Conservation buffers, areas of non-crop vegetation integrated into agricultural landscapes, enhance many agroecosystem services. Among other benefits, these buffers provide habitat for wildlife and beneficial organisms such as pollinators and pest enemies. However, non-crop vegetation in agricultural settings can also be a source for crop pests. One buffer strategy, fallowing strips of land adjacent to crop fields, provides critical habitat for threatened wildlife species, but fallow strips may increase in-field weeds by creating a reservoir of weed seeds that spreads into the adjacent crop. Fallow buffers also offer few resources for beneficial organisms that provide important pest management services. It has been suggested that conservation buffer practitioners move away from fallowing towards plantings of native forbs and grasses. Planted buffers provide more resources for beneficial organisms while maintaining good habitat conditions for wildlife. It is not clear, however, how these planted buffers augment or diminish weed dynamics in the agricultural landscape.

Ecological weed management, an integration of many indirect weed management strategies, can be of critical importance to organic growers who are restricted from using conventional weed management tools. The first two studies of this dissertation examined multiple field edge strategies to determine if they have an impact on ecological weed management in organic cropping systems. The first study investigated how the bank of weed seeds in the soil (the ‘weed seedbank’) changed over time in relation to field buffer management, distance away from the field edge, and crop type. Results showed how planted buffers, especially those with native warm season grasses, can reduce the number of weed
seeds that enter the seedbank.

The second study examines how ground dwelling, seed eating organisms (‘weed seed predators’) to the different field buffer management schemes. The results from this study showed that field buffers had little to no influence on weed seed predators or on the seed predation services they provide. However, crop type did heavily influence these organisms as well as seed removal. Fields with crops that provide dense cover, such as hay, experienced more weed seed predation than fields that were more open and had less vegetative cover, such as harvested maize fields. The results from this weed seed predation experiment, however, may reflect conditions that are specific to our system and to the southeastern United States.

One important aspect of the Southeast in relation to weed seed predators is the recent introduction of the invasive red imported fire ant, _Solenopsis invicta_. Although this ant is now present in high numbers inside southeastern U.S. agricultural fields, it is not clear if they are contributing to weed seed predation services. The third study of this dissertation used video monitoring in an agricultural field to collect direct evidence of _Solenopsis invicta_ and their impact on summer annual weeds. The video data suggest that this ant is not contributing to weed seed predation services. It is not clear from the video data, however, what impact these invasive ants have on native weed seed predators.
Conservation Buffers and Ecological Weed Management in Southeast Organic Cropping Systems: Weed Seedbanks and Weed Seed Predators

by
Aaron Freeman Fox

A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Crop Science

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2013

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DEDICATION

To all of my previous mentors who believed in me and who inspired me along the way:

Grant Gentry, Dan Sullivan, Dave Wilson, Paul Hepperly and Laura Lee Lienk

And to my wife, Lani, who inspires me every day.
BIOGRAPHY

Aaron Freeman Fox was born on the Presidio of San Francisco and grew up in Greensboro, North Carolina. He attended Tulane University in New Orleans and spent his junior year of college at the Universidad Complutense de Madrid in Spain. He received his Bachelor of Science in Ecology and Evolutionary Biology with a minor in Spanish along with a departmental award in 2005. After graduation Aaron worked in the cloud forests of Ecuador on a Biological Surveys and Inventories project looking at juvenile Lepidoptera and their parasitoids. Wanting to continue working on biological research projects but looking for a more applied direction, Aaron began a research internship at the Rodale Institute in Kutztown, Pennsylvania. At Rodale, Aaron found his passion for sustainable agriculture. He then moved to San Francisco to work on native plant restoration and environmental education through an internship with the Golden Gate National Parks Conservancy. From there, Aaron moved to the Monterey Bay area of California to serve as an AmeriCorps volunteer with the Watershed Institute at CSUMB. As an AmeriCorps member, Aaron worked on volunteer outreach and education programs focused on school gardens, childhood nutrition, and environmental restoration. Aaron moved back to North Carolina in 2008 to begin work on his doctoral studies under the guidance of Dr. Chris Reberg-Horton and Dr. David Orr. Aaron plans to develop a research and teaching career that enhances the economic and environmental sustainability of food production and that nurtures the next generation of leaders in sustainable food systems. Aaron married Lani Michelle Clough in 2013.
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I would like to extend my gratitude to my advisors, Chris Reberg-Horton and David Orr. Dr. Reberg-Horton’s support and guidance has helped me become an independent researcher, and this project would not have been possible without the resources and expertise of his Organic Cropping Systems laboratory. Dr. Orr is an encouraging mentor who has helped me become a creative researcher and an engaging teacher. I would also like to thank my other committee members, Chris Moorman and Steve Frank. Dr. Moorman has always been available for advice and guidance. Dr. Frank has always motivated me to think beyond the original scope of the project. Along with my committee members, Dr. Michelle Schroeder-Moreno, Dr. Yasmin Cardoza, and Dr. Julie Grossman have been incredibly helpful in guiding me towards a successful graduate career.

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The completion of this dissertation would not have been possible without the help of numerous colleagues and NC State University community members. Scott Wells and I started graduate school together and he has been a constant force throughout my graduate
experience. The weed seedbank work would not have happened without him. Brenda Penny was also critically important in making sure that the weed seedbank study was successful. Carrie Brinton, George Place, and Adam Smith in the Organic Cropping Systems lab as well as Matt Samsel, Matt Miller, Geoff Balme, Sriyanka Lahiri and Sam Marshall in the Biological Control lab were all incredibly inspiring people to work with and many of them were critically important to the successful completion of this dissertation. It took the following army of workers to complete this project: Caroline Sifford, Johanna Martinez, Ryan Van Cleave, Esdras Carbajal, Boo Blount, Wendi Jang, Luke Thornburg, Lynn Stillwell, Angel Cruz, Erika Larsen, Caleb Knox, Dan Parr, Abbey Kates, Joel Argueta, David Pompilio Calderon, Wesley Hancock, Sonisa Thapa, Eric Isley, and John Beck. Special thanks goes to Amy Hoffner without whom this project would never have gotten off the ground and to Elizabeth Cooper who stuck with us through thick and thin.

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CHAPTER 1

*Amaranthus* and *Digitaria* Seedbank Dynamics Associated with Conservation Buffers
Adjacent to Organic Crop Fields

1. Introduction

Vegetative buffers along the edges of crop fields reduce erosion (Yuan et al. 2009), limit herbicide runoff (Krutz et al. 2005), and provide habitat for wildlife (Palmer et al. 2005; Moorman et al. 2012; Plush et al. 2013), pollinators (Wratten et al. 2012), and predators of pests (Jonsson et al. 2008). However, farmers may view vegetative buffers as reservoirs for pests (Marshall 1989, 2002; Klein 1997). Weed abundance is often greatest near field edges (Marshall 1989; Wilson and Aebisher 1995; von Arx et al. 2002; Jelinek 2004), which may be due to weedy field borders contributing to in-field weeds (Theaker et al. 1995). Because vegetated field borders are perceived to negatively affect crop production, many extension publications recommend treating field borders with herbicides (e.g. Boyd 2008; Klein et al. 2012).

Buffers of fallow, unmanaged volunteer vegetation have particular benefits for quail and other animals that depend on early successional habitat (Gray and Teels 2006; Riddle et al. 2008). However, many of the plant species in these fallow areas are agronomic weeds (e.g. Burger 2005; Conover et al. 2009; Moorman et al. 2012). Patches of these weeds, when allowed to produce seeds, can quickly grow and spread over subsequent seasons (Thill and Mallory-Smith 1997; Beckie et al. 2005).

Preventing weed seeds from entering the soil is an important weed management strategy because the weed seedbank is the main source of new weeds in agricultural fields.
Increases in the weed seedbank lead to greater management costs in subsequent seasons (Hartzler and Roth 1993; Taylor and Hartzler 2000). Conservation buffers that limit the growth of the weed seedbank while still providing habit for wildlife would be more attractive to growers than fallow buffers. Vegetative buffers planted in non-weedy species may be better at preventing the spread of weed seeds because agronomic weed species in these buffers are suppressed through interspecific competition (Marshall and Moonen 2002). Planted buffers also provide better habitat than fallow buffers for parasitoids of crop pests and pollinators because the plantings can provide necessary nectar resources for these organisms (Olson and Wäckers 2007).

The influence of planted buffers on the weed seedbank is especially important to certified organic growers in the United States who are legally required to have distinct buffer zones next to adjoining non-organic land and are required to develop habitat for beneficial organisms. We compared multiple field edge strategies, including planted buffers, to determine their affect on summer annual weed seeds in the seedbank. We hypothesized that planted buffers would contribute fewer weeds seed to the seedbank than fallow buffers and would reduce the spread of weed seeds into adjoining crop fields.

2. Methods

Our research was conducted at the Center for Environmental Farming System’s (CEFS) Organic Research Unit (ORU) in Goldsboro, NC during 2009, 2010, and 2011. Nine fields (2.5 – 4 ha each) in the ORU were used for this study. The experimental design was a split-plot design with multiple levels of nesting. The hierarchy of variables was crop type, field buffer type, distance into the crop field from the buffer (repeated measure) and year.
(repeated measure). Each factor was randomly assigned within the level above except for the two repeated measures (see Fig. 1). The crops followed a typical organic rotation for the southeastern United States: soybeans (*Glycine max*) followed by maize (*Zea mays*) followed by hay. Each crop was planted in three of the nine fields every year. The first two years the hay crop consisted of orchardgrass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.). The third year the hay crop consisted of sorghum sudangrass (*Sorghum bicolor* subsp. *drumondii* (Stead.) de Wet ex Davidse), cowpea (*Vigna unguiculata* (L.) Walp.), and forage soybean (*Glycine max* (L.) Merr.).

Four experimental field buffer types were established around the borders of each field in the spring of 2008. All field buffers were approximately 91.44 x 9.14 m (0.08 ha). The four buffer treatments were 1) planted native-warm season grasses and native prairie flowers (hereafter, NWSG/Flowers), 2) planted native prairie flowers only (hereafter, Flowers Only), 3) fallow, unmanaged vegetation (hereafter, Fallow), 4) unmanaged vegetation mowed 2–3 times per month (hereafter, Mowed). The flower species used for the Flowers Only and NWSG/Flowers buffers were lance-leaved coreopsis (*Coreopsis lanceolata* L.), purple coneflower (*Echinacea purpurea* (L.) Moench), black-eyed Susan (*Rudbeckia hirta* L.), butterfly milkweed (*Asclepias tuberosa* L.), common milkweed (*Asclepias syriaca* L.), swamp sunflower (*Helianthus angustifolius* L.), white heath aster (*Symphyotrichum pilosum* (Willd.) G.L.Nesom), and showy goldenrod (*Solidago speciosa* Nutt.). The perennial bunch grasses planted in the NWSG/Flowers buffers were indiangrass (*Sorghastrum nutans* (L.) Nash) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash).
Planted field buffers were established in the spring of 2008. Throughout the 2008 growing season, planted borders were mowed 5–6 times to reduce weed competition and to promote stand establishment. Following the 2008 growing season, no further management was performed on planted border treatments. Fallow borders were first tilled in the autumn of 2007 and then vegetation was permitted to grow undisturbed for the duration of the study.

2.1 Soil Sampling and Weed Seed Recovery

Soil sampling for weed seedbank analysis was done in the winter (January/February) of 2009, 2010, and 2011 after summer annual weeds had shed their seeds and before winter annuals reached maturity. Mohler and Callaway (1995) argue that seedbanks sampled at this time of year reflect long-term population trends rather than the effect of a particular event. Samples from the winter of 2009 were used as a baseline for comparison with 2010 and 2011 samples. Soil augers, 10 cm diameter by 25 cm deep, were used to collect soil samples at measured intervals along transects extending from the middle of each experimental field buffer into the adjacent crop field. Three soil cores were taken from each transect interval and thoroughly mixed into one composite sample. The first set of cores was taken from the center of the field buffer and the second set at the interface of the field buffer and the crop field (6 m from the field buffer center). The remaining three sets of soil cores were taken at increasing distances away from the field buffer center (12 m, 20 m and 35 m away from the field buffer center). In total there were 180 composite soil samples collected per year.

Soil samples were transported to the laboratory in a cooler. They were then dried in a forced air oven at 40°C for 48 h. After drying, a 1 kg subsample (roughly one third) of each
composite sample was removed for further processing and analyzing. These subsamples were processed in an elutriator (protocol modified from Gross and Renner 1989) with 2 mm and 500 µm sieves. Samples from the two sieves were dried at 40°C for 24 hours. The 500 µm sieved samples were further processed in a South Dakota Seed Blower to remove chaff and seed husks and to separate samples into more manageable units. Using a dissecting microscope, weed seeds were removed from these units and an unimbibed seed crush test (Sawma and Mohler 2002) was used to determine seed viability. Viable weed seeds were identified to genus and counted.

2.2 Estimating changes in the weed seedbank and statistical analysis

The percent change in the weed seedbank for the most prominent summer annual broadleaf and grass genera was estimated by taking the log number of seeds for 2010 and 2011 and comparing each to the log number of seeds from 2009: \( Y = \log(z_a) - \log(x) \) Where \( Y \) is the percent change, \( x \) is the number of baseline seeds from 2009 and \( z_a \) is the number of seeds from year ‘a’.

An analysis of variance was conducted, which included multiple layers of nesting and two repeated measures, using the GLIMMIX procedure of SAS® Version 9.2 (SAS 2008). The year factor was used as the random residual factor. Means were separated with a Fisher’s protected LSD test \((P < 0.05)\).

3. Results and Discussion

Thirty-two thousand two hundred eighty-four viable summer annual weed seeds were removed from all three years’ soil samples. Fourteen summer annual weed genera were detected in our system’s seedbank. Of these genera, *Amaranthus* was the most common
broadleaf genus \( \text{mean} = 45.3 \text{ seeds/kg sample } SE = 5.4 \), and \textit{Digitaria} became the most common grass genus \( \text{mean} = 32.0 \text{ seeds/kg sample } SE = 4.8 \).

Although \textit{Amaranthus} contributions to the seedbank were high inside many of the fallow field buffers during the first year (Fig. 2), the overall trend was a reduction of \textit{Amaranthus} in the seedbank within all field buffers (Fig. 2) (Fig. 3). Also, \textit{Amaranthus} contributions to the seedbank was not statistically different between buffer treatments \( (P = 0.6) \) (Table 1).

The natural succession of agricultural lands left to fallow quickly transition from herbaceous annuals to a system dominated by perennial species (Keever 1950; Meiners et al. 2002). Because up to 93% of \textit{Amaranthus} seeds can lose their viability after twelve months in the soil seedbank (Taylorson 1970) a reduction in above ground \textit{Amaranthus} plants due to successional processes may lead to reductions in the number of \textit{Amaranthus} seeds in the soil. Similarly, herbaceous broadleaf summer annuals can be reduced by mowing (Donald et al. 2001), and by planting non-crop species such as those in our planted field buffer (Wratten et al. 2012). These reductions in plant numbers combined with the rapid decay of \textit{Amaranthus} seeds in the soil may explain why we saw \textit{Amaranthus} seedbank reductions within all field buffer types.

Distance into the crop field affected \textit{Amaranthus} contributions to the seedbank \( (P < 0.001) \) (Table 1). As distance away from the field buffer center increased, \textit{Amaranthus} contributions to the seedbank increased (Fig. 3). This trend was not an artifact of a difference between field buffer and in-field seedbanks because in-field seedbanks analyzed separately also had a statistically significant distance effect \( (P = 0.04) \), and the greatest seedbank
additions were often seen at the end of the transect (Fig. 3). However, there was also an interaction between crop type and distance from the field buffer ($P=0.001$) because *Amaranthus* contributions to the seedbank in soybean fields were much greater than in maize and hay fields ($P\leq 0.02$) (Fig. 3). Weed populations can become severe in organic soybeans (Cavigelli et al. 2008). Weed management is limited in soybean because the most common intra-row weed management tool for organic soybean production, postplant rotary hoe cultivation, can damage soybean crops if used too often and weather delays compromise its efficacy (Place et al. 2009). These same limitations are not present in other organic crops, and maize and hay in our study had lower *Amaranthus* seed contributions than soybean (Fig. 3). For many distances along the transect, the seedbank was reduced in maize and hay (Fig. 3). There are a number of reasons why maize and hay did not have the same increases in *Amaranthus* seeds as soybean. Maize in our system is harvested in August, soybean in late November, and the earlier harvest may prevent some *Amaranthus* weed seed production and reduce the contribution to the seedbank. Also, greater *Amaranthus* seedbank additions in soybean than maize may come from better organic weed management options for maize. Extension personnel in our area state that organic growers have fewer weed problems with maize than soy because, unlike soy, in-row weeds in maize can also be managed with furrow burial. Finally, including a hay crop into organic rotations has been shown to reduce summer annual weeds and weed seedbank inputs due to the hay’s phenological differences from annual row crops (Porter et al. 2003; Teasdale et al. 2004; Cavigelli et al. 2008). Thus, *Amaranthus* populations were lower in hay compared to soybean due to reduced soil disturbance and greater shading from the hay.
The trends for *Digitaria* seeds within the system’s seedbank were different from *Amaranthus*. *Digitaria* increased in the seedbank for all field buffer types (Fig. 4). Also, field buffer type influenced *Digitaria* seedbank increases (Fig. 4). There was a field buffer×distance interaction for the changes in *Digitaria* seeds (*P* < 0.001) (Table 1). The Mowed field buffer type had much higher *Digitaria* seed additions in the field buffer (*P* ≤ 0.009) and field interface (*P* ≤ 0.03) distances than the other field buffer types (Fig. 4). Frequent mowing can inhibit some weeds but can select for low growing weeds such as *Digitaria* (Green et al. 2006). Also, during the growing season the Mowed field buffer were mowed once every two weeks, but during the fall, mowing only occurred monthly and seed heads formed. These *Digitaria* seed heads subsequently contributed to the surrounding seedbank.

The seedbank within the NWSG+Flowers field buffer had less *Digitaria* additions than all other field buffers (*P* ≤ 0.02). The perennial bunch grasses planted in the NWSG+Flowers field buffer, especially *Sorghastrum nutans*, are highly productive and outcompete herbaceous annual weeds (Wilsey 2010). The vegetative cover of the NWSG+Flowers field buffers was dominated by *Sorghastrum nutans* with very few annual grass species while the other field buffers contained more *Digitaria* and *Cynodon* grass species (Plush et al. 2013).

The general trend for all field buffer types was that as distance away from the field buffer increased *Digitaria* contributions to the seedbank decreased (Fig. 4). The statistically significant distance effect (*P* < 0.001) (Table 1) for *Digitaria* was not an artifact of differences between field buffer and in-field seedbank dynamics because in-field seedbanks
analyzed separately also had a statistically significant distance effect ($P<0.001$).

There was no significant crop effect for *Digitaria* ($P=0.66$) (Table 1). *Digitaria* was not as successful as *Amaranthus* in the crop fields, especially in soybean. Fu and Ashley (2006) found *Amaranthus* to be more competitive than *Digitaria* in some instances perhaps due to better adaptations to limited water resources. *Amaranthus*, the dominant weed in our cropping system, may have outcompeted *Digitaria* in the crop fields. *Amaranthus* may have been more competitive because it emerges earlier than *Digitaria* and it can grow above the crop canopy.

Overall, the field buffers planted with native warm season grasses were the most effective at reducing weed seed inputs. These results confirm previous studies that showed how diverse plantings along crop field edges suppress weedy herbaceous species (Van der Putten et al. 2000; Asteraki et al. 2004). Our results also contribute to the idea that conservation buffers can provide multiple ecosystems services while having little or no negative impact on crop yield (Stamps et al. 2008). Weed seeds of the dominant broadleaf summer annual, *Amaranthus*, although high in some of the field buffers, did not move into the crop fields from the field buffers, but instead was high in the crop fields due to soil disturbance and inadequate weed management. High *Digitaria* seed contributions at the crop/field buffer interface, however, were likely due to high populations of *Digitaria* in many of the field buffers, especially the Mowed type. Although mowing can reduce some summer annual weed populations and prevent seed heads from forming, this is an active process that requires timely management. Planted buffers on the other hand, especially those that include
native warm season grasses, can passively suppress weeds and prevent weeds seeds from spreading into the crop fields.
Table 1. F and P values of statistical tests of the effects of crop, field buffer type, distance from the field buffer center, distance by crop interaction, distance by field buffer interaction, and field buffer by crop interaction on percent change of two prevalent weed genera in the weed seedbank. P-values are considered significant at $\alpha < 0.05$

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<th>Distance X Field Buffer</th>
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Fig. 1. Map of the experiment at the Center for Environmental Farming Systems in 2009. Crop type, which was randomly assigned to field, served as the main plot with field buffer type as the subplot. Each field has all four buffer types randomly assigned around the edges (4 = frequently mowed grassy buffer; 3 = unmanaged, fallow vegetation; 2 = a mix of planted native prairie flowers; 1 = planted native prairie flowers plus native-warm season grasses).
Fig. 2. Untransformed mean number of *Amaranthus* seeds per kg soil sample taken from the center of each field buffer type ± SE.
Fig. 3. Percent change of estimated least-square mean log number of *Amaranthus* seeds from log baseline by log(+1) of the distance from the center of the field buffer. Data at zero on the x-axis represent the center of the field buffer, data at 0.75 the interface between crop field and field buffer, and subsequent data points represent ever increasing distances into the crop field. For All Crops combined, distance from the field buffer is a statistically significant effect for in-field data (P=0.04) as well as for in-field distances plus field buffer (P<0.001).
Fig. 4. Percent change of estimated least-square mean log number of *Digitaria* seeds from log baseline by log(+1) of the distance from the center of the field border. Data at zero on the x-axis represent the center of the field border, data at 0.75 the interface between crop field and field border, and subsequent data points represent ever increasing distances into the crop field. For All Habitats combined, distance from the field border is a statistically significant effect for in-field data ($P<0.001$) as well as for in-field distances plus field border ($P<0.001$).
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CHAPTER 2
Crop and Field Border Effects on Weed Seed Predation

1. Introduction

Preventing weed seeds from entering the soil is an important weed management strategy because the weed seedbank is the main source of new weeds in agricultural fields. Increases in the weed seedbank lead to greater management costs in subsequent seasons (Hartzler & Roth 1993; Taylor & Hartzler 2000). Westerman et al. (2003b) found postdispersal weed seed predation accounts for greater losses to seedbanks than aging, microbial decay or even disturbances like cultivation. Estimates of annual weed seed losses due to granivory by epigeal vertebrates and invertebrates typically range from 33 to 90% (Baraibar et al. 2012; Harrison et al. 2003; O’Rourke et al. 2006). Even with these high predation rates, determining the ultimate impact of predation on weed emergence and competition with crops has been challenging. Estimate as low as 13% reduction in subsequent emergence (White et al. 2007) to projections of eliminating the need for herbicides (Bohan et al. 2011) have been published.

Despite the potential benefits of weed seed predation, the literature is not conclusive on how to best conserve weed seed predators to create a consistent and dependable weed management strategy. Increasing vegetative diversity surrounding crop fields through set aside programs may enhance this ecosystem service. Menalled et al. (2000) showed that fields adjacent to complex border vegetation experienced increased seed predation. However, other studies have been unable to replicate these results possibly due to small plot sizes (Kollmann & Bassin 2001; Marino et al. 1999).
Due to the diversity of granivores that contribute to weed seed predation services in agroecosystems conservation strategies may not prove effective in all scenarios. Organisms that provide weed seed predation services have various, sometimes opposing habitat requirements (Westerman et al. 2003a). However, despite the vast number of weed seed predator species that occur in an agricultural setting, specific groups of granivores typically dominate. Ants are the predominant granivore in tropical agroecosystems (Chauhan et al. 2010; Risch & Carroll 1986) and in semi-arid areas such as Australia (Jacob et al. 2006) and Spain (Baraibar et al. 2009). Crickets (Ichihara et al. 2011; Westerman et al. 2008), rodents (Booman et al. 2009; Daedlow et al. 2007), or ground beetles (Carabidae) (Fischer et al. 2011; Menalled et al. 2007; Ward et al. 2011) perform weed seed predation services in temperate zones.

The conditions in the humid subtropical climate of the Southeast United States (i.e. the warm temperate zone) raise particular issues for how weed seed predators will respond to increasing vegetative diversity in the agricultural landscape. Warmer average temperatures and infrequent ground freezes in this climate zone mean that many insects are active all year long (Keller 1986; Kimura 1988) and weed seed predators may not seek winter refuge sites. Furthermore, while an earlier study confirmed carabid beetles are the predominant weed seed eating invertebrate in the Southeast (Brust & House 1988) a more recent study found the invasive fire ant, Solenopsis invicta Buren, is now the dominant weed seed predator in the region (Pullaro et al. 2006). These non-native, diurnal, social ants will not respond to conservation strategies the same way as native, nocturnal, solitary ground beetles.
This study was undertaken to examine how field border management in the southeastern U.S. affects seed predator abundance and weed seed predation services. Managed habitats along fields are increasingly present due to cost share programs designed to enhance wildlife habitat in the region, particularly for quail. Multiple field border types were tested, varying in vegetative diversity and management practices. This project was part of a multidisciplinary effort to find a crop field border conservation strategy that maximizes ecosystems services. Our objectives were to determine how vegetative diversity along field borders affects seed predator abundance and seed predation rates and 2) if this effect changes with crop type.

2. Methods

Research was conducted at the Center for Environmental Farming System’s (CEFS) Organic Research Unit (ORU) in Goldsboro, NC during 2009 and 2010. Nine fields (2.5 – 4 ha each) in the ORU were used for this study. The experimental design was a split-plot design with multiple levels of nesting. The hierarchy of variables was crop type, field border type, distance (repeated measure), weed species, and month (repeated measure). Each factor was randomly assigned within the level above except for the two repeated measure factors (distance and month). The crops in these fields followed a typical organic rotation for the southeastern United States: soybean (*Glycine max*) followed by maize (*Zea mays*) followed by hay. Each of these crops was planted in three of the nine fields every year. The first year the hay crop consisted of orchard grass (*Dactylis glomerata* L.) and white clover (*Trifolium repens* L.). The second year the hay crop consisted of sorghum sudangrass (*Sorghum bicolor* subsp. *drummondii* (Steud.) de Wet ex Davidse), cowpea (*Vigna unguiculata* (L.) Walp.),
and forage soybean (*Glycine max* (L.) Merr.). The maize was harvested prior to the start of the experiment each year, and the soybeans were harvested after the last trial each year.

Four experimental field border types were established around the borders of each field in the spring of 2008. The first was a frequently mowed grassy border (Mowed). The other three represented various levels of vegetative diversity, 1) unmanaged vegetation (Fallow), 2) a mix of planted native prairie flowers (Flowers Only), and 3) planted native prairie flowers plus native-warm season grasses (NWSG/Flowers). The flower species used for the Flowers Only and NWSG/Flowers borders were lance-leaved coreopsis (*Coreopsis lanceolata* L.), purple coneflower (*Echinacea purpurea* (L.) Moench), black-eyed Susan (*Rudbeckia hirta* L.), butterfly milkweed (*Asclepias tuberosa* L.), common milkweed (*Asclepias syriaca* L.), swamp sunflower (*Helianthus angustifolius* L.), white heath aster (*Symphyotrichum pilosum* (Willd.) G.L.Nesom), and showy goldenrod (*Solidago speciosa* Nutt.). The perennial bunch grasses planted in the NWSG/Flowers borders were indiangrass (*Sorghastrum nutans* (L.) Nash) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash).

Seed predation assays were conducted during the first two weeks of October and November both years; a September trial was added the second year. Seed predation was measured with point estimates using weed seeds glued to 10×15 cm cards (Westerman et al. 2003b; Davis and Raghu 2010). Twenty-five seeds of three prevalent weed species, redroot pigweed (*Amaranthus retroflexus* L.), broadleaf signalgrass (*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster), or sicklepod (*Senna obtusifolia* (L.) H.S.Irwin & Barneby) were adhered to each card. Seeds were bought from Azlin Seed Service (Leland, MS). Weed
free soil was sprinkled on each card to remove any stickiness. Three cards, each carrying a different weed species, were placed in every exclosure cage.

Exclosure cages followed the design of Davis and Raghu (2010). Each cage consisted of a coarse wire mesh cylinder (20 cm tall by 20 cm in diameter) with a square base and top (40cm by 40cm) made of the same material. The coarse wire mesh had 10 cm by 10 cm openings. The tops were covered with aluminum window screen (mesh size <.25 cm) and had a 20 cm by 20 cm opening in their centers covered with a square piece (23 cm by 23 cm) of removable aluminum window screen secured with Velcro ® strips around its perimeter.

Three types of cages were used for this experiment. The first (ALL) included only the basic 10 by 10 cm coarse wire mesh which allowed all seed predators, including vertebrates, inside to feed on the seeds. The sides and base of the second cage type (INV) were covered with 1.25 by 1.25 cm hardware cloth, which excluded vertebrates, such as mice and birds. However, the cage could easily be entered by invertebrates (Gallandt et al. 2005). The third type of cage (NONE) served as a control and used aluminum window screen (mesh size <.25 cm) to exclude all seed predators.

Each of the three cage types were placed at measured intervals along transects extending from the middle of each experimental field border into adjacent crop fields. The first set of cages was placed at the interface of the field border and the crop field (6m from the field border center). The second set of cages was placed 12 m from the field border center, and the third set 35 m from the field border center. The third set was placed so the cage was >40 m from a neighboring field border type. In total there were 324 total cages per trial each with three seed cards (972 total seed cards per trial).
After two weeks of exposure in the field all of the cards were collected, placed individually into labeled plastic bags, and returned to the laboratory. The number of seeds remaining on each card was counted. Any missing seeds from the cards in the control cages (NONE) were attributed to abiotic factors. Seed predation rates for invertebrates, \( M_i \), were calculated using Abbott's correction formula: \( M_i = (C_i - R_i) / C_i \), where, \( R_i \), is the number of seeds remaining on the INV card, and \( C_i \) is the average number of seeds (by crop and date) remaining on the NONE cards (Abbot 1925). Predation rates for vertebrates, \( M_v \), were calculated with a similar equation, \( M_v = (R_i - R_a) / R_i \), where \( R_a \) is the number of seeds remaining on the ALL card, and \( R_i \) is the number of seeds left on the INV card for that field and field border type at the same distance along the transect. The fraction of seed predation by invertebrates was assumed to be equal in the ALL and INV cages. Following Saska et al. (2008), if \( R_i \) ever exceeded \( C_i \) or if \( R_a \) ever exceeded \( R_i \) by more than 5:4 then the data point was thrown out and if by 10:9 then that rate was converted to zero. Without these deletions the results would show that organisms were adding seeds to the cards, an unlikely scenario. Data exceeding these ratios occurred irregularly, but most commonly in the harvested maize fields where there was comparatively low biotic activity.

Catches from pitfall traps (10 cm diameter) were used to measure the relative abundance (i.e. activity-density [Spence & Niemelä 1994]) of ground dwelling invertebrate weed seed predators. Traps were placed along the transect extending from the middle of each experimental field border. One trap was placed in each of the following locations: in the center of the field border (0 m), at the interface of the field border and crop field (6m from the field border center), and 12, 20, and 35 m from the field border center. Traps were made
of two nested 450 mL plastic containers. To prevent rain overflow, the inside container had holes around the top rim and the outside container had holes in the base. These traps were sealed with a 10cm diameter Petri dish bottom and buried in the ground so that their tops were flush with the soil surface. Traps were opened at the beginning of each month’s predation assay, filled with 50% ethylene glycol solution to a depth of 5cm, and resealed after 96 hours. Invertebrate specimens from each trap were preserved in alcohol and brought back to the lab to be identified and counted. Ground beetles (Carabidae) were identified to species while crickets (Gryllidae) were identified to genus. Ants were counted as one group because the vast majority of the ants in these fields are red imported fire ants. Identifications were confirmed by David Stephan of the North Carolina State University Plant Disease and Insect Clinic (Raleigh, NC).

In September 2010, crop canopy light interception was measured next to each exclosure cage in one soybean field. Fifteen-second measurements of photosynthetically active radiation (PAR) were measured between 10:00 and 14:00 on a cloudless day (Pearcy 1991; Heggenstaller et al. 2006). Below-canopy measurements (PAR$_B$) and above-canopy measurements (PAR$_A$) were both taken with a quantum bar sensor (LI-191SA, LICOR Inc.). Light interception was calculated as (PAR$_A$-PAR$_B$)/(PAR$_A$). These numbers were compared to total number of seeds removed from each cage.

**2.1 Statistical Analysis**

The experimental design, which included multiple layers of nesting and two repeated measures, was analyzed using the GLIMMIX procedure of SAS© Version 9.2 (SAS 2008) to conduct an Analysis of Variance. The month factor was used as the random residual factor.
Point estimates from 2009 and 2010 were analyzed separately due to significant differences between years. Data was not transformed because it met the requirements for normality and homeoscedasticity. Seed predation rates by vertebrates (M_v) and invertebrates (M_i) were analyzed separately because the two variables lack independence (Davis and Raghu 2010). Means were separated with a Fisher’s protected LSD test (P < 0.05).

3. Results

3.1 Field Border Vegetation Effects on Seed Predation

For all three weed seed species field border vegetation treatments did not affect seed removal rates by vertebrates or invertebrates (Table 1). This was not an artifact of testing seed predation in the crop field, away from the border, because there was only one incidence of a border×distance interaction --- *S. obtusifolia* removal by invertebrates in 2009 (*P* =0.03) (Table 1). However, mean separation procedures did not show any border×distance combination greater or less than any other (at \( \alpha =.05 \)).

There was a significant month×field border type interaction for *A. retroflexus* removal by invertebrates in 2009 (*P* =0.027), but this was not due to differences between field border types. *A. retroflexus* removal rates were lower in the Fallow and Mowed field borders during November than in October (*P* =0.002, Fallow; *P* =0.002, Mowed) while the other two field border types did not differ between these months (*P* =0.07, NWSG+Flowers; *P* =0.83, Flowers Only).

3.2 Crop Effects on Seed Predation

Crop type affected seed removal rates (Table 1). During 2009, hay experienced higher removal than maize for *A. retroflexus* (*P* =0.025, vertebrates; *P* = 0.02,
invertebrates) and *U. platyphyla* (*P* = 0.002, vertebrates; *P* = 0.0012, invertebrates) and for vertebrate removal of *S. obtusifolia* (*P* < .001). 2009 vertebrate removal of *S. obtusifolia* was greatest in hay, followed by soybean, and was the lowest in maize fields (Table 2). Crop differences were not as strong when we changed the hay species composition in 2010 (Table 1 and Table 2).

The difference in hay composition between 2009 and 2010 also changed how vertebrate seed removal was affected by distance from the field border. Distance did not influence vertebrate removal of *U. platyphyla* (*P* = 0.68) or *S. obtusifolia* (*P* = 0.22) seeds in the 2009 hay fields where removal rates of both species were high relative to the other crop fields (Table 2). However, in the 2009 annual row crops, maize and soybeans, vertebrate removal rates of *U. platyphyla* and *S. obtusifolia* were affected by the distance away from the field border (*U. platyphyla* *P* = 0.004, maize; *P* = 0.05, soybean) (*S. obtusifolia* *P* = 0.03, maize; *P* < .001, soybeans). During 2009, vertebrate seed predation was greatest near the field borders in the annual row crops (Fig. 1). This crop×distance effect was not present in 2010 despite the fact that vertebrate removal of *U. platyphyla* seeds was highest closer to the field borders in maize and soybean fields but not in hay fields (Table 1 and Fig. 1). The interaction may not have been significant during 2010 because of lower seed removal in the hay fields compared to the previous year ---vertebrate removal rates of *U. platyphyla* seeds in 2010 hay fields (mean = 0.55 SE = 0.08) were lower than in the 2009 hay fields (Fig. 1).

3.3 Activity-Density of Weed Seed Predators

The three invertebrate weed seed predators most commonly detected in the pitfall traps over the 2 years of this study were red imported fire ants, *Solenopsis invicta* (*mean* = 9.5
SE=0.56), field crickets, *Gryllus* sp. (*mean*=4.5 *SE*=0.38), and the ground beetle, *Harpalus pensylvanicus* (*mean*=1.6 *SE*=0.13). Mean activity-density between years was different for *S. invicta* (*P*<.001) and *Gryllus* (*P*<.001), but not for *H. pensylvanicus* (*P*= 0.34) (Table 3). *Solenopsis invicta* activity-density was higher during 2010 than 2009 (2009 *mean*= 3.4 *SE*= 1.4; 2010 *mean*= 13.5 *SE*= 1.5; *P*<.001), and *Gryllus* activity density was higher during 2009 than 2010 (2009 *mean*= 6.7 *SE*= 0.70; 2010 *mean*= 2.2 *SE*=0.70; *P*<.001).

The month of the pitfall trapping affected the activity-density of all three weed seed predators in both years (Table 3). *H. pensylvanicus* and *Gryllus* activity density were highest in October (*H. pensylvanicus* *mean*= *SE*=) (*Gryllus* 2009 *mean*= 12.5 *SE*= 1.1; 2010 *mean*= 4.4 *SE*= 0.78) (Fig. 2). *S. invicta* had the highest (*P*<.001) activity density during September in 2010 (*mean*=22.0 *SE*= 1.8), a month we did not collect in 2009. However, *S. invicta* activity-density was also high in October both years (2009 *mean*= 5.2 *SE*= 1.8; 2010 *mean*= 12.3 *SE*= 1.8) (Fig. 2).

Activity-density for *H. pensylvanicus* was greater in the field border (*P*<.001) and adjacent to the border (*P*<.001) than further into the crop field. There was no difference between activity-density for *H. pensylvanicus* in the center of the field border and adjacent to the field border (*P*= 0.08). *Gryllus* sp. had much higher activity density in the field border than any pitfall in the crop field during 2010 (*P*<.001), but there was no distance effect in 2009 (*P*=0.34) (Table 3) nor any crop×distance interaction (*P*=0.3). Within the crop field, 2009 *S. invicta* activity-density was higher adjacent to the field borders than 20 m (*P*=0.02) or 35 m (*P*=0.01) away from the field border center. During 2010 the activity-density of *S. invicta* adjacent to the field border (6 m away from field border center) was greater (*P*<.001)
than pitfall captures 35 m away from the center of the field border. Activity-density differences between 6 and 12 m ($P=0.002$), as well as between 6 and 20 m ($P=0.02$), were also significant in 2010 for *S. invicta*. The activity-density of *S. invicta* inside the field border was greater during 2010 than at the 35 m distance ($P=0.02$), but was not different from any of the other distances (at $\alpha=.05$).

*Solenopsis invicta* activity-density was not influenced by field border type, but there were instances where the other two common invertebrate weed seed predators showed significant differences in activity-density among field border types (Table 3). The effect field border type had on *H. pensylvanicus* activity-density was due to a month×distance×field border type interaction ($P=0.001$). *H. pensylvanicus* activity-density spiked in October inside the Mowed field border ($mean= 15.2 \ SE=2.5 \ P= 0.02$). Activity-density was especially high inside the Mowed border during October 2010 ($mean=20.2 \ SE=3.5$), and had a marginal significant difference ($P=0.053$) during October 2009 ($mean=10.2 \ SE=3.5$). *H. pensylvanicus* activity-densities were not higher in the Mowed field border during any other month ($P\geq0.50$). There was no effect of field border type on *H. pensylvanicus* activity-density inside the crop field ($P=0.35$) (Table 3).

The activity-density of *Gryllus* during 2010 also differed among the field border types ($P=0.003$) (Table 3), primarily as a result of a spike in field cricket pitfall catches inside the Mowed field border during October ($mean=35.8 \ SE=2.1 \ P<.001$). No other activity-density estimate for *Gryllus* sp. inside the Mowed field border was statistically different than any other field border type during the other months of 2010 ($P\geq0.185$). Field border type was not
significant for *Gryllus* sp. activity-density inside the crop fields *(P=0.35)* (Table 3).

### 3.4 Effects of Crop Type on Weed Seed Predators

Crop type did not affect the activity-density of any of the three invertebrate weed seed predators inside the field borders (Table 3). However, in the crop fields the activity-density of *H. pensylvanicus* and *Gryllus* sp. differed between crops (Table 3) (Fig. 3). *H. pensylvanicus* pitfall catches inside the fields were greater in hay than in maize *(P= 0.01)* and were greater in soybeans than in maize *(P= 0.01)* (Fig. 3). During 2009 *Gryllus* activity-density was greater in hay fields than in maize fields *(P < .001)* and soybean fields *(P < .001)* (Fig. 3). During 2010 *Gryllus* activity-density was greater in soybean fields than in maize fields *(P=0.03)* and marginally greater in hay fields than in maize fields *(P=0.052)*. *S. invicta* had the lowest activity-density in the hay fields in both years (Fig. 3), but captures varied among individual fields of each crop type (Table 3) (Fig. 3).

The correlation between canopy light interception and total seed removal was significant for *the seeds removed from the* (ALL) cages *(r =0.636, P =0.03)*, but it was not significant for the seeds removed from the (INV) cages *(P=0.48)*.

### 4. Discussion

Increasing vegetative diversity along field borders did not enhance weed seed predation rates in crop fields nor did it increase the activity-density of prevalent invertebrate weed seed predators. None of the experimental habitats influenced seed predation rates for any of the three weed species. Although we found three taxa of invertebrate weed seed predators, both native and non-native, none of the field border types increased any of these organisms’ activity-densities.
Our findings contrast starkly with other studies, especially those that have found greater carabid beetle numbers associated with perennial bunch grasses (MacLeod 2004; Menalled et al. 2000; Thomas et al. 1991). This conflict suggests either a regional difference, differences in the structure of the landscape at our location, or differences in production practices from other studies.

Ground-dwelling insects were still active in November at our site. Although sampling in this study did not continue through the winter, others have recorded activity of flies and even minute parasitic wasps through the winter in the humid subtropics (Keller 1986; Kimura 1988). It is not out of the question that weed seed predators could also be active throughout the winter and may not need to seek long-term refuge as they do in more temperate climates.

Also, the southeastern U.S. is more varied than many other production regions, both in terms of more natural habitat scattered amongst production fields and a greater diversity in crop rotations (Lubowski et al. 2006). Many of the fields in our study were surrounded by woodlands and non-crop vegetation. Using a buffer analysis in ArcGIS® (ESRI 2011), we determined that the area surrounding all fields had > 20% non-crop vegetation at the 1-km scale. Non-crop vegetation provides nesting, refuge, and alternative food sites (Bianchi et al. 2006). Twenty percent non-crop vegetation in agricultural landscapes may be a threshold level for other beneficial organisms, such as parasitoids, to have an impact on pest management (Thies et al. 2003). However, availability of non-crop habitat may reduce the effect field level habitat manipulations can have on biodiversity and ecosystem services.
(Concepción et al. 2008; Tscharntke et al. 2012). Increasing vegetative diversity in already diverse landscapes may prove ineffective in enhancing weed seed predation services.

Our results may also contrast with other studies because previous research (e.g. Bohan et al. 2011; Menalled et al. 2000) was conducted in fields that used herbicides. Organic fields have larger resource bases for weed seed predators because of increased weed diversity and abundance (Bengtsson et al. 2009). Thus, weed seed predator activity-density may already be at a maximum in organic fields without conservation efforts. In fact, inverse density-dependent relationships have been seen for seed predation rates relative to background seed density (Westerman et al. 2008).

Unlike field border type, crop type had a large effect on weed seed predation. The reduced weed seed removal rates and lower activity-density of native invertebrate weed seed predators in the harvested maize fields support the hypothesis that increased cover enhances weed seed predation (Heggenstaller et al. 2006; Meiss et al. 2010). The increase in vertebrate weed seed predation rates with increased crop canopy light interception, especially in the dense hay fields, provides further evidence that increased crop cover facilitates greater seed predation. The most abundant vertebrate weed seed predator in our fields was the house mouse, *Mus muscus* L. (Moorman et al. In Press). Foraging house mice and other rodents depend on overhead vegetation to provide protection from predators (Orrock & Danielson, 2009; Davis & Raghu, 2010).

Although *Solenopsis invicta* had the highest invertebrate activity-density, these high ant numbers did not correlate with increased overhead cover or high seed predation rates. The highest *Solenopsis invicta* activity-densities were seen in the harvested
corn fields and in the warmest month, September. Thus, red imported fire ant population levels were most likely spatially dependent on greater disturbance intensity (Pereira et al. 2010) and temporally dependent on elevated soil temperatures (Porter & Tschinkel 1987). Results from our seed predation assays correspond better with the activity-densities of the native invertebrate seed predators, especially _Gryllus_ sp. The activity-densities of native seed predators were high in October, and the especially high seed predation in the 2009 hay fields corresponded with elevated numbers of _Gryllus_ sp.

5. Conclusion

Although agroecosystem weed population regulation by granivores holds great promise, manipulating vegetative diversity along the edges of crop fields does not appear to be a viable conservation strategy for these organisms in the southeastern U.S. However, our results showing a large effect of vegetative cover on seed predation is in accordance with other research (Heggenstaller et al. 2006; Meiss et al. 2010) and should be promoted as a component of ecological weed management programs. Efforts to accomplish this denser cover, though, such as delaying harvest or delaying hay cutting, are not likely to be adopted due to economic constraints. Other suggested approaches, such as eliminating or delaying fall tillage (Gallandt et al. 2005) or using hay species that provide denser cover (Meiss et al. 2010) may be more tenable approaches that work with growers’ rotations and economic restraints.
Table 1. F and P values of statistical tests of the effects of month, crop, field border treatment, distance from the field border habitat center, distance-border interaction, and crop-distance interaction on weed seed removal by vertebrate and invertebrate predators for three weed species. P-values are considered significant at $\alpha$<.05.

| WEED SPECIES | Weed Seed Predator | Month 2009 |  | Month 2010 |  | Crop 2009 |  | Crop 2010 |  | Field Border Type 2009 |  | Field Border Type 2010 |  | Distance from the Field Border 2009 |  | Distance from the Field Border 2010 |  | Distance X Field Border 2009 |  | Distance X Field Border 2010 |  | Distance X Crop 2009 |  | Distance X Crop 2010 |  |
|--------------|-------------------|------------|---|------------|---|----------|---|----------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|------------------------|---|
| A. retroflexus | Vertebrate | 15.55 | <.001 | 7.08 | 0.001 | 11.7 | 0.03 | 0.93 | 0.4 | 0.96 | 0.42 | 0.3 | 0.83 | 0.16 | 0.85 | 1.49 | 0.23 | 0.58 | 0.74 | 0.61 | 0.72 | 0.75 | 0.56 | 1.06 | 0.38 |
|               | Invertebrate | 14.31 | <.001 | 22.63 | <.001 | 7.1 | 0.03 | 4.36 | 0.02 | 0.73 | 0.54 | 0.7 | 0.56 | 1.04 | 0.36 | 0.00 | 0.998 | 1.98 | 0.09 | 1.38 | 0.22 | 2.36 | 0.06 | 1.41 | 0.23 |
| U. platyphylla | Vertebrate | 5.59 | 0.02 | 10.67 | <.001 | 26.69 | 0.002 | 4.42 | 0.07 | 0.3 | 0.82 | 0.2 | 6.8 | 0.01 | 3.38 | 0.036 | 1.04 | 0.4 | 1.05 | 0.4 | 3.03 | 0.02 | 1.42 | 0.23 |
|               | Invertebrate | 6.8 | 0.01 | 85.87 | <.001 | 27.83 | 0.002 | 23.26 | <.0001 | 0.82 | 0.49 | 2.31 | 0.09 | 0.23 | 0.79 | 0.12 | 0.88 | 0.52 | 0.79 | 1.09 | 0.38 | 2.86 | 0.03 | 0.62 | 0.65 |
| S. obtusifolia | Vertebrate | 9.37 | 0.003 | 0.06 | 0.94 | 26.61 | <.001 | 3.03 | 0.11 | 0.52 | 0.67 | 0.7 | 0.55 | 7.19 | 0.001 | 1.84 | 0.16 | 0.55 | 0.77 | 1.29 | 0.27 | 2.7 | 0.03 | 1.03 | 0.39 |
|               | Invertebrate | 10.6 | <.001 | 3.14 | 0.045 | 2.26 | 0.17 | 1.13 | 0.38 | 1.02 | 0.4 | 0.14 | 0.94 | 1.82 | 0.17 | 0.15 | 0.86 | 2.55 | 0.03 | 0.58 | 0.75 | 2.99 | 0.03 | 0.44 | 0.78 |
Table 2. Estimated least-square mean removal rates of weed seeds per two week interval in different crops. Within year, weed species, crop, and predator type means with the same letter are not significantly different according to Fisher’s protected LSD ($\alpha = .05$).

<table>
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<th>Weed Species</th>
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<tr>
<td></td>
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<td>0.07 c</td>
<td>0.1 a</td>
<td>0.12 a</td>
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<tr>
<td></td>
<td>S. obtusifolia Soy</td>
<td>0.15 a</td>
<td>0.21 b</td>
<td>0.15 a</td>
<td>0.19 a</td>
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<td>0.31 a</td>
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Table 3. F and P values of statistical tests of the effects of month, crop, field border type, and distance from the field border center on activity-densities of three prevalent weed seed predators. P-values are considered significant at *a* < .05.

<table>
<thead>
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<th>WEED SEED PREDATOR</th>
<th>Pitfall Trap</th>
<th>Year</th>
<th>Month</th>
<th>Crop</th>
<th>Field Border Type</th>
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<td><em>Harpalus pensylvanicus</em></td>
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</table>

‡ Analysis does not apply

† All Formicidae (ants) are considered *Solenopsis invicta*
Fig. 1. Estimated least-square mean vertebrate removal rates of *Urochloa platyphyla* seeds per two week interval in different crops. Error bars represent the standard error of the mean (SE). Within year, means with the same letter are not significantly different according to Fisher’s protected LSD ($\alpha = 0.05$).

*These least-square means are also statistically significant from each other ($\alpha = 0.05$).
Fig. 2. Estimated least-square mean activity density for three prevalent invertebrate weed seed predators during four months. Error bars represent the standard error of the mean (SE). ‡ All Formicidae (ants) are considered Solenopsis invicta.
Fig. 3. Estimated least-square mean activity-density for three prevalent invertebrate weed seed predators in three crops. Error bars represent the standard error of the mean (SE). Within years and predator, means with the same letter are not significantly different according to Fisher’s protected LSD ($\alpha = 0.05$).

‡ All Formicidae (ants) are considered *Solenopsis invicta*. *Crop not a significant main effect.*
References


CHAPTER 3
Video Analysis to Determine How *Solenopsis invicta* (Hymenoptera: Formicidae) Affects Predation of Summer Annual Weed Seeds

1. Introduction
Nonnative, invasive species are responsible for extensive ecological change and economic loss (Holway et al., 2002; Pimentel et al., 2005). Invasive species have been cited as the second most important cause of global species extinction after habitat destruction (Wilcove et al., 1998). Alien species populations often experience high growth in their new habitats and can be detrimental to native organisms due to increased predation, competition, and other factors (Kenis et al., 2009). However, nonnative organisms can also provide economic and ecological benefits (Schlaepfer et al., 2011) and can enhance ecosystem services (Pejchar and Mooney, 2009).

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is an example of a nonnative invasive species that has been cited for both the damage it inflicts and the benefits it provides. Native to South America, the RIFA is now found in Taiwan (Chen et al., 2006), New Zealand (Corin et al., 2008), Australia (McCubbin and Weiner, 2002), and throughout the southern United States (NAPIS, 2012). RIFA costs the U.S. $6 billion dollars annually from damage and control costs (Drees and Lard, 2006). These invasive ants are aggressive, have a powerful sting, and have negative impacts on wildlife (Allen et al., 2004), beneficial arthropods (Eubanks, 2001), and human health (Vinson, 1997). RIFA, however, are also known to prey on a number of important pest organisms, including corn earworm, *Helicoverpa zea* (Diaz et al., 2004), beet armyworm *Spodoptera exigua* (Diaz et al., 2004),

Seeds can make up a portion of the materials brought back to the nest by RIFA foragers (Vogt et al., 2002), and one study indirectly showed that RIFA may be an important weed seed predator in agricultural settings (Pullaro et al., 2006). However, there is currently no direct evidence that RIFA are destroying weed seeds in agricultural fields. Typically, crickets (Ichihara et al., 2011; Westerman et al., 2008), rodents (Booman et al., 2009) or ground beetles (Carabidae) (Fischer et al., 2011; Menalled et al., 2007; Ward et al., 2011) perform weed seed predation services in temperate zones, and RIFA may negatively impact these organisms. RIFA introductions reduce carabid beetle numbers (Galarraga, 2003) and negatively affect rodent populations (reviewed in Allen et al., 2004). To be considered beneficial, RIFA would need to compensate for these negative impacts on native weed seed predators by consistently destroying an equivalent or higher number of seeds than the granivores they displace.

It is critical to understand RIFA’s influence on seed predation, an important ecosystem service that helps regulate weed populations (White et al., 2007). Westerman et al. (2003b) found postdispersal weed seed predation accounts for greater losses to weed seedbanks than aging, microbial decay or even disturbances like cultivation. Research in the conservation of weed seed predators, however, has produced conflicting results (Menalled et al., 2006). Weed seed eating organisms have differing responses to farming practices and conservation efforts (Westerman et al., 2003a). In order to enhance weed seed predation, identification of the predominant weed seed predators in a system is essential. Frank et al.
(2007) showed how video monitoring equipment can elucidate important agroecosystems services by directly identifying key species. We used video recordings to conduct the first direct-observation experiment of RIFA’s impact on weed seed predation. Our objectives were to determine if: 1) RIFA remove or eat summer annual weed seeds inside agricultural fields and determine if 2) RIFA alter the impact of other weed seed predators.

2. Methods

Our experiment was conducted in 2011 at the Caswell Research Station in Kinston, NC, during autumn, when summer annual weed seeds are shed. The study was conducted in one 4 ha field where soybeans (Glycine max (L.) Merr.) had been drill planted into wheat stubble in May and were harvested in November, one month after the end of the experiment. The field was surrounded on three sides by urban development and on the fourth side by a wooded riverbank. Six plots, each 30 m by 30 m and each >30 m apart from each other and from the field edge were randomly divided into 2 groups: high fire ant density (untreated) or suppressed fire ant density (treated). Treated plots were established with biweekly applications of Amdro®, a commercially available insecticide bait. The active ingredient of Amdro® is hydramethylnon, a slow-acting insect poison that disrupts energy production in cells (Valles and Koehler, 1997). Slow-acting baits work well on social insects because they can be brought back to the nest and shared with the colony (Stringer et al., 1964). Amdro® has little effect on small mammals (EPA-OPP, 1998). While some research has reported Amdro® to have minimal effects on non-target arthropods (Hu & Frank, 1996; Eubanks et al., 2002), other studies show that it negatively affects crickets and other ground dwelling insects (Plentovich et al., 2010).
2.1 Invertebrate population estimates

Amdro® was applied at 7.5kg/ha starting August 25, 2011 and reapplied biweekly until October 7, 2011 (4 total applications), and two different trap types were used to monitor populations of potential weed seed predators. One “hotdog trap”, consisting of a 2.5 cm piece of an all-beef hotdog in the bottom of a 50 ml test tube, were placed 10 m from the center of each plot for 45 minutes at 11:00 h prior to treatment and weekly thereafter to estimate RIFA abundance. Hotdog traps were also deployed at 11:00 h for 45 minutes on October 14, 15, and 16 to coincide with video monitoring and pitfall trapping. Each pitfall trap consisted of two nested 450 mL plastic containers, and was used to measure the relative abundance (i.e., activity-density [Spence & Niemelä, 1994]) of ground dwelling invertebrates including RIFA. One 10 cm diameter pitfall trap per plot was placed 10 m from the center of each plot in the opposite direction from the hotdog traps. These traps were sealed with a 10 cm diameter Petri dish bottom and buried in the ground so that their tops were flush with the soil surface. Traps were opened for 24 hours prior to Amdro® treatment (August 24-25) and for 24 hours every day during October 14, 15, and 16. Following deployment, all hotdog traps were sealed, put on ice, returned to the laboratory and stored in a freezer until ants were identified and counted. Invertebrate specimens from pitfall traps were preserved in alcohol and brought back to the lab to be identified and counted. Crickets (Gryllidae) were identified to genus. Ants were counted as either Solenopsis invicta or “other”. Ground beetles (Carabidae) were identified to species, but because of low catch numbers they are reported as one group.
2.1.1 Statistical analysis

To determine if Amdro® treatments were successful, RIFA numbers from hotdog traps were compared between treated and untreated plots using t-tests before treatment, one week after treatment, two weeks after treatment and during October 14-16. Similarly, pitfall traps in treated and untreated plots were also compared with t-tests. P-values <0.05 were considered significant.

2.2 Weed seed predator video monitoring

Weed seed predation was monitored with video cameras to determine which organisms were eating and removing seeds in treated and untreated plots. Each of the six plots had one video camera in its center, 15 m from the plot edge. The experiment was conducted on three consecutive days, October 14 - 16, 2011. Five seeds of three common weed species, redroot pigweed (*Amaranthus retroflexus* L.), broadleaf signalgrass (*Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster), and sicklepod (*Senna obtusifolia* (L.) H.S. Irwin & Barneby), were monitored from 12:00 to 15:00 h and from 20:00 to 23:00 h. These three weed species are common summer annual weeds in our system, and they represent small (*A. retroflexus* >1 mm), medium (*U. platyphylla* ~2mm), and large sized seeds (*S. obtusifolia* ~5 mm). All six video cameras recorded at the same time. Sony® DCR-TRV280 digital video camera recorders were used. These cameras have a nighttime recording option that uses infrared LED bulbs. The cameras were positioned on-top of 20 cm tall exclosure cages made of coarse wire mesh with 10 by 10 cm openings which allowed all seed predators, including vertebrates, inside to feed on the seeds (Davis & Raghu 2010), but prevented larger organisms, such as deer, from disrupting the experiment. The cameras were
positioned so that they focused on a 10 cm diameter arena consisting of a Petri-dish covered in weed seed-free soil with seed of the three weed species sprinkled on top. The recordings were viewed in the lab. Each time a seed predator entered the arena, both the visit and duration were recorded. Any weed seeds that were eaten or removed from the arena were counted as destroyed and the organism responsible was noted.

2.2.1 Statistical analysis

The total number of seeds destroyed on the video tapes in treated and untreated plots and during daytime and nighttime were compared with t-tests. The time prevalent epigeaic organisms spent in treated and untreated plots and during daytime and nighttime were also compared with t-tests. Because of unequal variances two-sided Satterthwaite approximations were used. P-values <0.05 were considered significant.

3. Results

3.1 Invertebrate population estimates

Of the ants collected, 93% were RIFA. The Amdro® bait successfully reduced ant numbers in the treated plots. Before the first bait application, RIFA numbers were not different between plots according to both the hotdog traps (P =0.8) and the pitfall traps (P =0.85) (Table 1). However, one week after each Amdro® treatment, the hotdog trap RIFA numbers in the treated plots (mean= 3.7/ trap) were forty-five times lower than the untreated plots (mean= 161.5) (P =0.03) (Table 1). RIFA consistently recolonized the treated plots, though. RIFA numbers still appeared lower two weeks after Amdro® application in treated plots (mean = 111.3) than in untreated (mean = 264.3) plots, but there was no statistical difference (P =0.2) (Table 1). We used this trend to conduct video monitoring on October 14
– 16, one week following the last Amdro® treatment, to insure differences in RIFA numbers between treated and untreated plots. During video monitoring, hotdog traps showed that RIFA numbers in treated plots (mean=0.17) were much lower than in untreated plots (mean=170.6) \((P <.001)\) (Table 1). Pitfall catches, however, did not show any difference between RIFA numbers in treated and untreated plots \((P =0.3)\) during these three days (Table 1), but few RIFA were caught in the pitfall traps \((average = 4 \text{ RIFA/trap})\), and video data confirmed the results of the hotdog traps.

The activity-density of other epigeaic invertebrates was reduced in the treated plots. Pitfall catches of \textit{Gryllus} sp. were not different between plots before the Amdro® bait was first applied \((P =0.9)\) (Table 1). However, activity-density of \textit{Gryllus} sp. was five times lower in the treated plots \((mean= 1.7)\) compared to the untreated plots \((mean= 8.5)\) \((P <.001)\) when pitfall traps were deployed during October 14, 15, and 16 (Table 1). Only eight carabid beetles were recorded in all the pitfall traps during the entire sampling period \((average = 0.27 \text{ carabids/trap})\), so no comparisons were made with this sparse data.

3.2 Weed seed predator video monitoring

Video monitoring captured forty-eight ants visiting the arena, thirty-one \textit{Gryllus} visits, thirteen visits by house mice (\textit{Mus musculus} L.), and only four carabid appearances. Average time spent in the arena by ants was greater in the untreated plots than the treated ones \((P =0.03)\) and greater during the day than at night \((P =0.02)\) (Fig. 1). \textit{Gryllus} sp. also had greater average time in the untreated than in the treated plots \((P =0.007)\) but no difference between night and day \((P =0.3)\) (Fig. 1). Although house mice were seen more often in treated plots than untreated ones, the average time spent in arenas was statistically
areal between the two ($P=0.8$) (Fig. 1). House mice were only seen during the nighttime hours, 20:00 to 23:00 (Fig. 1).

Forty-six weed seeds were either removed from or eaten in the arenas (i.e., ‘destroyed’). Thirty-one of these seeds were broadleaf signalgrass, eleven were sicklepod seeds, and four were pigweed seeds. *Gryllus* sp. and house mice were the predominant weed seed predators. *Gryllus* more commonly removed seeds from the arena ($total=17$ seeds) than ate them in the arena ($total=8$). House mice only ate seeds in the arena ($total=20$ seeds). The video cameras only captured one instance of an ant eating or removing a seed. On October 16 during the daytime, one ant was seen removing one pigweed seed. *Gryllus* sp. destroyed the other three pigweed seeds captured on video. *Gryllus* also destroyed 17 broadleaf signalgrass seeds and five sicklepod seeds. House mice ate 14 broadleaf signalgrass seeds and 6 sicklepod seeds. No carabid beetles were seen removing or eating weed seeds on any of the videos.

Among all seeds and seed predator taxa, total weed seed destruction was greater in the untreated plots ($P=0.04$) (Fig. 2). This was because all of the seeds destroyed by *Gryllus* sp. were in the untreated plots (Fig. 2). There was no statistical difference between treated and untreated plots for seeds destroyed by mice ($P=0.8$) (Fig. 2). Although mice ate seeds exclusively at night, there was no significant difference between total weed seeds destroyed during the day versus at night ($P=0.06$) because crickets destroyed seeds during both times (Fig. 2).

4. Discussion

Our results suggest RIFA are not significant predators of summer annual weed seeds.
in agricultural fields. While ant numbers were high in untreated plots, video monitoring recorded only a single seed being removed by ants. Although RIFA are known to change their diets seasonally (Cassil and Tschinkel, 1999; Stein et al., 1990), this experiment was conducted when summer annual weed seeds are available to weed seed predators (Westerman et al., 2009). Even if RIFA eat other seeds at other times of the year, they still would not affect summer annual weed populations.

It is less clear what effect RIFA had on other epigeaic organisms. Weed seed predation rates (i.e. number of seeds removed per unit of time) in this study were similar to previously reported numbers (see review in Westerman et al., 2003). Our video monitoring recorded *Gryllus* sp. eating and removing weed seeds in plots with high RIFA numbers. RIFA and *Gryllus* were occasionally recorded simultaneously in the same arena but never directly interacted with each other. Hooper (1976) showed that in non-agricultural habitats RIFA reduced the density of other invertebrate groups but not the density of *Gryllus*. RIFA may even enhance the weed seed destruction caused by *Gryllus*. Porter and Savignano (1990) reported that another group of crickets, *Neonemobius mormonius* (Scudder), significantly increased after the introduction of RIFA in native Texas vegetation, perhaps due to feeding on RIFA refuse or due to reductions in native competitors. Mice were also present in our abundant ant plots and we found no statistical difference in the number of seeds eaten by mice in treated and untreated plots. Although RIFA may be harmful to small mammals (Allen et al. 2004), Holtcamp et al. (1997) showed that deer mice, *Peromyscus maniculatus*, were able to successfully forage for seeds in the presence of RIFA. Perhaps house mice similarly compensated for the presence of RIFA. It should be noted, however, that house
mice are an invasive species themselves, brought to the Americas by European settlers (GISD, 2010).

One common group of native weed seed predators that we did not observe preying on seeds was carabid beetles. A previous study showed that the greatest activity density of *Harpalus pensylvanicus*, a common weed seed eating carabid beetle, occured in October in North Carolina (Fox et al., *In Press*). Also, a study conducted before the introduction of RIFA to North Carolina, used direct observations to determine that carabid beetles were the most significant weed seed predator in this area (Brust and House, 1988). The absence of carabid weed seed predation in our study could be due to a number of factors, including the presence of RIFA. Carabid numbers have been shown to decline after RIFA introductions (Galarraga, 2003). However, our study site was isolated from other agricultural fields and natural areas. Diverse landscapes are critical for carabid life cycles because these areas provide refuge and nesting sites (Aviron et al., 2005). Other studies should be conducted to determine if poor carabid presence in our fields was due to interactions with RIFA or due to low habitat availability.

The application of hydramethylnon (Amdro®) bait on our treated plots, a common RIFA control measure, reduced *Gryllus* sp. and overall weed seed predation. Although RIFA did not consume weed seeds in our study, eliminating this invasive insect may have non-target effects on other organisms providing important ecosystem services. Hydramethylnon application may ultimately help RIFA populations because these ants can quickly recolonize areas after chemical treatments (Adams and Tschinkel, 2001). Programs aimed at eradicating
nonnative invasive taxa can sometimes cause more ecological harm and be more costly than the invader itself (e.g. Bergstron et al., 2009).

While seed predation can occur in high numbers in the presence of RIFA it is clear from our study that attributing this seed predation to RIFA is mistaken. Identifying key weed seed predators is often done using indirect, corollary evidence (e.g. Pullaro et al., 2006; Fox et al., In Press), but our study shows how direct evidence is important to better elucidating weed seed predation. Although visual gut content analysis and molecular tools have been used to identify seed eating invertebrates (Harper et al., 2005; Moulton, 2011), these tools have many limitations (Moulton, 2011). Our research used video recordings to easily identify the weed seed predators in our system and to quantify seed predation events. Similar video technology has proved useful for identifying insect pest predators in vineyards (Frank et al., 2007), blueberry and field crops (Grieshop et al., 2012), and in agricultural field margins (Merfield et al., 2004). The information from video monitoring studies helps us concentrate conservation biological control measures on critical species, increasing the chances of successfully enhancing ecosystem services.

To enhance weed seed predation services in areas invaded by RIFA, our study shows that management decisions should neither focus on conserving or eliminating these invasive ants. Instead, efforts should focus on conservation measures that promote effective weed seed predators. Denser overhead cover has been shown to increase weed seed predation (Fox et al., In Press, Heggenstaller et al., 2006; Meiss et al., 2010) and has the added benefit of reducing soil temperature, which should decrease RIFA activity (Porter and Tschinkel,
1987). This denser cover can be achieved by delaying or eliminating fall tillage (Gallandt et al., 2005) or by using hay species that provide greater ground cover (Meiss et al., 2010).

Acknowledgements

This research was funded by a graduate student research grant from USDA Southern Sustainable Agriculture Research and Education (project number GS10-091). We wish to thank Dr. Micky Eubanks at Texas A&M University and Dr. Ed Vargo at NC State University for their advice on this project. We thank Elizabeth Cooper and Wendi Jang for assisting with video analysis. Lani Fox helped with statistical analysis.
Table 1. Mean number of potential weed seed predators per trap in 45 minute hotdog traps and 24 hour 10cm diameter pitfall traps deployed before and after Amdro® treatments, and during video monitoring (Oct. 14-16). Significantly lower numbers in treated plots versus untreated plots are noted with asterisks ($P < .05$) and triple asterisks ($P < .001$).

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<th><strong>HOTDOG TRAPS</strong></th>
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<td><em>Gryllus sp.</em></td>
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<tr>
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<td>UNTREATED</td>
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<td>2 Weeks After Treatment</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>October 14-16</td>
<td>5.4 ($\pm 1.9$)</td>
<td>5.6 ($\pm 1.5$)</td>
</tr>
</tbody>
</table>
Fig. 1. Mean time (s) common weed seed predators were recorded per hour in a 10 cm diameter arena. Asterisks represents significantly ($P < .05$) greater time according to two-sided $t$-tests using Satterwaithe approximations.
Fig. 2. Total number of weed seeds removed or eaten in arenas during October 14 - 16, 2011. Asterisk represents significantly $(P < .05)$ higher seed destruction according to two sided $t$-tests using Satterwaithe approximations.
References


