0.1745	0.3593	0.0307	0.4222	0.4778	0.0030	0.0123	0.9662
LSD	NS	NS	NS	12.2	NS	NS	12.7	5.6	NS

Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[z] Denotes non-treated control.

Table 2. Legume dry weight in greenhouse as influenced by defoliant treatment.

Defoliation treatment	Run 2			Run 3		
	Clover	Pea	Vetch	Clover	Pea	Vetch
	g 100 plants^{-1}					
NTC[z]	0.83 a	5.09 a	1.58 a	1.19 ab	4.90 a	1.64 a
Carfentrazone	0.64 b	4.84 a	1.38 ab	1.09 abc	4.59 ab	1.42 ab
Phosphorotrithioate	0.70 ab	4.73 a	1.22 bc	1.43 a	4.58 ab	1.53 a
Ethephon plus Cyclanilide	0.58 b	4.14 b	1.04 cd	0.91 bc	4.28 ab	1.20 bc
Urea Sulfate/Ethephon	0.63 b	4.53 ab	0.77 ef	0.83 c	4.24 ab	1.13 bc
Ethephon Phosphonic Acid	0.57 b	3.97 b	0.99 de	0.96 bc	4.14 b	1.11 bc
Thidiazuron	0.65 b	2.65 c	0.57 fg	0.85 bc	2.68 c	0.89 cd
Thidiazuron plus Diuron	0.38 c	2.56 c	0.52 g	0.36 d	2.23 c	0.62 d
P > F	0.0015	0.0001	0.0001	0.0020	0.0001	0.0010
LSD	0.17	0.58	0.23	0.35	0.67	0.31

Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$.

[z] Denotes non-treated control.

Field Experiment. A three-way interaction of site, by cover crop, by overseed timing was present for cover crop germination, and TDZ plus diuron did not affect cover crop germination (Table 3). Clover and pea germination were not affected at Rocky Mount during 2010 by overseed timing; however, germination of both species was affected by overseed timing in Clayton (Table 4). In Clayton, the lowest germination count was recorded in both clover and pea when the cover crops were overseeded immediately prior to defoliation application. Intermediate levels of cover crop germination were noted when overseeding took place two weeks prior to cotton defoliation. Cover crop germination at Clayton tended to be higher than Rocky Mount. This difference was attributed to higher soil moisture at Clayton. Clayton received 19.3 cm of rain compared to Rocky Mount, which received 7.7 cm of rain during the cover crop overseeding period; furthermore, Clayton received heavy rains totaling 13.0 cm rain during a five day rainy period, 12 days after the first overseeding date. Some of the overseeded clover and pea seed washed into row middles and alley-ways following these heavy rain events; batches of clover and pea seedlings accumulated where water ponded. Heavy rain events were soon followed by unseasonably low air temperatures prior to the second overseeding date; cold and saturated soil conditions had the potential to reduce cover crop germination as observed at Clayton. The lack of germination response to TDZ plus diuron is different than expected based on greenhouse experiments. Defoliation application techniques may explain the lack of cover crop germination response observed in the field. Greenhouse experiments were designed to evaluate a worst case scenario where the defoliation treatments were sprayed directly on the cover crop seed, covered, and watered to move the defoliant into the rooting zone; whereas, field defoliation applications were a foliar spray designed to provide good cotton leaf coverage with minimal contact with soil.

Cover crop dry weight was affected by cover crop species; in addition, an interaction of site by overseed timing was present for cover crop dry weights (Table 3). Crimson clover dry weight, 7,780 kg ha^{-1}, was higher than Austrian winter pea, 5,240 kg ha^{-1}, when pooled over site, overseed timing, and defoliation treatment. Cover crop dry weight was not affected by overseed timing in Rocky Mount during 2010; however, cover crop dry weight was higher when overseeded 14 days prior to defoliation compared to seeding at defoliation and 14 days after defoliation in Clayton in 2011 (Table 5). Soil moisture and air temperature in Clayton during 2011 promoted earlier germination and growth due to higher rainfall and accumulated growing degree days. Cover crops overseeded 14 days prior to defoliation accumulated 276 and 464 degree days, base 50 (DD50), more than cover crops overseeded at defoliation at Rocky Mount and Clayton, respectively. They also received 473 and 628 more DD50's than cover crops seeded at 14 days after defoliation at Rocky Mount and Clayton, respectively. Cover crop germination and dry weight were sensitive to overseed timing when soil moisture and temperature were highly variable. Defoliation treatment did not reduce cover crop dry weight in the field experiment, opposite of what was observed in greenhouse experiments but consistent with cover crop germination field results. The lack of response was attributed to defoliation interception by cotton leaves.

Table 3. P > F for cover crop germination, cover crop dry weight, plant height, nodes per plant, bolls per plant, and lint yield.

Treatment factor	Cover crop germination	Cover crop dry weight	Plant height	Total nodes	Number of Bolls	Lint yield
	p value					
Site	0.0021	0.2307	0.0096	0.0458	0.0574	0.8266
Cover Crop (CC)	< 0.0001	< 0.0001	0.0286	0.0169	0.6652	0.1683
Site x CC	< 0.0001	0.6817	0.1433	0.3150	0.0402	0.8310
Overseed Timing (Time)	< 0.0001	< 0.0001	0.1620	0.1868	0.0935	0.4645
Site x Time	< 0.0001	< 0.0001	0.0672	0.4548	0.0352	0.0247
CC x Time	< 0.0001	0.3620	0.5774	0.2880	0.3336	0.3082
Site x CC x Time	< 0.0001	0.5484	0.0269	0.3059	0.0808	0.2610
Defoliation (Defol)	0.3714	0.0764	0.9112	0.2568	0.3204	0.5271
Site x Defol	0.2333	0.6724	0.6387	0.6977	0.9599	0.7396
CC x Defol	0.6173	0.0666	0.0060	0.0645	0.1646	0.0686
Site x CC x Defol	0.9473	0.7652	0.2031	0.9268	0.7397	0.6838
Time x Defol	0.4050	0.3391	0.3418	0.5920	0.7549	0.4034
Site x Time x Defol	0.2881	0.5313	0.3058	0.0553	0.2564	0.3252
CC x Time x Defol	0.2756	0.6183	0.2589	0.7197	0.7131	0.4058
Site x CC x Time x Defol	0.2199	0.9193	0.9591	0.4114	0.3466	0.2752
Coefficient of variation (%)	42	24	9	5	20	13

Table 4. Cover crop germination as influenced by overseed timing, cover crop, and site.

Cover crop overseed timing relative to cotton defoliation	Rocky Mount 2010		Clayton 2011	
	Clover	Austrian Pea	Clover	Austrian Pea
	Plants m^{-2}			
14 days before defoliation	82 a	35 a	219 b	61 ab
At defoliation	54 a	35 a	98 c	23 b
14 days after defoliation	81 a	40 a	419 a	81 a

Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Data is pooled over defoliation treatment.

Table 5. Cover crop dry weight 10 days before cotton planting, immediately before termination, as influenced by cover crop species, overseed timing, and site.

Cover crop overseed timing relative to cotton defoliation	Rocky Mount 2010	Clayton 2011
	kg ha^{-1}	
14 days before defoliation	6,350 a	10,000 a
At defoliation	5,760 a	5,670 b
14 days after defoliation	5,350 a	5,910 b

Means within site followed by the same letter are not different according to Fisher's Protected LSD at $p < 0.05$. Data is pooled over cover crop and defoliation treatment.

An interaction of cover crop by defoliation and site by cover crop by overseed timing was present for cotton plant height (Table 3). In addition, cotton plant height and node production were affected by cover crop species (Table 3). Cotton planted in clover was taller, 73.2 cm, and contained more nodes, 15.8, when compared to cotton planted in pea, 70.3 cm and 15.3 nodes. Taller cotton plants and higher node production were consistent with higher biomass observed in clover. When cotton was planted into clover in 2010 and 2011, plant height responded positively to cover crop biomass as expected (Tables 5 and 6). Conversely, cotton grew taller when Austrian winter pea was overseeded at defoliation in Rocky Mount during 2010 compared to overseeding 14 days prior to and after defoliation (Table 6). The opposite occurred in Clayton during 2011 when shorter cotton was observed when Austrian winter pea was overseeded at defoliation compared to overseeding at earlier and later dates. Cotton planted in Austrian winter pea in 2010 did not respond to cover crop biomass levels as expected. No explanation can be given for this unexpected cotton response in 2010; however, we can only speculate that the inoculant did not consistently establish itself on the Austrian winter pea across the overseed dates. Additional testing should be considered before selecting Austrian winter pea as cover crop candidate for cotton.

An interaction of site by cover crop and site by overseed timing was present for total bolls per plant (Table 3). A higher number of total bolls were recorded on cotton planted into crimson clover in Clayton during 2011 when clover was overseeded 14 days prior to defoliation compared to later overseeding times; however, overseed timing did not affect total bolls when planted into Austrian winter pea or into clover at Rocky Mount during 2010 (Table 7). Even though the number of bolls per plant was different depending on cover crop seeding time, there was no correlation between boll number and lint yield. Lint yield was only affected by the interaction of site and overseed timing (Table 3). Lint yield was lower when the cover crops were overseeded at defoliation in Rocky Mount compared to overseeding at 14 days prior to and 14 days after defoliation. Contrary to Rocky Mount, lint yield was lower when cover crops were overseeded 14 days after defoliation in Clayton compared to earlier overseeding dates (Table 8). Lint yield and cover crop biomass were consistently higher, regardless of site, when cover crops were overseeded 14 days prior to defoliation. An early overseeding time allowed the cover crops to accumulate more degree-days, produce more biomass, and ultimately increase lint yield. Early establishment is aided by high soil moisture and warm temperatures. Ranells and Wagger (1996) reported higher crimson clover and hairy vetch spring dry matter following favorable fall conditions in North Carolina. These favorable conditions were characterized by 60 % higher monthly rainfall, and 6 °C higher monthly mean temperatures in the six weeks following cover crop plantings. Legume cover crop establishment in standing cotton in Texas was highly dependent on rainfall events or irrigation. Small seeded legumes, clovers and medics, provided high dry matter yields and in the spring following high winter precipitation only. Larger seeded legumes, hairy vetch and Austrian winter pea, proved to be less dependent on timely rainfall after planting (Keeling et. al., 1996).

Table 6. Cotton plant height as influenced by cover crop overseed timing, cover crop species, and site.

Cover crop overseed timing relative to cotton defoliation	Rocky Mount 2010		Clayton 2011	
	Clover	Austrian Pea	Clover	Austrian Pea
	cm			
14 days before defoliation	70.9 a	64.1 b	80.4 a	77.4 a
At defoliation	67.8 a	72.3 a	78.6 a	69.5 b
14 days after defoliation	65.7 a	65.0 b	76.3 a	73.6 ab

Means within a column followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Data is pooled over defoliation treatment.

Table 7. Number of bolls per plant as influenced by cover crop termination timing and site.

Cover crop overseed timing relative to cotton defoliation	Rocky Mount 2010		Clayton 2011	
	Clover	Austrian Pea	Clover	Austrian Pea
	Number of bolls plant^{-1}			
14 days before defoliation	8.1 a	7.0 a	10.5 a	9.9 a
At defoliation	8.8 a	6.9 a	7.5 b	9.3 a
14 days after defoliation	7.2 a	7.6 a	8.4 b	8.9 a

Means within site followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Data is pooled over defoliation treatment.

Table 8. Lint yield as influenced by cover crop timing and site.

Cover crop overseed timing relative to cotton defoliation	Rocky Mount 2010	Clayton 2011
	kg lint ha^{-1}	
14 days before defoliation	1,090 a	1,090 a
At defoliation	1,000 b	1,090 a
14 days after defoliation	1,110 a	990 b

Means within site followed by the same letter are not different according to Fisher's Protected LSD at $p \leq 0.05$. Data is pooled over cover crop and defoliation treatment.

SUMMARY

Defoliation with TDZ plus diuron has little to no effect on crimson clover or Austrian winter pea germination, cover crop biomass accumulation, cotton plant height, cotton total nodes, total bolls, and lint yield in the field. Although cover crop germination was influenced by overseed timing, site, and cover crop species, variability in cover crop germination had little or no effect on cover crop dry weight and cotton lint yield. Cover crop overseed timing was the most important factor affecting cover crop dry weight and lint yield. The highest cover crop dry weights and cotton yields were obtained when cover crops were overseeded 14 days prior to defoliation. Based on this two-year study, overseeding of legume cover crops should be completed as early as possible, preferably prior to defoliation, or during extended periods of high soil moisture and warm soil to promote legume germination and seedling growth. If early overseeding dates are not an option, growers may still consider overseeding cover crops later, as long as they understand that cover crop biomass will decline with growing degree days and cotton may require additional in-crop nitrogen if the level of nitrogen supplied to cotton from the cover crop is inadequate. In a companion experiment, lint yield of cotton following crimson clover and hairy vetch with 4,400 kg ha^{-1} shoot biomass equaled lint yield of cotton without a cover crop plus 70 kg N ha^{-1} of liquid UAN. The net returns justified establishing crimson clover and hairy vetch winter cover crops based on nitrogen savings alone (Foote et.al., 2014). Weed suppression experiments in Georgia estimated that 4,900 kg ha^{-1} biomass of winter cover crop would provide 50% control of *Amaranthus palmeri* in non-disturbed cotton row middles and 80 % *A. palmeri* control was possible above 10,000 kg ha-1

cover crop biomass (Webster et. al., 2013). Selection of cover crop species is important and should be matched to soil type, suitability to overseed establishment, and prevailing weather trends. Crimson clover produced more biomass than Austrian winter pea even though it did not translate into higher yield. Given equal lint yields, the cover crop species that produces more biomass is preferred because it is more likely to reduce early season weed competition (Reddy et al., 2006, Webster et. al., 2013). Previous research also indicates that legume cover crop species perform differently under different soil types. Hairy vetch prefers sandier soils and yet will tolerate more poorly drained soils than crimson clover (Crozier et al., 2011; Parr et al., 2011).

In this experiment, we assumed that defoliant behavior in the greenhouse typified behavior in most North Carolina fields. More specifically, TDZ plus diuron had the greatest potential of all the cotton harvest aids tested to reduce legume cover crop germination and growth in the field. There is no reason to dispute this assumption; however, further experimentation with combinations of defoliants with multiple modes of action is recommended. In an associated experiment conducted adjacent to this experiment in the same field in Clayton during 2011, crimson clover germination and growth were reduced. Even though the overseeding rate, overseeding date, cotton defoliation date, biomass sampling date, and cultural practices were nearly the same, crimson clover dry weight was approximately 50% lower in the associated experiment. Reduced clover growth may have been caused by the different cotton defoliation strategy. In that experiment, a broadcast application of the combination of phosphorotrithioate plus ethephon phosphonic acid plus TDZ was used as the defoliation treatment.

Further research may also be warranted to better determine the effects of defoliants on overseeded winter cover crops by overseeding all cover crops on the same day into cotton of different ages with the same defoliation strategy. Cotton should be planted at three different dates so that overseeding on the same day would approximate 14 days before defoliation, at defoliation, and prior to harvest. By staggering cotton planting dates, overseeded cover crops would effectively experience three different defoliation regimes and thus eliminate any environmental affects due to temperature and soil moisture variability. However, this experiment should be conducted in a southern region of the cotton producing belt since four to five weeks of cotton ages rarely exist in North Carolina due to limited and unpredictable late season degree day accumulation.

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