Invasion, bioenergy, and natural enemies of insect pests:
Ecological and agricultural tradeoffs in two study systems

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

James O. Eckberg

IN PARTIAL FULLFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Dr. Gregg A. Johnson, Dr. Donald L. Wyse

November 2015

Copyright Page

James O. Eckberg 2015 ©

Johnson, Barb and Jerry Galowitz, Timm, Joe, and Chelsey Bockman, and Andy

Montain.

Dedication:

I dedicate this dissertation to my grandparents, Olaf and Aleda Kaasa, for their

encouragement, support, and wisdom. They continue to be an inspiration in my life.

Abstract:

Agriculture is at the intersection of major global challenges for the 21st century. There is a growing need to increase food security and production. Mounting demand for bioenergy, biomaterials, and naturalized areas for recreation is also placing pressure on food production systems. At the same time, the intensive practices credited for improving production have degraded the environment, eroded ecosystem services and threaten the potential to sustain further increases in yield. There is an incredible need to understand the tradeoffs inherent in diversifying and optimizing farming systems for food and bioenergy production in ways that also support ecosystem services. This thesis explores tradeoffs in two systems. The first is an integrative system that seeks to manage perennial crop bioenergy, natural enemies, and pests. The second is a study of the invasion risk of selectively breeding switchgrass for bioenergy production.

In chapter 1, I describe the community of hoverflies (Syrphidae) in soybeans. The original objective of this research was to test the predation and biological of hoverflies in perennial crop-soybean systems. Hoverfly adults in our region feed exclusively on nectar and pollen and the larvae prey only on aphids. Coupling flowering perennial crops adjacent to soybean aphid populations was expected to greater enhance hoverfly populations and biological control of soybean aphid. However, across two years of research hoverflies remained at very low density in soybeans. Upon further evaluation of the literature it became clear that there was little information on the abundance and community composition of hoverflies in soybeans. Therefore, I collaborated to develop a manuscript describing the abundance of hoverflies, their performance on soybean aphid, and the possible ecological causes for their low abundance.

In chapter 2, I evaluated the spillover of tent caterpillars onto willow bioenergy crops. The prevailing notion is that perennial crops will support natural enemies, yet little information is available on the pests of perennial crops. I developed a manuscript describing the spatial dynamics of tent caterpillar spillover onto willow bioenergy crops.

In chapter 3, I tested the natural enemies and bioenergy production of integrative perennial cropping systems. Producing natural and bioenergy crops is a major goal of integrative cropping systems. Yet little information is available on the tradeoffs between crops and cropping structure in terms of supporting natural enemies and producing bioenergy. Across three years I quantified both theoretical ethanol yield and natural enemies in perennial crops and found significant tradeoffs but also some potential synergies in the ability to achieve both of these objectives.

In chapter 4, I explore the emerging ecological risk associated with producing switchgrass biomass for bioenergy. This research is critical to better understanding the tradeoff between producing bioenergy and minimizing invasion potential of switchgrass. As a first step to understanding invasiveness in chapter 4, I tested the biomass and competitiveness of switchgrass cultivars and wild populations in the greenhouse. I discuss strategies for minimizing invasiveness while promoting biomass production.

In chapter 5, I characterize the germination and dormancy of switchgrass cultivar and wild population as well as the factors contributing to dormancy. Though the focus of this manuscript is primarily on breeding, the implication is that populations with greater germination could have greater emergence in natural areas which, in some situations, may contribute to greater weediness.

These chapters highlight several important ecological and agricultural tradeoffs in integrative perennial agriculture: crop production and weediness, natural enemies, or pests. Such information is important to designing and optimizing agricultural systems for agricultural production in ways that also support ecosystem services.

Table of Contents

List of Tables

List of Figures

<u>Chapter 1 Figures</u>

Syrphid avg (± standard error) adult abundance across four soybean fields (4 ha per field)

Syrphid avg (± standard error) larvae and pupae density across four soybean fields

Syrphid abundance and aphid abundance across four soybean fields

Chapter 2 Figures

Map of experimental site showing spatial distribution of willow plots, their defoliation, proximity to the nearest host tree and its defoliation.

Relationship between host tree defoliation and herbivory on willow.

The proportion of willow with more than 10% insect defoliation versus proportion of willow surviving to the end of the growing season.

Insect defoliation on host trees in relation to habitat type and tree size (volume, m^3).

Chapter 3 Figures

Chapter 4 Figures

Chapter 5 Figures

Mean (± SE) a) germination and b) average seed mass (based on samples of 50 seeds) of

eight wild and four cultivar populations of switchgrass.

Mean (± SE) germination of switchgrass populations in relation to cold-moist

stratification.

Mean seed mass and origin (cultivar, wild) versus germination for eight wild and four

cultivar populations of switchgrass

Relationship between mean germination of each switchgrass population and the increase

in germination after cold-moist stratification

CHAPTER 1

Field Abundance and Performance of Hoverflies (Diptera: Syrphidae) on Soybean Aphid

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a major pest of soybeans in most of the soybean growing regions of North America, causing up to a 40% reduction in yield (Ragsdale et al. 2007, 2011). Since its detection in 2000 and subsequent outbreaks in North America, there has been a concerted effort to describe the soybean aphid predator community, quantify the extent to which it exerts biological control, and understand the role of habitat in mediating those interactions (Fox et al. 2004, Rutledge et al. 2004, Costamagna and Landis 2006, 2007, Mignault et al. 2006, Costamagna et al. 2007, 2008, Gardiner et al. 2009, Gagnon et al. 2011, Koh et al. 2013). There are several significant predators of soybean aphid including ladybird beetles (Coleoptera: Coccinellidae), the insidious flower bug *Orius insidiosus* Say (Hemiptera: Anthocoridae), predatory midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae), and lacewing species (Neuroptera: Chrysopidae and Hemerobiidae) (Desneux et al. 2006, Costamagna and Landis 2007, Donaldson et al. 2007, Costamagna et al. 2008, Gardiner et al. 2009). Predation from these and other arthropods can exert strong biological control, often providing adequate population suppression to keep soybean aphids below the economic threshold and thus protecting soybean yield and reducing insecticide use (Costamagna et al. 2007, Landis et al. 2008, Gardiner et al. 2009, Heimpel et al. 2013).

Another potentially important predator group of soybean aphids are aphidophagous hoverflies (Diptera: Syrphidae: Syrphinae) (Vockeroth 1992, Kaiser et al.

2007, Noma and Brewer 2008). Adult syrphids were among the most common aerially dispersing predators collected by sticky cards in soybean fields sampled across four states in the upper Midwest (Schmidt et al. 2008, Gardiner et al. 2009). However, syrphid larvae (the predatory stage) are much less abundant than other predators, such as coccinellids and anthocorids, on soybean plants, comprising an estimated 0.1 to 8% of the predator community (Rutledge et al. 2004, Costamagna and Landis 2006, Donaldson et al. 2007, Gardiner et al. 2009, Noma et al. 2010). Few studies have quantified syrphid species composition throughout the soybean growing season. Among those, two studies conducted in southern Michigan shortly after soybean aphid was first detected (2003-2006) showed that seven syrphid species oviposit and develop to adult stage on out-planted soybean aphid colonies (~1,000 aphids per plant), *Allograpta obliqua* (Say) was the most common (Kaiser et al. 2007, Noma and Brewer 2008). However, parallel observations at the same sites showed that larvae were not nearly as abundant in soybeans stands (less than 0.1 larva per plant) as compared to out-planted soybeans (up to six larvae per out-planted soybean), and *Toxomerus marginatus* (Say) was more common than other syrphid species in stands than out-planted soybeans (Noma and Brewer 2008). Beyond these experiments little is known about the species composition, abundance, or performance of syrphids in soybeans, despite their significance as aphid predators in other crops (Smith et al. 2008). More information on species composition from other parts of the soybean growing region would strengthen our ability to make inferences on their potential role in the biological control of soybean aphids.

Performance of syrphids feeding on aphids is fundamental to syrphid population growth and biological control yet this information is lacking for soybean aphids. While

there are at least seven syrphid species that can develop on soybean aphid (Kaiser et al. 2007, Noma and Brewer 2008), the rate of syrphid development on soybean aphid remains unknown. The relatively low abundance of syrphids in soybeans implies that syrphids may perform poorly on soybean aphid. While poor syrphid larval performance can result from secondary defense compounds (Vanhaelen et al. 2002), soybean aphids are considered to have low resistance and are suitable for several predator and parasitoid species (Mignault et al. 2006, Desneux et al. 2009, Hopper et al. 2013). Thus, a better understanding of syrphid larval development on soybean aphids would enhance our knowledge of syrphid performance on soybeans aphids.

The current study 1) employed four adult sampling techniques (vacuum-suction, sweep nets, yellow sticky cards and timed observations) to quantify and describe seasonal abundance and species composition of adult syrphid flies, 2) counted syrphid larvae and pupae on soybean plants during two years, and 3) conducted aphid feeding trials in the laboratory to estimate larval performance of the most dominant syrphid on soybean aphid from objective 1. Performance on soybean aphid was compared to two native aphid-plant associations: *Aphis nerii* Boyer de Fonscolombe reared on *Asclepias incarnata* L. and *Aphis monardae* Oestlund reared on *Monarda fistulosa* L. The *A. nerii – A. incarnata* association could be mildly toxic and thereby unsuitable for syrphids, however, cardenolides were shown to be virtually absent in *A. nerii* feeding on *A. incarnata* (Martel and Malcolm 2004) and parasitoids are able to complete development on this aphid – plant association (Desneux et al. 2009). Taken together, information on larval performance and field abundance provides timely information on the potential importance of syrphids as biological control agents.

Materials & Methods

Abundance and Composition of Hoverflies.

Research Site. Field sampling was conducted at the University of Minnesota Rosemount Research and Outreach Center in east-central Minnesota, USA, from four 4-ha (10-acre) soybean *Glycine max* (L.) fields: Field A (Center of field: 44°44'16.64" N, 93°05'33.06" W), Field B (44°44'02.34" N, 93°03'55.71" W), Field C (44°42'24.46" N, 93°04'26.04" W), and Field D (44°41'47.07" N, 93°03'24.65" W). Average distance between all plots was 3.4 km (minimum distance = 1.7 km, maximum = 5.5 km). All sampling was conducted within a 45 x 50 m or smaller area in the center of each 4-ha field. Sampling near the middle of relatively large soybean fields was intended to minimized effects from surrounding habitats and provide a better representation of the syrphid community in soybeans. Fields were planted with the soybean cultivar Pioneer 91M51 (susceptible to soybean aphid) on 30 May to 31 May 2012 and 13 June to 14 June 2013 and maintained under standard agronomic practices including applications of the herbicide glyphosate, sethoxydim, quizalofop p-ethyl, and fluthiacet-methyl. There were no insecticide or fungicide applications to the field or in a pre-treatment to seeds.

Timed Observations- 2012. Timed observations were performed in 2012 to directly observe adult syrphid abundance. The timed observations involved 5 min of visual searching along a diagonal transect through a 300 m^2 area at the center of each field. Every 30 s the observer advanced along the transect to a new stationary position where they searched a 1 m radius. Syrphid adults were identified to genus using characters from Vockeroth (1992). From 19 July to 5 September seven and eight weekly

timed observations were conducted for fields A, B and C, D, respectively (two samples were not collected).

Sticky Cards. In 2012, the abundance and composition of adult syrphids was sampled with unbaited yellow sticky cards, PHEROCON® AM No-Bait Traps, (Trécé, Inc. Adair, Oklahoma, USA) placed approximately 5 cm above the mean soybean canopy height on a 2.74 m tall fiberglass post. Protruding wires were attached to the top of the post to minimize bird perching activity. At each plot, four sampling locations were established within a 75 m^2 area at the center of the field. Sticky cards were placed on the posts for 7 d intervals from 8-June (eight d after planting) to 21 September (11 d before harvest). Syrphids were identified to genus and species by J.C.L. using keys from Vockeroth (1992). Identifications were further confirmed by referencing identified specimens in the University of Minnesota Insect Collection, UMSP (Saint Paul, Minnesota, USA). All syrphids were identified to species and counted on traps collected every other week (four fields x four posts per field x eight sampling periods; N = 127, one trap was lost).

Vacuum-Suction Sampling. In 2012, Arthropods were collected using a vacuum-suction sampling device created by modifying a Toro PowerVac™ T25 gasoline-powered handheld leaf blower/vac with the addition of a layer of fine-mesh no-see-um netting (openings of 0.65×0.17 mm; Quest Outfitters, Sarasota, Florida, USA) secured over the vacuum opening with a rubber band. A 56 cm long piece of flexible plastic tubing with an 81 cm^2 circular opening was attached to the vacuum to collect arthropods. Sampling was conducted by running the leaf blower at full throttle (maximum air speed 257 kmph) while placing the flexible tubing over soybean plants to vacuum for 2 min, approximately

a 20 m length row, for each sample. After each sample was collected, the no-see-um mesh pouch and its entire contents were placed in a plastic bag and immediately frozen in a portable Engel MT15 freezer (Engel, Jupiter, Florida, USA) before adult syrphids were identified to species and counted. Every other week from 26 June to 18 September 2012, three samples were collected along transects at each field within a 500 m^2 area near the center of the field, for a total of seven sampling periods (four fields x three samples/field x seven sampling periods; N = 84).

Sweep Net Sampling. Sweep net sampling was conducted during the 2012 and 2013 growing seasons. Each sample consisted of 20 figure-eight sweeps conducted over approximately a 20 m length transect of soybeans with a 38 cm diameter sweep net. All sweep net contents were placed directly into Whirl-Pak® bags (Fisher Scientific, Pittsburgh, Pennsylvania, USA) containing >95% ethanol and all syrphids were identified to species and counted. Every other week from 28 June to 20 September 2012 and 19 June to 25 September 2013 three samples were collected at each field within a 500 m^2 area near the center of the field, for a total of seven sampling periods in 2012 (four fields x three samples/field x seven sampling periods; N = 84) and eight sampling periods in 2013 (four fields x three samples/field x eight sampling periods; N = 96).

Visual Observations of Larvae and Pupae. Some life stages of syrphids can be difficult to sample using the above described methods due to their habit of taking refuge in protected areas of the plant. Therefore, hoverfly larvae and pupae were quantified in 2012 and 2013 by thorough visual inspection of the entire soybean plant within a 0.25 m^2 sample quadrat. Once every week from 1 June to 21 September 2012 and 6 June to 26 September 2013 four quadrats were sampled at each field within a 500 m^2 area near the

center of the field, for a total of 17 sampling periods in both 2012 and 2013 (four fields x four samples/field x 17 sampling periods; N = 272 per yr).

Visual Observations of Larvae in Relation to Aphid Density. Syrphid larvae and soybean aphids (total number of alates and apterae) were quantified in 2012 and 2013 by visually inspecting randomly selected soybean plants along two concentric grids within a 45 by 50 m area in the center of each 4-ha soybean field. Observations were made weekly from 26 July to 13 September 2012 and from 1 July to 2 October 2013. Plant size was estimated by counting the number of trifoliates per plant.

T. marginatus Performance on *A. glycines*, *A. monardae*, and *A. nerii*.

A sweep net was used to collect adult *T. marginatus* from a 0.21 ha restored tallgrass prairie at the University of Minnesota, Saint Paul, Minnesota, USA (44.98^o N, 93.23^o W). Adult *T. marginatus* were identified and introduced into plexi-glass/mesh cages (approximately 30 x 30 x 46 cm) with either *A. glycines* (on *G. max*), *A. monardae* (on *M. fistulosa*), or *A. nerii* (on *As. incarnata*) and a mixture of fresh-cut native and non-native forbs from the restored prairie (e.g. crownvetch *Securigera varia* (L.), early sunflower *Heliopsis helianthoides* (L.), tickseed *Coreopsis* sp., white clover *Trifolium repens* L., and spiderwort *Tradescantia* sp). Caged flies were monitored for mating and ovipositions and leaves with eggs were cut from the plant and isolated in a Petri dish. Eggs were collected from the same aphid-plant associations as used in the feeding trials except eggs from *A. monardae* cages were used for feeding trials of *A. nerii* (larvae were not available from *A. nerii* cages at the start of the trials though adults oviposited in the *A. nerii* cage).

7

Syrphid eggs were observed for larval emergence. Newly hatched larvae were fragile and therefore left undisturbed on leaves for 24 h (day one). Leaves and larvae were kept in a growth chamber at 25°C with a 16:8 h light: dark photoperiod for the entire experiment, except during examination and feeding. On day two, larvae were each transferred to a separate host plant leaf (the petiole cut was placed into a 0.6 ml microcentrifuge tube, containing deionized water, covered by a parafilm layer) with 30-50 aphid nymphs measuring 0.5-1.0 mm in length. Every 24 h until pupation, larvae were transferred to a fresh leaf with typically 30-100 aphid nymphs so that live aphids were available in excess (*ad libitum*). More aphids were generally provided to larvae in later stages of growth when there were higher feeding rates. Upon pupation of syrphid larvae, excess aphids were removed and the pupae monitored daily until eclosion of the adults. Three replications were consecutively performed with: 10 larvae fed *A. glycines* (28 June to 21 July 2012), three larvae fed on each of *A. glycines*, *A. monardae*, and *A. nerii* (22 July to 5 August 2012); and two larvae fed *A. glycines* and five fed on each of *A. monardae* and *A. nerii* (19 August to 4 September 2012). Total sample size was therefore 15 larvae fed *A. glycines*, eight larvae fed *A. nerii*, and eight larvae fed *A. monardae*.

The following metrics of *T. marginatus* performance were measured: number of individuals that successfully pupated and the time (d) from egg hatch to successful pupation. Among the successfully pupated individuals, the number which subsequently emerged as adults and time (d) to emergence was recorded.

Analysis.

Generalized linear mixed models (glmmPQL) from the open source statistical software R (R Development Core Team 2005) were used to model seasonal change in

abundance detected by sticky cards, timed observations, sweep nets, and larval surveys. Underlying Poisson and binomial distributions were assumed for count and binary data, respectively. The generalized linear mixed model underestimated the raw mean larval and pupal abundance in 0.25 m^2 quadrats. We therefore analyzed abundance with a linear mixed model (lme in the statistical software R) when syrphids were present (11 July to 7 September; N = 288) and present those p-values which were similar to the p-values from the generalized linear mixed model. The linear mixed model was also used to analyze syrphid to aphid ratio. Differences in abundance among the two most common species, pupae versus larvae, and years, as well as year-dependent differences in life stage or species abundance (two-way interaction), were treated as fixed effects and tested using the *t*-statistic. Polynomial terms (i.e. linear, quadratic, cubic) were tested for significance (*P*<0.05) and fit to approximate change in abundance over time. Variation between plots, repeated samples within plots, and sub-sampling were treated as random effects.

Larval performance in the laboratory was also analyzed using mixed models in the statistical software R. Time (d) of development from egg hatch to pupation and pupation to emergence was analyzed with linear mixed effect models (lme) which included replication as a random effect. Proportion of individuals that pupated and the proportion of pupae that emerged as adults were analyzed with a generalized linear mixed-effects model (glmer) including replication as a random effect. Results of ANOVA are presented in Table 1 for proportion pupated or emerged (likelihood ratio test based on Chi-square distribution) and time to pupation or emergence (*F*-test). Table 1 presents the average (± standard error) performance and statistical comparisons among aphids for all three replications. A second analysis was performed for replications two

9

and three when all aphid species were tested concurrently, but the results did not differ

qualitatively from the tests involving all three replicates.

Results

Abundance and Species Composition of Adult Syrphids.

Timed Observations. In 2012, a total of 11 adult syrphids were observed

equivalent to an average 0.37 adult syrphids (± 0.11 SE) per five min or one syrphid

every 13.6 min (N = 30 timed observations; Fig. 1a). *Toxomerus* was the dominant genus

observed, comprising 91% of all observed adult syrphids (Fig. 1a). The observation of

one *Eristalis* sp. adult comprised the remaining 9%.

Sticky Cards. In 2012, a total of 124 adult syrphids were collected, an average

0.98 syrphids (± 0.17) per sticky card (average abundances by plot: A = 1.00, B = 0.72, C

= 0.97, and D = 1.22; N = 127 sticky cards; Fig. 1b). There were significantly more *T.*

marginatus than *T. geminatus* (Say) (t_{126} = 2.36, *P* = 0.020) and together these species

comprised 83.1% of all syrphids (Fig. 1b). In total, eight hoverfly species were collected

and identified from the four soybean fields with the following relative abundances: *T.*

marginatus (56.5%), *T. geminatus* (26.6%), *Eristalis stipator* Osten Sacken (5.6%),

Eupeodes americanus (Wiedemann) (5.6%), *Eristalis tenax* (L.) (2.4%), *Sphaerophoria*

philanthus (Meigen) (1.6%), *Sphaerophoria contigua* Macquart (0.8%), and *Helophilus*

latifrons Loew (0.8%).

Sweep Net Sampling. In 2012, a total of 12 syrphid adults were collected, an

average of 0.14 syrphids (± 0.05) per sweep net sample (average abundances by plot: A =

0.10, B = 0.19, C = 0.10, and D = 0.19; N = 84 sweep net samples; Fig. 1c). In 2013, a

total of six syrphid adults were captured, an average of 0.06 syrphids ($\pm$ 0.02) per sweep net sample (average abundances by plot: A = 0.00, B = 0.04, C = 0.17, and D = 0.04; N = 96 sweep net samples; Fig. 1d). There were significantly more *T. marginatus* than *T. geminatus* in sweep nets (t_{179} = 7.88, *P* < 0.001; Figs. 1c-d) but no detectable differences in abundance among years (t_{165} = 1.19, *P* = 0.23) or species by year interaction (t_{178} = 0.96, *P* = 0.34).

Vacuum-Suction Sampling. Vacuum-suction sampling captured zero adult syrphids in 2012 (N = 84 samples).

Abundance of Syrphid Larvae and Pupae.

Sweep Net and Vacuum-Suction Sampling. Sweep netting captured zero syrphid larvae and pupae in 2012 (N = 84). In 2013, three syrphid larvae and one pupa were collected, an average 0.031 larvae ($\pm$ 0.018) and 0.010 pupae ($\pm$ 0.010) per sweep net sample (N = 96). Vacuum-suction sampling captured one larvae and zero pupae in 2012, an average of 0.012 larvae ($\pm$ 0.012) per vacuum-suction sample (N = 84).

Visual Observation of Larvae and Pupae. There were more larvae than pupae (t_{287} = -3.26, *P* = 0.0012) and there was marginal statistical support (t_{286} = -1.97, *P* = 0.0502) that this difference was larger in 2013 versus 2012. In 2012, 17 syrphid larvae and 10 pupae were observed, an average density of 0.063 larvae ($\pm$ 0.018) (average densities by plot: A = 0.074, B = 0.029, C = 0.074, and D = 0.074) and 0.037 pupae ($\pm$ 0.011) (average densities by plot: A = 0.029, B = 0.00, C = 0.100, and D = 0.015) per 0.25 m^2 quadrat (N = 272 quadrats; Fig 2a). In 2013, 37 larvae and nine pupae were observed, an average density of 0.136 larvae ($\pm$ 0.033) (average densities by plot: A = 0.382, B =

0.029, C = 0.088, and D = 0.044) and 0.033 pupae (± 0.014) (average densities by plot: A = 0.132, B, C, and D = 0.000) per 0.25 m^2 quadrat (N = 272 quadrats; Fig. 2b).

Visual Observation of Syrphid Larvae and Aphids per Soybean Plant. In 2012, 424 plants were inspected and a total 15,315 soybean aphids were counted (total number of aphids per plot A = 4,061, B = 885, C = 8,479, D = 2,402). In 2013, 903 plants were inspected and a total 103,608 soybean aphids were counted (total number of aphids per plot A = 54,032, B = 24,423, C = 20,514, D = 4,639). There were significantly more syrphid larvae in 2012 versus 2013 (t_{1292} = 2.08, *P* = 0.038). An average of 0.014 syrphid larvae (± 0.006) were observed per plant in 2012 (six syrphid larvae in total for 2012) versus 0.021 syrphid larvae (± 0.006) per plant in 2013 (19 total observed larvae; Fig. 3b). Average abundances of syrphid larvae per plot in 2012 were: plot A = 0.018, B = 0.0096, C = 0.019 and D = 0.0096 and in 2013 plot A = 0.038, B = 0.017, C = 0.013, D = 0.017.

Peak soybean aphid density was observed on 23 August 2012 (131.9 aphids per plant) and 21 August 2013 (798.0 aphids per plant). Peak syrphid density was observed on 23 August 2012 (0.0312 larvae per plant) and 15 August 2013 (0.125 larvae per plant) (Fig. 3b). The ratio of syrphids to aphids varied from 0.0 to 0.0018, with the highest values per yr occurring on 23 August 2012 and 4 September 2013 (Fig. 3c). The frequency of syrphids per soybean plant varied from to 0.00 to 0.094, with the highest values per yr occurring on 23 August 2012 and 15 August 2013 (Fig. 3d). There were no significant differences in the syrphid to aphid ratio (t_{1293} = -0.45, *P* = 0.65) or the frequency of syrphids per soybean (t_{1292} = 1.55, *P* = 0.12) among years.

T. marginatus Performance on *A. glycines*, *A. nerii* and *A. monardae*.

Syrphid Performance. Among the *T. marginatus* fed *A. glycines*, 80% pupated and of those 75% emerged as adults. Pupation and emergence to adult stage occurred in 12.3 and 4.8 d, respectively (Table 1). Proportion larvae maturing to the adult stage as well as the time to maturation were similar for *T. marginatus* fed *A. glycines* versus *A. nerii* or *A. monardae*. No statistically significant differences were detected for any performance measures (Table 1).

Discussion

In this study, the syrphid community was comprised of eight species, dominated by *T. marginatus* and *T. geminatus*. However, syrphid abundance was relatively low in soybean fields. Laboratory feeding trials indicated that *T. marginatus* larvae perform equally on *A. glycines* as on native aphids. We first provide a discussion of syrphid community composition and abundance based on our collection methods. Then we consider the potential for syrphids to exert biological control on soybean aphids and discuss the factors potentially influencing syrphid populations in soybeans.

Sticky cards and sweep nets indicated that *T. marginatus* was the most common species followed by *T. geminatus*. Timed observations further confirmed that the genus *Toxomerus* was dominant in these soybean fields. Consistent with our study, Noma and Brewer (2008) reported for southern Michigan that *T. marginatus* larvae were relatively common compared to other syrphid species in soybean stands but their larval density was low (Noma and Brewer 2008). Conversely, in those same fields, out-planted soybeans with elevated soybean aphid density suggested the syrphid community was dominated by *A. obliqua*, not *T. marginatus* (Noma and Brewer 2008). We contend that our sticky card,

sweep net, and timed observation surveys as well as the larval density surveys of Noma and Brewer (2008) on soybean stands are likely to be more representative of the syrphid community occurring in soybeans fields. Thus, while *T. marginatus* occurs at low density they may be one of the more common species of syrphids to occur across the soybean growing region.

Direct observations and counts showed that syrphid larval densities, and their ratio to aphids, are extremely low in soybeans. Averaged across years and plots there were 0.03 larvae per soybean plant when aphids were present and a ratio of 0.00021 larvae to aphids (equivalent to one larva per 4,762 aphids; Fig. 3). Even when aphids reached their highest densities in 2012 (16 to 23 August) and 2013 (30 July to 28 August), the ratio of syrphids to aphids usually stayed well below 0.001 and syrphid larvae were present on no more than 10% of the soybean plants (Fig. 3). Our estimates of larval density from searching 0.25 m^2 quadrats further confirmed the low density of syrphid larvae (Fig. 2).

Our observations of syrphid larval density are consistent with the general finding that syrphids are less abundant compared to other predators of soybean aphids. In a related study by Rutledge et al. (2004) syrphids represented less than 1% of the total insect predator community and other predators including *O. insidiosus* and *Harmonia axyridis* Pallas were more abundant than syrphids. The highest larval density of syrphids observed in this study (0.13 syrphids per soybean plant on 15 August 2013) was less than one-tenth of the peak abundance of *O. insidiosus*, 1.7 adults and nymphs per plant (Rutledge et al. 2004). In southern Michigan, syrphid larval density was similarly low

throughout the season in soybean stands across two sites (Noma and Brewer 2008, Brewer and Noma 2010).

Our study provides insight on the potential biological control of soybean aphids. Syrphid larvae were observed to be vastly outnumbered by aphids. Laboratory feeding studies show that one syrphid can kill 132-507 aphids during larval development depending on the syrphid species, environmental conditions, and aphid species (Soleyman-Nezhadiyan and Laughlin 1998, Hopper et al. 2011). Hopper et al (2011) reported that individual *T. marginatus* killed 132 lettuce aphids (*Nasonovia ribisnigri* Mosley) over the larval stage. Predation rates can be 50% lower in field than under laboratory conditions (Tenhumberg 1995). Thus, the low syrphid density observed in the field combined with estimates of predation from the literature suggests there is limited potential for syrphids to exert biological control on soybean aphids.

Our results contrast other agricultural systems in which syrphids are highly abundant in aphid colonies (Pineda and Marcos-García 2008, Gontijo et al. 2012) and larvae can be important predators exerting strong population-level control on aphids (Chambers and Adams 1986, Tenhumberg 1995, Tenhumberg and Poehling 1995). For example, the primary syrphid species in soybeans, *T. marginatus*, also complete their development on a major aphid pest of organic California lettuce, the non-native *N. ribisnigri* (Hopper et al. 2011). *T. marginatus* is the dominant syrphid species feeding on *N. ribisnigri*, comprising 39% of the syrphid community followed by syrphids *A. obliqua*, *Sphaerophoria* sp., and *Eupeodes* sp. (Smith and Chaney 2007) which were also detected in our soybean fields. However, unlike in soybeans, syrphid larvae are highly abundant in field populations of *N. ribisnigri* reaching average high densities of 2.75 – 9.08 larvae per

lettuce plant, far greater than the peak 0.13 larvae observed per soybean plant in this study, and syrphids provide significant population suppression of *N. ribisnigri* (Smith et al. 2008).

A question which arises from this study is why are syrphids not as abundant in soybean aphids as they are in other agricultural systems (Gontijo et al. 2012)? In our study, *T. marginatus* performed well by feeding on soybean aphids in the laboratory, equally well as on two native species of aphids. However, some caution should be exercised in the comparison of *T. marginatus* performance among aphids as our conclusions are based on a relatively small sample size (N=31). Soybean aphids are suitable prey unlike some aphids (e.g. *Cavariella theobaldi*) which can be toxic to syrphids (Ruzicka 1975). Further, syrphids occurred more commonly, often more than two syrphids per plant, on out-planted soybean plants when the larvae were allowed to develop in the laboratory (Kaiser et al. 2007, Noma and Brewer 2008, Brewer and Noma 2010). In out-planted soybeans surrounded by grassland, syrphids were over 400% more abundant than coccinellids or *Orius insidiosus* (Kosmala 2013). However, syrphids occurred at low density in the larger soybean stands (i.e. four ha) of the current study and in other large field studies (e.g. Gardiner et al. 2009). The potential importance of scale-dependent ecological effects is further implied by the observation that syrphid abundance was greater on out-planted soybeans in smaller (0.01 – 0.02 ha) versus larger (0.5 – 1.0 ha) soybean plots (Noma and Brewer 2008). Thus, syrphids can perform well on soybeans and soybean aphids but their potential for population growth appears more limited in the context of larger soybean stands.

Multiple factors may limit syrphid populations in soybean stands. While pupation rates in the laboratory were 80%, the occurrence of only 35% as many pupae versus larvae in the field implies successful pupation in the field is much lower. One potentially limiting factor to syrphids could be intraguild predation from coccinelids such as *Harmonia axyridis* and *Coccinella septempunctata* and chrysopids such as *Chrysoperla* sp. (Hindayana et al. 2001, Alhmedi et al. 2010, Ingels and De Clercq 2011), all of which often occur abundantly in soybeans (Gardiner et al. 2009) and are known intra-guild predators of several other taxa in soybeans (Gardiner and Landis 2007, Chacon and Heimpel 2010, Gagnon et al. 2011). For example, *Coleomegilla maculata* have been observed feeding on syrphid pupae in alfalfa (Wheeler 1977). Further, dipteran protein likely to include syrphids has been detected in the guts of three coccinellid species (*C. septempunctata*, *C. maculata*, and *H. axyridis*) known to prey on soybean aphids (Moser et al. 2011). Finally, when coccinellid abundance was low, syrphids were much more common on out-planted soybeans than was observed in the current study (Kosmala 2013). Plant traits of soybeans such as pubescence (trichomes) may also limit syrphid performance. Pubescence was shown to reduce movement and performance of syrphids in other crops (Verheggen et al. 2009). It is perhaps no coincidence that syrphids are major predators of aphids in crops which lack dense pubescence (Chambers and Adams 1986, Nieto et al. 2006, Gontijo et al. 2012). Thus, while the ecological mechanisms limiting syrphid populations in soybeans remain unclear, the current body of literature suggest syrphids can be added to the list of predators and parasitoids (e.g. *Binodoxys kellogensis*, *Lysiphlebus testaceipes, C. maculata*) which can complete development on soybean aphids (Mignault et al. 2006, Brewer and Noma 2010) but have remained at low

density in soybean stands (Schmidt et al. 2008, Brewer and Noma 2010, Noma et al. 2010).

In conclusion, the syrphid community dominated by *T. marginatus* showed a low abundance of larvae and pupae in four soybean fields across 2 yrs. The high developmental performance of *T. marginatus* on soybean aphid is consistent with the hypothesis that ecological factors (e.g. intraguild predation, syrphid-soybean interactions) limit populations of syrphids. A potential extension of this study would be to further characterize such factors and quantify their effects on syrphid abundance and predation in soybeans.

Life Stage Development	*Aphis glycines*	*Aphis nerii*	*Aphis monardae*	Sample Sizes[a]	Test Statistic	*P*-value
Proportion larvae that pupated	0.80 (± 0.10)	0.88 (± 0.12)	0.75 (± 0.15)	15, 8, 8	$\chi^2 = 0.42$	0.81
Proportion pupae that emerged	0.75 (± 0.13)	0.57 (± 0.19)	0.83 (± 0.15)	12, 7, 6	$\chi^2 = 1.21$	0.55
Proportion larvae that pupated and emerged	0.60 (± 0.13)	0.50 (± 0.18)	0.63 (± 0.17)	15, 8, 8	$\chi^2 = 0.299$	0.86
Days from egg hatch to pupation	12.33 (± 1.13)	10.71 (± 0.47)	11.00 (± 0.73)	12, 7, 6	$F_{2,20} = 0.069$	0.93
Days from pupation to adult emergence	4.78 (± 0.15)	5.00 (± 0.00)	4.60 (± 0.40)	9, 4, 5	$F_{2,13} = 0.995$	0.40

Table 1: *Toxomerus marginatus* avg (± Standard error) larval performance on *Aphis glycines*, *A. nerii*, and *A. monardae*.

Test statistics and corresponding *P*-values are presented for χ^2 tests (Likelihood Ratio test performed via glmer in R) and *F*-tests (ANOVA performed using lme in R).

[a]Samples sizes are shown in order for *A. glycines*, *A. nerii*, and *A. monardae*.

Figure Legends

Fig. 1. Syrphid average (± SE) adult abundance across four soybean fields (4 ha per field) based on (a) 5-minute timed observations in 2012, (b) yellow sticky cards in 2012, and (c) sweep nets in 2012 and (d) 2013 in Rosemount, MN. Based on a generalized linear mixed model, the curves represent best fit polynomial terms for a single continuous variable (total syrphidae across time) or two continuous variables (*Toxomerus marginatus* versus *T. geminatus* across time). Dashed curves with double-dots, dashed curves, and solid curves represent total syrphids, *T.* marginatus and *T. geminatus*, respectively.

Fig. 2. Syrphid average (± SE) larvae and pupae density across four soybean fields (4 ha per field) during (a) 2012 and (b) 2013 in Rosemount, MN. Curves represent best fit polynomial terms from a linear mixed model that compared syrphid pupae to larvae in 2012 and 2013. Dashed curves and solid curves represent pupae and larvae, respectively.

Fig. 3. Soybean aphid (a) and syrphid larvae (b-d) average (± SE) abundance per soybean plant during 2012 and 2013 across four soybean fields (4 ha per field) in Rosemount, MN. Syrphid larvae abundance shown as (b) abundance per soybean plant, (c) ratio of syrphids to aphids, and (d) frequency of larvae per soybean plant (proportion of plants with one or more syrphids). Curves in b-d represent best fit polynomial terms for date based on a (generalized) linear mixed model that compared syrphid abundance, ratio of syrphids to aphids, and frequency of larvae in 2012 (solid line) versus 2013 (dashed line).
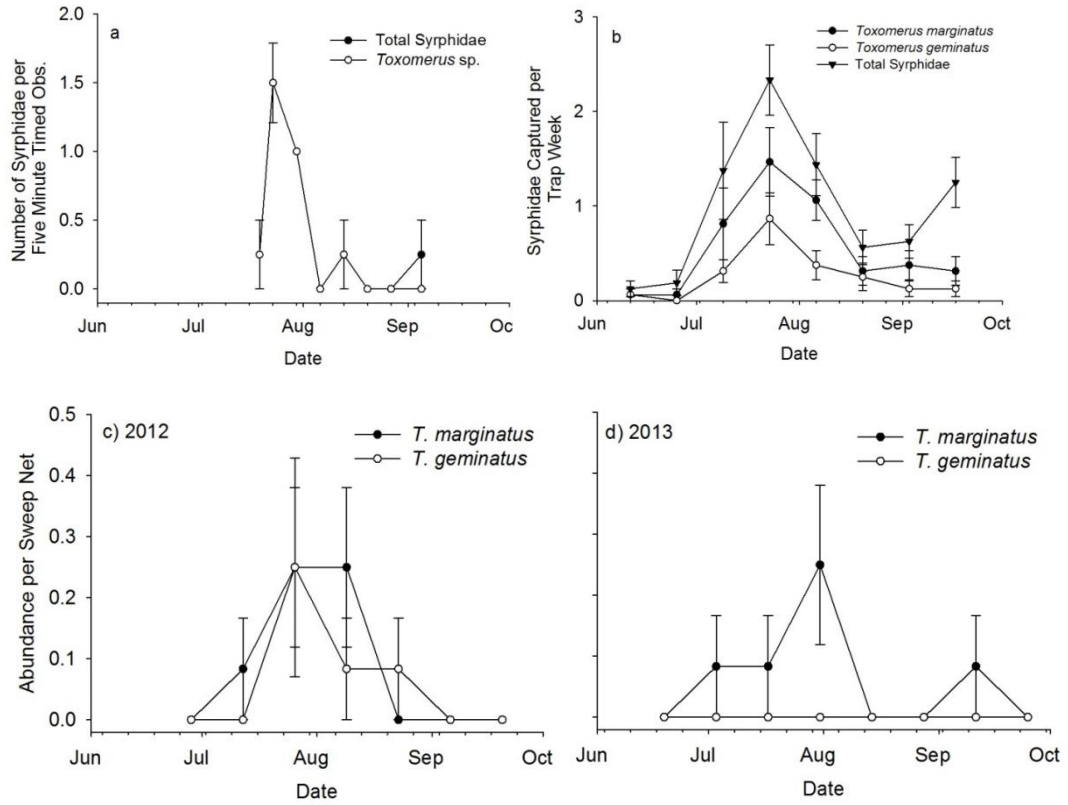
Figure 1
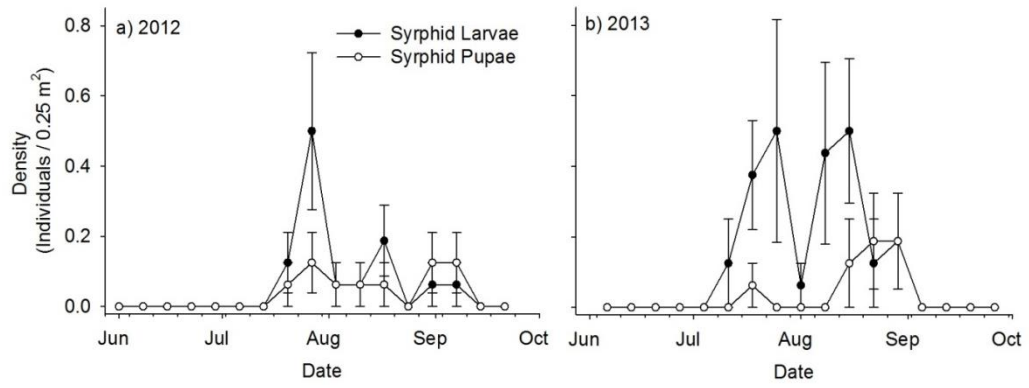
Figure 2
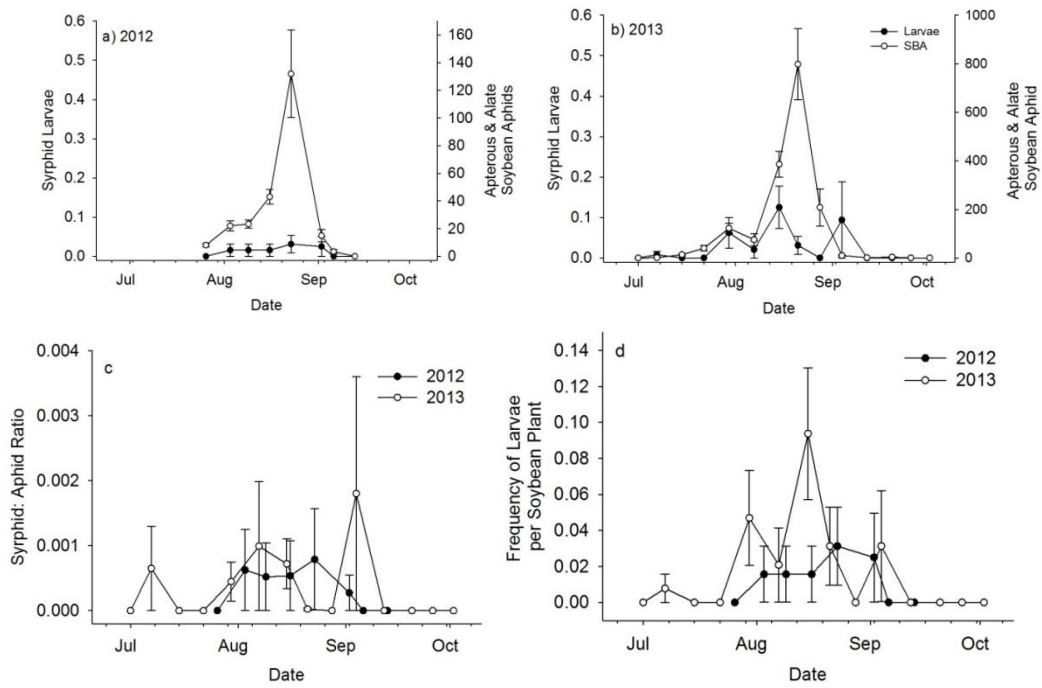
Figure 3

CHAPTER 2

Spillover of tent caterpillar (*Malacosoma americanum*) herbivory onto willow bioenergy
crops in an agricultural landscape

Introduction

Integrative perennial cropping systems for food and biomass production are posed to
drive a second green revolution in the U. S. (Runck et al., 2014; Jordan et al., 2007;
Heaton et al., 2013). There is growing demand for advanced biofuels from perennial
crops (Holzmueller & Jose, 2012). Meanwhile, recent advances in genomics and breeding
are leading to the rapid development of perennial food crops that may soon have
competitive yields with conventional systems (Runck et al., 2014). The emergence of
perennial biomass and food crops on the landscape will drastically change agro-
ecosystems presenting new challenges and opportunities for insect pest management
(Weih et al., 2008). Perennial crops provide relatively stable environments as well as
alternative prey and floral resources; introduction of such crop diversity could therefore
enhance biological control of some insect pests and contribute to greater sustainability in
agriculture (Dalin et al., 2011; Andow, 1991; Weih et al., 2008). However, the identity,
dynamics, and management of the insect pests on perennial crops themselves are largely
unknown (Landis & Werling, 2010) but are expected to be fundamentally different from
pest dynamics in annual crops. One such perennial crop that shows promise both for
biomass production and enhancement of biological control services is short rotation
coppice (SRC) willow, *Salix spp.*(Dalin et al., 2011; Keoleian & Volk, 2005).

Little is known about the potential impacts of insect pests on willow biomass crops in the U.S. (Coyle et al., 2005; Landis & Werling, 2010). Much more is known from Europe where defoliating insects (e.g. leaf beetles; Coleoptera: Chrysomelidae) can damage over 85% of leaves (Kendall et al., 1996) and cause up to 68% leaf loss (Sage & Tucker, 1997). Leaf defoliation can reduce willow biomass production by 32-70%, an effect that can persist into subsequent three-year rotations (Bell et al., 2006; Björkman et al., 2000). Further, stem-feeding aphids (*Tuberolachnus salignus* Gmelin and *Pterocomma salicis* L.) can reduce stem and root biomass of willow by more than 50% (Collins et al., 2001). Native and non-native insects are known to attack native willow in North America (Hochwender & Fritz, 2004; Hjältén & Price, 1997; Broberg et al., 2001; Bach, 2001; Solomon & Randall, 1978; Wade & Breden, 1986) but little is known about insect herbivore damage and impacts on the performance of non-native hybrid willow in this region. The potential for willow damage is significant given the high susceptibility of some willow clones to several insect species, especially willow sawflies in the genus *Nematus* (Hymenoptera: Tenthredinidae), occurring in the U.S. (Nordman et al., 2005). As hybrid willow plantations are expanded in North America, colonization of willow crops is expected (Strong et al., 1977). Environmental context (i.e. density of natural versus plantation stands) was shown to mediate impact by the blue willow beetle (*Phratora vulgatissima* L.) (Coleoptera: Chrysomelidae) in Sweden (Dalin et al., 2009). It is critical to better understand the insects and environmental contexts that may lead to impacts on willow in the U.S.

There have been observations of eastern tent caterpillars (*Malacosoma americanum* Fabricius) (Lepidoptera: Lasiocampidae) utilizing willows as an alternative

food source (Fitzgerald, 1995) but the spatial dynamics or consequences of such herbivory for willow are unclear. Eastern tent caterpillars have periods of rapid population growth that result in outbreak densities and density-dependent dispersal of late instar caterpillars to alternate food sources after degradation of the host tree (Fitzgerald, 1995; Rieske & Townsend, 2005). On a local scale (1-10 meters) insect herbivores are known to spillover from host plants to nearby palatable plant species with consequences for plant performance (Andersen & Louda, 2008). However, the associated susceptibility hypothesis posits that spillover effects could occur on a landscape level, especially within an agricultural community (Barbosa *et al.*, 2009). Spillover dynamics for tent caterpillars may occur on a spatial scale greater than 10 meters as the caterpillars are known to travel up to 7 meters in 30 minutes (Rieske & Townsend, 2005). An open question with broad ecological application centers on identifying landscape characteristics and configurations which are conducive to the spillover of tent caterpillars onto willow biomass crops.

The spillover dynamics of insect pests into an adjacent area is dependent upon the insect's natural history, behavior and mobility. The eastern tent caterpillar is a gregarious, central-place forager (Fitzgerald, 1995). The moths primarily lay their eggs on trees within the *Rosaceae* family including *Crategus* spp., *Malus* spp., *Prunus pensylvanica* L. f., *P. serotina* Ehrh., and *P. virginiana* L. (Fitzgerald, 1995). The pharate larvae overwinter in egg masses on trees and emerge with their host tree's bud break (Neal et al., 1997). Caterpillars build silken tents on their host tree and feed on its leaves until pupation. If caterpillars defoliate their host tree before they reach pupation, they disperse from the host tree in search of alternative food sources (Fitzgerald & Edgerly, 1979). When caterpillars find palatable leaves, they establish a pheromone recruitment trail to

signal siblings to a suitable food source (Fitzgerald & Peterson, 1983). This process may enhance the efficiency in searching for distant food sources. Large instars (primarily 6th instars) can disperse to their new food source and not return to their tent, leading to heavier defoliation of the new food source (Fitzgerald et al., 1988).

Outbreak population densities of eastern tent caterpillars that defoliate their host tree could subsequently spillover onto adjacent willow stands and cause severe defoliation. This has the potential to negatively affect growth and establishment in willow stands nearby host trees, leading to a decrease in biomass production. Here, we investigate leaf herbivory and caterpillar abundance on willow in relation to proximity of the nearest tent caterpillar host tree and its degree of defoliation. This study provides insight on the spatial dynamics of tent caterpillar spillover from host trees across an agricultural landscape. Such information will improve management of herbivores in willow cropland by helping identify landscape positions where willow are susceptible to tent caterpillar invasion.

Materials and Methods

Study site, plots, and plant material

We quantified tent caterpillar abundance and leaf herbivory in eight experimental willow production plots at the University of Minnesota Rosemount Research and Outreach Center in southeastern Minnesota, USA during an outbreak of eastern tent caterpillars (Figure 1). The willow was a hybrid cross of *Salix purpurea* and *S. miyabena*, 'Millbrook' obtained from a commercial nursery (AA Willow, Fredonia, New York USA). The experimental design involved four planting treatments: prairie polyculture,

willow monoculture, willow-prairie alley configuration, and control (corn) surrounded by four hectares (200 x 200 m) of corn planted on April 25-May 17th, 2011. Each treatment was randomly distributed within four blocks (four replications per treatment) in a randomized complete block design. Each plot was at least 0.5 km away from any other plot. The position of three pairs of plots was changed after randomization for logistical reasons, to enhance spatial representation of treatments, or to improve direct comparison among treatments within block. We focus on the willow-prairie and willow monoculture treatments in the current study on willow herbivory by tent caterpillars (map of alley and willow monoculture in Figure 1).

On May 16-17th (2011), we planted four willow plots as 15 x 20 m monocultures (412 willow stakes on average per plot) and four in an alley configuration consisting of two 13.3 x 7.5 m willow monocultures bordering a 7.5 x 13.3 m prairie polyculture planting on the east and west side (289 willow stakes per plot). One-third of the willow stakes at one alley plot were not planted until June 8th, after tent caterpillars were first observed feeding on willow, and we therefore do not include those willows in the estimate of willow herbivory.

Observations of tent caterpillars and leaf herbivory
We first observed tent caterpillars (*M. americanum*) feeding on willow on June 5, 2011 and by June 9th approximately 25% of planted willows were defoliated in one plot (J. O. Eckberg, personal observation). This prompted us, on June 14-16th, 2011, to score leaf removal from insect chewing on all established willows in the study (N = 2,682 willows) as no damage (0% leaf removal from insect chewing), minor damage (1-10% leaf

removal), moderate to substantial damage (>10% leaf removal), or complete defoliation (98-100% leaf removal). We subsequently analyzed herbivory frequency as the proportion of willow per plot with more than 10% leaf removal. To quantify total tent caterpillar abundance per plot, from June 14 to June 29th, we counted and removed all caterpillars every one to three days until we observed zero caterpillars. On June 13th, tent caterpillars were removed from two plots without being quantified. We removed tent caterpillars to ensure establishment of the willow stands for a subsequent study on the insect predators in willow that provide biological control for soybean aphids (Peterson et al, unpublished manuscript). Our study thus presents a conservative estimate of tent caterpillar abundance and associated herbivory.

On June 16-17th we searched the field margins associated with each willow plot and measured the distance (m) from each willow plot to the nearest tree with any *M. americanum* tents. We quantified the number of tents and scored the tree as completely defoliated (≥ 98% leaf loss) or not (≤ 97% leaf loss). If the nearest tree with tents was not completely defoliated we expanded our search along the field margin until we located such a tree. However, if the nearest tree was completely defoliated, we did not search farther for the nearest non-defoliated tree. We located the nearest completely defoliated tree with tents for seven of eight plots; in the only exception we recorded the farthest distance searched relative to the willow plot (385 m) as a conservative estimate of distance to the nearest defoliated tree. We reviewed pictures of each tree with tents and queried an observer blind to willow herbivory patterns among plots for a second categorical assessment of defoliation; assessment results were consistent with those performed in the field. On July 6-7th we measured host tree height and width in two

perpendicular directions to quantify tree size as volume assuming a cylindrical shape. We

also classified the habitat in the 5 meters surrounding each host tree as predominately

open (i.e. grassland, little to no tree cover) or closed (i.e. mixed forest edge).

To estimate willow survival at the plot level we quantified willow stand number

in monoculture and alley plots at the end of the season (September 1 to 8th, 2011) and

divided by the initial number of willow counted on June 14-16th. Because this was the

establishment year for willow, we coppiced all willow on November 18th, 2011 and

quantified coppiced willow dry biomass for a subsample of 24 willow per alley plot and

36 willow per monoculture plot representing approximately 8.5% of willow in each plot

(N = 240 willow). Willow was dried at $60^{o}C$ for 280 hrs to obtain constant dry mass. To

evaluate the potential effect of tent caterpillars on willow growth in the two years after

coppice we randomly selected two sections of eight willows in each plot and measured

their stem diameter at 30 cm from the plant base on September 10 to 13, 2012 and

September 23 to October 2, 2013.

Statistical analysis

We used the open-source software R to analyze all data (R Development Core Team,

2014). The effects of single continuous variables were analyzed using regression (lm in

R). This included how distance of willow to the nearest defoliated host tree the

influenced abundance of tent caterpillars in willow, how abundance of tent caterpillars

influenced willow herbivory, and how willow herbivory affected willow stand survival.

We used ANCOVA to analyze the interactive effect of discrete and continuous variable

(lm in R). These included how complete host tree defoliation (yes, no) and their

proximity influenced willow herbivory as well as how host tree volume (m^3) and surrounding habitat influenced host tree percent defoliation. Prior to ANCOVA of the latter analysis we performed backward elimination to determine which variables significantly influenced percent defoliation on host trees including host tree habitat (open, closed), tents per host tree, and host tree volume (m^3). We present marginal effects (*t*-statistics) from backward elimination and sequential effects (*F*-statistics) for all other analyses. We present R^2 adjusted for the number of parameters in each model. We used residual plots to evaluate the homogeneity and normality of variance; data transformation was not necessary to meet the assumptions of any tests. We employed AIC (Akaike's Information Criterion)- based model selection to evaluate linear versus non-linear (quadratic or decay) terms that best fit the data for each test (Akaike, 1974; Hilborn & Mangel, 1997).

Results

Willow herbivory and proximity to a defoliated host tree

We observed significantly more herbivory on willow (>10% defoliation) when host trees (tree with a tent) were closer and the host tree was itself severely defoliated (Figure 2A; host tree proximity* host tree defoliation interaction $F_{1, 8} = 16.67$, $p = 0.0035$). Host tree proximity, its defoliation, and the interaction of these factors accounted for 82% of the variation in willow leaf herbivory. Model selection (Akaike weights, w_i) strongly supported a non-linear relationship between tent host tree proximity and herbivory on willow (decay function $w_i = 0.991$, linear $w_i = 0.009$). We found no effect of treatment (willow monoculture versus alley) on willow defoliation ($F_{1, 7} = 0.16$, $p = 0.74$). Two

findings support the association between herbivory on willow and proximity to a defoliated host tree. First, there were more tent caterpillars on willow that were closer to a defoliated host tree (Figure 2B; R^2=0.52, $F_{1, 6}$ = 8.72, p =0.026) and the form of this relationship was non-linear (decay function w_i = 0.803, linear w_i =0.196). Second, greater tent caterpillar abundance was associated with a higher frequency of leaf herbivory (> 10% defoliation) on willow plants (Figure 2C; R^2 = 0.66, $F_{1,6}$ 14.72, p =0.0086). Thus, these data suggest that the proximity to the nearest defoliated host tree mediated the spillover and herbivory of tent caterpillars on willow.

Willow herbivory, survival and biomass

There was a negative linear relationship between the proportion of willow plants with more than 10% leaf defoliation and willow survival to September (adjusted R^2=0.63, $F_{1,6}$ = 12.70, p=0.012; Figure 3). This suggests defoliation greater than 10% was a relevant interaction intensity to evaluate the spatial dynamics of herbivory. We performed the regression without the point near 0.80 willow herbivory and this showed a similarly significant linear relationship (R^2=0.54, $F_{1, 5}$ = 8.18, p=0.035). There was, however, no detectable relationship between willow mortality and willow biomass (dry, g) per m^2 at the time of coppice ($F_{1, 6}$ = 0.051, p = 0.83) or willow stem area (mm^2) after one or two subsequent years of growth following establishment (2012, 2013; $F_{1,6}$ = 0.11, p = 0.75 and $F_{1, 6}$ = 0.44, p = 0.53, respectively). Furthermore, there was no relationship between willow herbivory in 2011 and biomass per m^2 at coppice ($F_{1, 6}$ = 0.0052, p = 0.95) or willow stem area in 2012 ($F_{1, 6}$ = 0.16, p = 0.71) or 2013 ($F_{1, 6}$ = 0.021, p = 0.89).

Host tree habitat and herbivory

There was significantly higher percent defoliation on smaller host trees (t = -2.62, p = 0.031) growing in open (97.9 ± 0.76% SE, n = 8) versus closed habitat (74.7 ± 9.9% SE, n = 3; t = 5.0, p = 0.0011; Figure 4). The interactive effect of host tree habitat and tree size was significant ($F_{1, 7}$ = 32.9, p <0.001); the entire model explained 95.3% of the variation (R^2) in percent defoliation (Figure 4). Defoliated host trees were all in the family Rosaceae. The trees near four willow plots were wild plums (*Prunus americana* Marshall), choke cherries (*Prunus virginiana* L.) were near two plots, and crab apple (*Malus* sp.) was near one plot. There was no defoliated host tree within 385 meters (the farthest within field search distance) of one plot.

Discussion

Key features of the landscape were shown to play an important role in the herbivory dynamics of willow crops. Proximity to caterpillar tents and the extent to which the caterpillars defoliated their host tree jointly influenced the spillover of tent caterpillar herbivory onto the willow bioenergy crop. Supporting this pattern, we observed more tent caterpillars on willow with increasing proximity to a defoliated host tree and as tent caterpillar abundance increased, so did herbivory on willow. These results are consistent with the associational susceptibility hypothesis; a recent meta-analysis found that the presence of palatable neighboring plants enhanced the herbivory of specialist insects on focal plants (Barbosa et al., 2009). While spillover of insect herbivory among palatable plants is typically shown to occur on a smaller spatial scale, the current study suggests associational susceptibility operates at the landscape level, a phenomenon for which

mechanisms have remained less clear (Barbosa *et al.,* 2009). Our study suggests that resource depletion caused the spillover of a relatively specialized herbivore across an extensive area. By embedding our willow plots in a landscape of corn and soybeans we inferred that numerous eastern tent caterpillars travelled over 100 meters to inflict major herbivory on a non-host food source, willow. While it is surprising to find caterpillars so far from the nearest tent, especially because in artificial arenas tent caterpillars moved only 12 meters in two hours (Rieske & Townsend, 2005), long distance travel has been observed for other species with highly injurious caterpillar stages. For example, the gypsy moth caterpillar can travel 125m away from a suitable food source (Doane & Leonard, 1975).

The habitat and size of the host tree mediated its herbivory, smaller host trees in open habitats were more defoliated than larger trees in closed habitats. Greater access to sunlight in open habitats can allow tent caterpillars to maintain a higher constant body temperature, leading to higher rates of growth (Knapp & Casey, 1986) and defoliation (Verdinelli & Sanna-Passino, 2003) of the host tree. Once the host tree is defoliated, tent caterpillars are known to search for secondary palatable plants. By the sixth instar they do not return to the original host tree (Fitzgerald et al., 1988). Thus, the association between habitat and host tree defoliation was likely significant to the dispersal of tent caterpillars and subsequent discovery and defoliation of nearby willow bioenergy crops. Such dynamics are likely to occur only during high density, outbreak years when such severe defoliation is expected (Rieske & Townsend, 2005; Barbosa *et al.,* 2009).

Herbivory reduced survival of willow during the establishment year consistent with other examples of insect herbivore-associated mortality of willow (Collins et al.,

2001; Bach, 2001). However, there was no effect of herbivory on biomass yield at the plot level during the time of coppice or in following years. This contrasts other studies which found partial and severe defoliation to have long-term negative effects on biomass yields (Bell et al., 2006). The defoliation in our study occurred early in the growing season, allowing apparently ample time for compensatory growth in surviving willow plants (Björkman et al., 2000; Nakamura et al., 2003; Kendall & Wiltshire, 1998). For example, Kendall and Wiltshire (1998) found that a single, early season defoliation event had smaller effects on biomass yields than defoliation later in the season. Further, our study may underestimate the effects of herbivory on willow mortality and biomass production because tent caterpillars were removed from plots before pupation. Thus, it remains unclear whether and how much tent caterpillar herbivory may reduce biomass production of willow. These data suggest that the population dynamics of tent caterpillars and spatial position of host trees on the landscape mediate the early survival and density of willow.

Tent caterpillars may not present a major and persistent barrier to willow cultivation because outbreaks are episodic and impacts may be limited to the establishment year of a willow plantation. On the other hand, the irregular time intervals between eastern tent caterpillar outbreaks and their gregarious foraging habits make it difficult to observe willow defoliation prior to severe damage and in some years damage may be more intense on willow (Fitzgerald et al., 1988) . Also, there is wide variation in susceptibility to herbivory among cultivars; cultivars other than Millbrook may be more or less susceptible to tent caterpillars (Kendall et al., 1996). To address this potential pest problem, less palatable cultivars could be identified and integrated into genetically

diverse stands of willow to confer associational resistance to willow stands and deter

large scale herbivory (Peacock & Herrick, 2000; Kendall et al., 1996; Barbosa et al.,

2009). In extreme cases of damage, insecticide treatments may be necessary. However,

broad spectrum insecticides can harm non-target, beneficial insect populations and

diminish the opportunity to utilize willow crops in integrated pest management. Our

study suggests pest sampling for newly established willow should target specific

landscape positions prone to spillover by tent caterpillars to provide early detection and

increase efficiency of pest management schemes.

The current study supports mounting evidence that landscape characteristics and

spatial dynamics are key determinants in the spillover of herbivorous insects across the

agricultural landscape (Gladbach et al., 2011; Rand et al., 2006; Dalin et al., 2009). This

has important implications for the strategic integration and landscape placement of

perennial crops into multi-crop agricultural systems (Heaton et al., 2013). Willow

production is sensitive to position on the landscape based on variation in terrain and soil

attributes (Thelemann et al., 2010). Our study suggests spatio-temporal variation in insect

populations may also be important to the selective placement and productivity of such

crops at the field or landscape level. For example, depositional landscape positions where

willow show increased productivity (Thelemann et al., 2010) are often adjacent to non-

arable, semi-natural habitats which may comprise host trees of tent caterpillars along with

a greater risk of defoliation to willow. Therefore, willow could be more susceptible to

spillover of tent caterpillar herbivory in landscapes with extensive semi-natural habitat as

in our case (Figure 1). This finding contrasts other studies that show less herbivory with

crop or landscape diversity due to reduced foraging efficiency and/or enhanced biological

control (Dalin et al., 2009; Gardiner et al., 2009). Our study reinforces the assertion that pest dynamics of perennial crops will be 'predictably unpredictable'(Landis & Werling, 2010). Understanding the dynamics of emerging pests on perennial crops will thus be critical to the successful establishment of such crops across a diversity of environments.

Figure Legends

Fig. 1: Map of experimental site showing spatial distribution of willow plots, their defoliation, proximity to the nearest host tree and its defoliation. Gray area represents semi-natural habitat, woodlands and grasslands. White indicates annual crops, primarily corn and soybeans. Willow plots are shown as closed circles for low willow herbivory (herbivory frequency < 0.20) or open circles for high willow herbivory (herbivory frequency >0.50). Host trees are indicated by a closed triangle for non-defoliated host trees or open triangle for severely defoliated host trees.

Fig. 2: Relationship between host tree defoliation and herbivory on willow. A) Distance to nearest defoliated or non-defoliated host tree versus the frequency of willow with more than 10% insect defoliation. Non-defoliated host trees: Willow defoliation = 0.035 + 3.1(1/distance); Defoliated host trees: Willow defoliation = -0.39 + 130.7(1/distance); R^2 = 0.82, residual σ^2 = 0.015 on 8 df. B) Distance to nearest defoliated host tree versus the number of tent caterpillars in each willow plot. Caterpillar abundance = -12.6 + 4824.3(distance); R^2 = 0.52, residual s^2 = 102.0 on 6 df. C) The number of tent caterpillars in willows plots versus the frequency of willow with more than 10%

defoliation. Willow defoliation = 0.052 + 0.019(caterpillar abundance); $R^2 = 0.66$,

residual $s^2 = 0.037$ on 6 df.

Fig. 3: The proportion of willow with more than 10% insect defoliation versus proportion

of willow surviving to the end of the growing season. Willow survival = 0.99 −

0.18(willow defoliation); $R^2 = 0.63$, residual $s^2 = 0.0019$ on 6 df.

Fig. 4: Insect defoliation on host trees in relation to habitat type and tree size (volume,

m^3). "Open" refers to shrub land, grassland, or otherwise non-forest edge habitat.

"Closed" are forest edges. The cut off for defoliated versus non-defoliated host trees (Fig.

2A) is more than 95% defoliation. Host trees in open habitat: % Defoliation = 99.3 −

0.58(tree volume); Host trees in closed habitat: % Defoliation = 88.6 − 4.6(tree volume),

$R^2 = 0.95$, residual $s^2 = 8.4$ on 7 df.
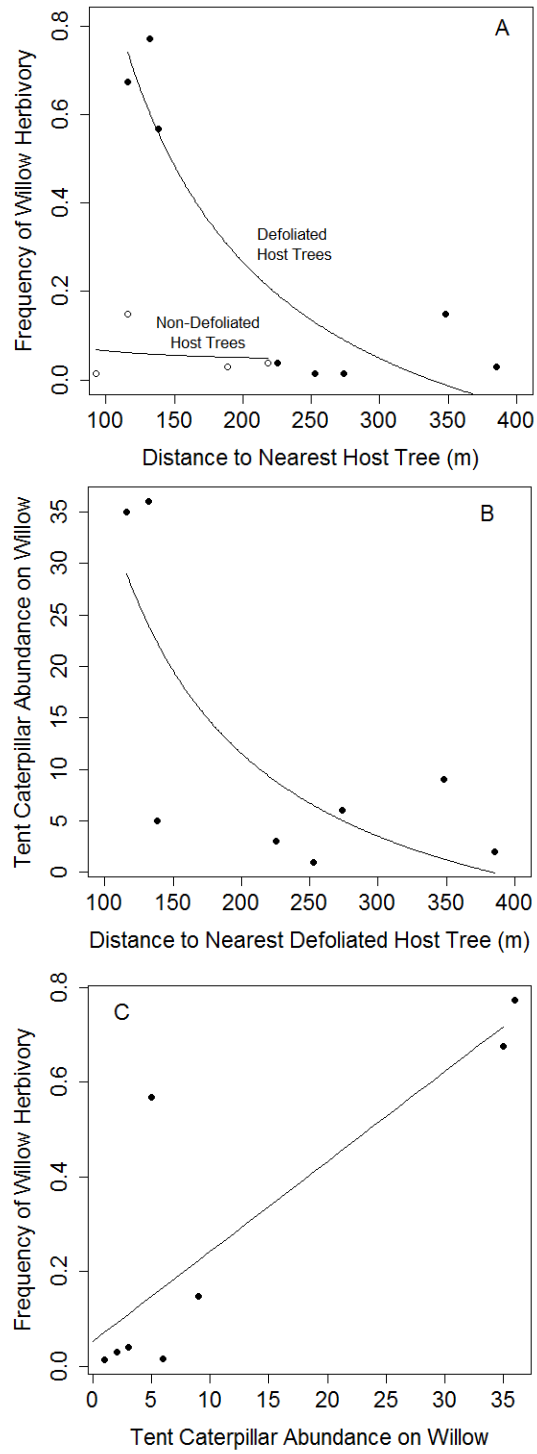
1 km
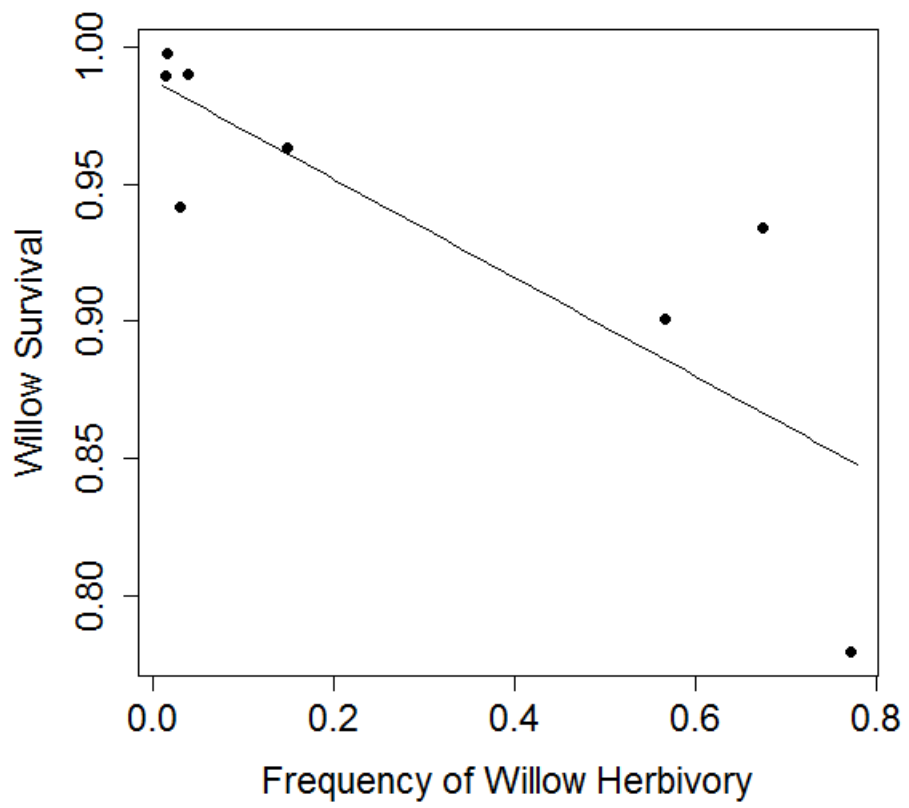
Figure 1
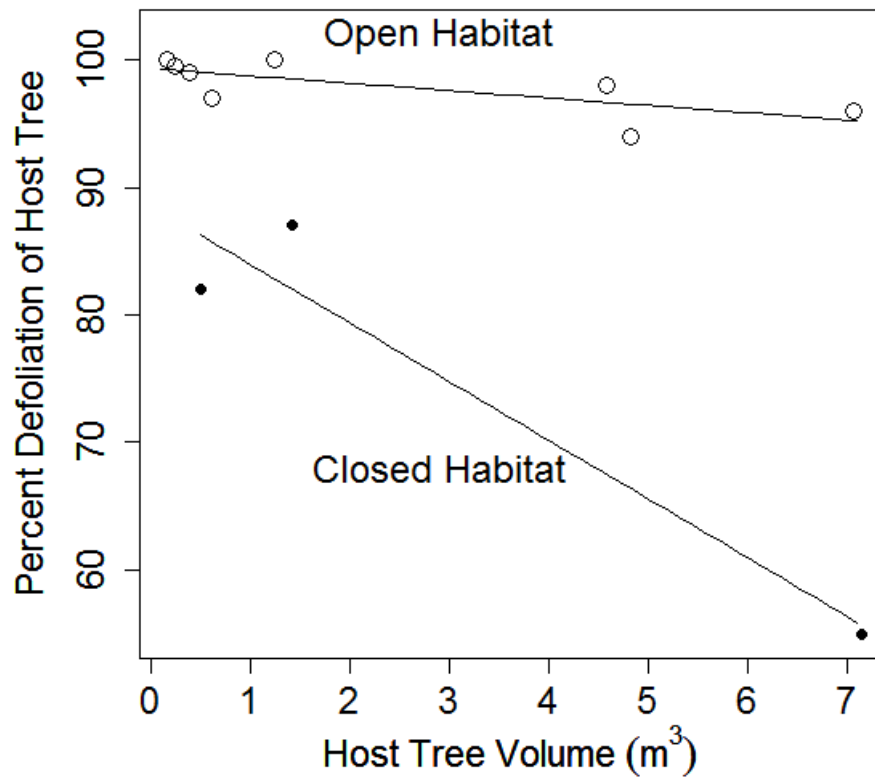
Figure 2

Figure 3

Figure 4

CHAPTER 3

Integrative Perennial Cropping Systems – Biomass Bioenergy and Natural Enemies of Insect Pests

Introduction

Agriculture is at the intersection of major global challenges for the 21st century. There is a growing need to increase food security and production while meeting the demand for bioenergy and biomaterials. At the same time, we are faced with significant environmental concerns around water quality, biodiversity, and sustainability resulting from intensive annual crop production practices (Pimentel et al., 1995). Addressing these problems will require us to re-think and re-design agriculture in a way that provides a diverse array of food, fiber, bioproducts, and bioenergy while strategically enhancing ecosystem services. Perennial crops are being suggested as a way to provide a sustainable source of biomass feedstock while enhancing ecosystem services including biological control of agricultural pests (Landis et al., 2008), wildlife habitat (Fargione et al., 2009), soil carbon sequestration (Anderson-Teixeira et al., 2009), and mitigation of the nitrogen and phosphorus in surface waters (Blanco-Canqui et al., 2004). Perennial biomass cropping systems can also be designed and integrated into conventional agriculture to directly support and add economic value to row-crop systems while generating biomass and ecosystem services.

The challenge to designing and optimizing perennial cropping systems for multiple ecological and agronomic objectives hinges on identifying and understanding potential tradeoffs and synergies among multiple interacting crops. Integration of

perennial and annual crops shows promise to meet these interconnected challenges by diversifying outputs, minimizing conservation-production tradeoffs, and improving system-level yields (Jordan et al., 2007; Heaton et al., 2013; Runck et al., 2014). By directly supporting conventional agriculture, such integrated systems can temper long-standing debates over biomass and conservation versus food production (Jordan et al., 2007). At the same time, careful selection and integration of multiple perennial crops in alley configurations can improve their productivity (Jose et al., 2004; Johnson et al., 2013). Thus, careful consideration of perennial crop selection, spatial configuration, and integration into conventional agricultural landscapes systems can help optimize a diverse suite of agricultural products and ecosystem services.

Perennial biomass crops may enhance biological control in row crops by provisioning habitat and floral resources to support natural enemies of row crop pests. Substantial evidence indicates that structural and species diversity can increase predator populations by provisioning pollen and nectar, hosting alternative prey, or creating refugia to minimize intraguild predation (Root, 1973; Andow, 1991; Finke and Denno, 2002). As posited by the enemies hypothesis (Root, 1973) such dynamics are conducive to stable predator populations leading to enhanced suppression of pest insects. A meta-analysis involving 552 experiments showed that crop diversification generally enhances insect predator populations and control of insect pests (Letourneau et al., 2011). Structural diversification (i.e. alley cropping) can also support natural enemy populations by increasing niche availability (Peng et al., 1993) and providing continuous habitat to mitigate the effects of annual row-crop harvest disturbance on predator population dynamics (Dalin et al., 2011) . For example, walnut-alfalfa alley crops provided

continuous cover between alfalfa harvests and this was associated with greater parasitism rates of the alfalfa weevil as compared to alfalfa monocultures (Stamps et al., 2009). Thus integration of perennial biomass crops and plant communities into agricultural landscapes could be an effective conservation practice for improving biological control of insect pests (Landis et al., 2000, 2005).

Our ability to efficiently deliver the benefits of diversified farming systems for biomass production and enhanced biological control is limited by the small body of information on how to select and integrate woody and herbaceous crops to support natural enemy communities (Landis and Werling, 2010; Gill et al., 2014) and produce biomass for bioenergy. Despite this, several emerging patterns suggest cropping systems can be designed for improved biomass production and natural enemy habitat. For example, floral resources can support insects beneficial to biological control with their efficacy depending on their design (Isaacs et al., 2009). Mixtures of species that provide flowers throughout the season can better supply floral resources to support beneficial insects (Isaacs et al., 2009). Such mixtures have been shown to generally support more natural enemies than less diverse crops or crops lacking flowers altogether (Gill et al., 2014). The value of flowering plant species to beneficial insects varies (Fiedler and Landis, 2007a) given differences in plant architecture and olfactory properties which mediate attractiveness and availability of floral resources to natural enemies (Wackers, 2004; Fiedler and Landis, 2007b). While floral resources of herbaceous crops can support greater natural enemy abundance than woody crops (Gardiner et al., 2010; Robertson et al., 2012; Gill et al., 2014), other factors such as harvest regime and disturbance frequency could become more important in biomass production systems. The lower

harvest frequency of woody compared to herbaceous crops could lead to greater predator populations (Dalin et al., 2011). Woody crops also produce more biomass than many herbaceous crops (Thelemann et al., 2010; Johnson et al., 2013). Integrating woody crops with diverse herbaceous crops could help optimize biological control and biomass production not only because integrative systems include high diversity herbaceous communities and productive woody monocultures but such systems could enhance structural diversity further improving habitat for natural enemies (Peng et al., 1993; Thevathasan and Gordon, 2004). Furthermore, woody crops can perform better in integrated systems by showing enhanced growth along the woody-herbaceous edge (Gamble et al., 2014), and woody crops can enhance the microclimate for herbaceous crops by reducing wind velocity and temperature leading to improved herbaceous yields (Jose et al., 2004). Despite these potential benefits, few have tested biomass production and natural enemies in integrated perennial cropping systems.

In this study, we test the efficiency of three perennial cropping systems (herbaceous polyculture, willow monoculture and integrated alley configuration of both crops) to provide floral resources and plant cover that supports natural enemies as well as biomass for ethanol. The biomass crops include hybrid willow, prairie polyculture and an alley configuration. These crops are highly productive and alley configurations including them has led to limited depression of herbaceous yields (Johnson et al., 2013; Gamble et al., 2014). The insects we focus on are all relevant to the biological control of the soybean aphid, a major pest of soybeans in the United States (Ragsdale et al., 2011). These include Syrphidae, spiders, Coccinellidae, *Orius insidiosus*, Nabidae, Geocridae, Chrysopidae, and Hemerobiidae (Rutledge et al., 2004; Desneux et al., 2006). We tested

1) the biomass and biofuel (theoretical ethanol) yields of SRC willow and prairie polyculture cropping systems, 2) the role of spatial position (interior versus edge) and planting configuration (i.e. continuous versus alley) on biomass yields and flower abundance, 3) the abundance of natural enemy groups in each crop, and 4) the influence of planting configuration (i.e. polyculture bordered by SRC willow versus soybeans) on the natural enemies of prairie polyculture. A better understanding of the performance of perennial cropping systems in terms of supporting natural enemies while producing biomass feedstock could illuminate design strategies that improve the integration of such crops into conventional agriculture.

Methods:

Site:

The study was conducted at the University of Minnesota Rosemount Research and Outreach Center in east-central Minnesota (44º 42' 50'' N, 93º 04' 32''W). The most common soil type was Waukegan silt loam followed by Kanarazni, Estherville, Udorthents, and tallula silt loams. Soil nutrients, organic matter, and pH were measured in 2011; on average there was 23 ppm P ($\pm$ 24 SD), 111 ppm K ($\pm$ 22 SD), 3.9 % organic matter ($\pm$ 0.6 SD), and 6.3 pH ($\pm$ 0.6 SD). In spring 2012 we fertilized SRC willow plots with low P (see below).

Experimental Design:

Treatments: The experiment included four treatments: herbaceous polyculture (Polyculture surrounded by soybeans, PS), SRC willow (Willow surrounded by soybeans,

WS), polyculture-willow alley configuration (Polyculture surrounded by willow, PW; Willow adjacent to polyculture and soybeans, WPS), and control (soybeans) and we focus on the perennial treatments in this study. Treatments were established in a randomized complete block design with four replications (blocks) per treatment. However, we changed the position of SRC willow with control plots in three blocks for 1) logistical reasons, 2) to improve comparison among perennial plots by increasing their proximity, and 3) to improve spatial representation within treatments. All perennial plots were separated by at least 0.4 km. All plots were 300 m^2. The herbaceous polyculture and SRC willow were 15 x 20 m; the length was oriented north to south. To establish a wide alley in the polyculture-SRC willow alley configuration these plots were 13.3 x 22.5 m with the length oriented east to west. The herbaceous polyculture (7.5 x 13.3 m) was bordered by SRC willow (each 7.5 x 13.3 m) on the east and west side.

Polyculture species mixture: We developed a polyculture mixture that could provision floral resources through the growing season to support natural enemies (Isaacs et al., 2009), produce significant biomass for bioenergy, and was appropriate for site conditions. Selected species were native to the same county or adjacent counties as the study site (Minnesota Department of Natural Resources- MNTaxa 2015), commercially available as seed, tolerant of a wide range of soil moisture and sunlight conditions (Prairie Moon Nursery Catalog, Winona, MN), and could establish and flower within the duration of the study (personal observations & personal communications: Keith Frederick, Minnesota Native Landscapes, Inc.). Among these species, we used literature (e.g. (Fiedler and Landis, 2007a)) and personal observations to select plants known to provision prey species (aphids) and/or floral resources to natural enemies of soybean aphids. And, we

48

selected several grasses and forbs that are highly productive (Mangan et al., 2011). The final species list included 13 forb and 3 grass species that flower throughout the growing season, May – October (Prairie Moon Nurse Catalog). The forbs included *Agastache foeniculum, Asclepias incarnata, Aster novae-angliae, Astragalus Canadensis, Coreopsis palmate, Eryngium yuccifolium, Helianthus maximilliani, Lupinus perennis, Monarda fistulosa, Petalostemum candidum, Rudbeckia hirta, Solidago rigida,* and *Verbena stricta*. The grasses included *Elymus Canadensis, Andropogon gerardii*, and *Sorghastrum nutans*.

The polyculture mixture was seeded at 5 g seed m^{-2}. This seeding rate was nearly 3X the rate used in tallgrass prairie restorations and one-third the rate used in diversity studies involving smaller plot size (Tilman et al., 2001; Mangan et al., 2011). The forb and grass component each represented 50% of the seed mass. Each forb species represented 3.8% and each grass species represented 16.7% of the total seed mixture mass. This represents a significant increase in the mass of forb seed used in typical tallgrass prairie restorations (~13% forb seed mass used by Minnesota Board of Water and Soil Resources & Department of Transportation).

Perennial Plot Establishment:

Plots were tilled to 10 – 13 cm and herbaceous polyculture areas were compressed with a brillion seeder to create a firm seed bed (15 – 16 May, 2011). Planting occurred 16 – 17 May, 2011. Herbaceous polyculture seeds were evenly distributed across the plots by hand, shallowly raked into the soil, and the seed bed was recompressed with the brillion to increase seed-to-soil contact. Oats (*Avena sativa* L.) were included as a cover

49

crop at a bulk rate of 3.1 g m^{-2} to suppress weeds during establishment of the polyculture. The three legumes (*Lupinus perennis*, *Astragalus canadensis* and *Dalea candidum*) were inoculated with 0.8 – 0.85 g Genus-specific *Rhizobia* inoculant per 100 m^2 (Prairie Moon Nursery, Winona, MN) applied in two inoculations, 9 May and 16 May. We applied a sugar solution (453 g sugar per 400 ml DI water) to adhere the inoculum and seeds. Inoculum and inoculated seeds were stored at ~3º C prior to planting. After planting we performed minor irrigation of the polyculture to facilitate establishment, an equivalent of approximately 0.25 cm in rainfall was sprayed on 8 – 9 June 2011. From 25 June – 22 August we used a brush cutter to cut annual weeds (i.e. *Amaranthus retroflexus*, *Chenopodium album*, *Setaria* sp., *Eriochloa villosa*) 4 – 6X in each herbaceous polyculture plot. The majority of established vegetation at the end of the first season represented our original seed mixture; minimal weed control was required during 2012 – 2014.

The SRC willow was established at the same time as herbaceous polyculture except two outer twin rows of a polyculture-willow alley plot planted two weeks later (8 June). The willow was a hybrid of the species *Salix purpurea* and *S. miyabena* ('99217-015', 'Millbrook') acquired from the State University of New York- College of Environmental Science and Forestry (Syracuse, NY) and Double A Willow Inc. (Fredonia, NY). The cuttings (15.3 cm) were planted to a depth of 13 cm leaving at least one leaf bud above-ground. Willow were planted in twin rows with 60 cm spacing within row, 75 cm between rows, and 150 cm between twin rows. There were six twin rows in the willow monoculture (20 m twin row length) and alley (13.3. m twin row length). Immediately after planting we applied pre-emergent herbicides: oxyfluorfen (0.11 g m^{-2})

and simazine (0.22 g m^{-2}) and on 11 July we cultivated between twin rows. Thereafter

(2011-2014) weeds were controlled by hand-pulling, cultivation along the edges of

perennial plots, and the grass-selective herbicide sethoxydim was applied to control

weedy grasses in willow. No herbicides were used to control weeds in the polyculture.

On 18 November we coppiced all willow. The following spring (11 May 2012) we

fertilized all willow at 5.15 g N m^{-2} as urea which is less than half the recommended rate

of 100 lb N per acre (Abrahamson et al., 2010). Half of the willow plots showed much

lower phosphorus (1 – 16 ppm Bray P) than the others (24 – 85 ppm P); we fertilized

willow in the low phosphorus plots with 11.7 g P m^{-2}. Fertilizer was incorporated 2-5 cm

into the soil of only half of each treatment, though we did not detect any effects of

incorporation on willow biomass ($F_{1, 2} = 0.025$, $P = 0.89$).

Surrounding Crops:

The perennial plots were surrounded by 4 ha (200 x 200 m) of soybeans during

the three years of observation (2012-2014) and corn in the prior year (2011). Soybeans

and corn were managed with standard agronomic practices for the upper Midwest US

except soybeans were not treated with insecticides. We planted the soybean cultivar

Pioneer 91M51 at 375,600 seeds ha^{-1} from 2012 – 2013 and Pioneer 91Y70 at 370,658

seeds ha^{-1} in 2014 (76 cm wide rows). We selected these cultivars because they can

support an experimental environment to study natural enemy-soybean aphid interactions

by having low soybean aphid antibiosis and high resistance to soybean cyst nematode and

soybean diseases, and they were similar to each other in maturity and structure. Soybeans

were treated with recommended rates and dilutions of the herbicides, primarily

glyphosate. To prevent drift of herbicide we hand-sprayed the first 12 soybean rows

around each perennial plot. We maintained a 2.4 m wide tilled path from the four hectare

perimeter to within 20 meters of each perennial plot to access plots. Corn (2011) was

planted as refuge, round-up ready – BT, or sweet and treated with recommended rates

and dilutions of herbicides. Bifenthrin insecticide was aerial sprayed on sweet corn

including one herbaceous polyculture plot in 2011. Given the potential for this to

adversely affect our treatment plots we investigated the potential effects on our results.

However, natural enemies in the sprayed plot did not differ significantly ($t = 0.38$, $P =$

0.71) from the other herbaceous polyculture in the following year (2012) when our

experimental data collection began.

Measurements

Plant Cover- Leaf Area Index: We measured leaf area index (LAI) of willow and

herbaceous polyculture approximately every 29 days from 10 May – 27 September, 2013

and 6 May – 7 October, 2014 (6 measurements each year). LAI was measured using the

AccuPAR ceptometer LP-80 (Decagon Devices, Inc., Pullman, WA) and external sensor

mounted above the canopy. Measurements were taken under daylight conditions (i.e. 20 –

70º zenith angle) for accurate LAI estimation. LAI was measured in four interior

locations of the SRC willow and herbaceous polyculture plot and in two interior locations

for each crop in the alley planting configuration. For SRC willow sampling locations, we

measured LAI directly under the twin row and space between twin rows on a randomly

selected side (east or west). We measured the same sampling locations in SRC willow yet

disturbance in herbaceous polyculture from research activities (e.g. sweep netting)

necessitated periodic relocation of samples to undisturbed vegetation in the immediate vicinity.

Species Composition- Biomass: We measured species composition of biomass in the herbaceous polyculture at the end of each growing season (2012, 2013, and 2014). In mid-September 2012 and 2013, we harvested spatially representative subplots in the interior of the herbaceous polyculture. Two 0.25 m^2 (0.5 x 0.5 m) areas were collected for alley and three for continuous herbaceous polyculture (N = 20 year^{-1}). One randomly selected edge sample was collected per plot (N = 8 year^{-1}). We increased our sampling effort in 2014 to include twice as many interior subplots (N = 39, one sample was lost) and four edge subplots per plot (N = 32); half of the 2014 samples were each collected early September and late September to early October. Approximately 95% of biomass was clipped and sorted to species in the field. Fallen leaves of primarily *H. maximilliani* were also collected and added to this species when they constituted over 5% of the subplot biomass, such leaves constituted a low percentage of the biomass of *H. maximilliani*. Biomass was dried at 35 ºC.

Species Composition- Flowers: We measured species composition of flowers in the herbaceous polyculture approximately every 15 days from June to September (2012) or October (2013, 2014). We identified and counted forb (2012 – 2014) and grass (2013 – 2014) flowers in 80 x 80 cm subplots. Counted grass flowers showed at least some pollen. In 2012 – 2013 we monitored two interior subplots in each alley plot and six interior subplots in each continuous herbaceous polyculture (N = 32 subplots). We monitored one edge subplot on the east and west side of each plot. In 2014, we re-allocated subplots to focus on the contrast in flowers between the interior versus plot

edge. Four interior subplots and four edge (two per side) were monitored in all plots (N = 32 subplots).

Biomass and Biofuels: We compared biomass and theoretical ethanol yield of SRC willow and herbaceous polyculture across three years. Herbaceous polyculture was harvested after senescence (November) in 2012 – 2013 using a carter harvester that removed biomass to a height of 10 cm. Evenly spaced 0.9 m wide strips were harvested across the length of plot interiors (3 strips per herbaceous polyculture plot and two strips per alley) along with a randomly selected edge (east or west) in each plot (N = 28 strips year^{-1}). Early snow prevented access to carter harvester into plots in 2014 so we hand-clipped three interior and two edge subplots (1 m^2) from each plot (N = 40 subplots). We determined dry mass in 2012 – 2013 by recording wet mass of the entire sample and percent moisture for a sub-sample and in 2014 dry mass was measured for entire samples. Biomass was dried at 60 ℃. Within five days of biomass sampling we similarly harvested biomass from the remaining plot area in 2012 – 2013.

After three years of growth post-coppice (13 November 2014), we harvested six contiguous willow (2 x 3 willow in a twin row) from the interior and edge of the monoculture and alley. Six contiguous willow (2 x 3 willow in a twin row) were harvested from the interior and edges adjacent soybeans of the willow monoculture (N = 12) as well as the interior and edges adjacent the herbaceous polyculture and soybeans in the willow alley (N = 24). Dry mass was determined by recording wet mass of the entire sample and percent moisture for a sub-sample that was dried at 60 ℃ until constant mass.

We determined hemicelluloses (xylose, mannose), pectins (arabinose, galactose), cellulose and lignin using the standard procedure NREL/TP-510-42618 (Sluiter et al.,

2010). We assumed a Mannose: Glucose ratio of 2:1 for all biomass which likely had little effect on cellulose estimation given the small concentration of mannose. We used the equations $1 - 6$ provided by Schmer et al (2012) to calculate theoretical ethanol yield for each sample collected from herbaceous polyculture (2012 – 2013: 28 samples year^{-1}, 2014: 40 samples) and five composite samples each representing biomass from all willow samples. Theoretical ethanol production (L m^{-2}) was determined from theoretical ethanol yield (L g^{-1}) by biomass production yield (g m^{-2}) (Schmer et al., 2012). Total ethanol production (L m^{-2}) was estimated for herbaceous polyculture and SRC willow for each plot and spatial position (interior, edge) across three years.

Statistical Analysis:

  Treatments were analyzed with mixed effects models in the statistical program R, version 3.1.3 (R Development Core Team, 2014). The treatments or fixed effects included: crop (SRC willow, herbaceous polyculture), cropping structure (continuous crop, alley configuration), and spatial position (interior, edge). Time (Julian date), and any significant higher-order polynomials, were treated as fixed effects for variables measured across the season. Block and plot within block were treated as random effects. Continuous variables (LAI, biomass yield, and theoretical ethanol yields) were analyzed with linear mixed effects models (R library nlme: lme) and square root (LAI) or log+1 (biomass) transformed as necessary to improve equality of variance. Count data (Flower number, natural enemies) were analyzed with generalized linear mixed models (R library MASS: glmmPQL) assuming an underlying poisson error distribution.

Results:

Spatial position and cropping structure effects on herbaceous species

Biomass composition: From 2012 – 2014, we compared herbaceous biomass in the interior of the polyculture portion of the alley treatment adjacent to willow (polyculture integrated with willow in alley configuration, PW) versus pure polyculture plots (polyculture soybeans, PS) for the six most dominant species constituting 85% of the total biomass. Each of these species constituted 6.2 – 24% of total biomass; all other species composed less than 3% of total biomass. *Rudbeckia hirta* was analyzed only in year 1 because it was dominant (44.2%) but not years 2 and 3 when it composed less than 1% of the total biomass. There was more *Aster novae angliae* ($F_{1, 11} = 21.8$, $P = 0.0007$; Figure 1) and *Monarda fistulosa* ($F_{1, 11} = 14.4$, $P = 0.0030$; Figure 1) biomass in PW whereas *Sorghastrum nutans* was more productive in PS ($F_{1, 11} = 10.5$, $P = 0.0079$; Figure 1). No significant differences were detected for *Helianthus maximiliani*, *Rudbeckia hirta*, *Andropogon gerardii*, or *Elymus canadensis*. Community herbaceous biomass was slightly greater in PW than PS (7.5% more), a non-significant difference ($F_{1, 11} = 0.94$, $P = 0.35$), suggesting a net-neutral effect of species-level differences in biomass.

In 2014, we detected a significant interactive effect of spatial position (interior, edge) and treatment (PW, PS) on herbaceous biomass ($F_{1, 61} = 16.1$, $P = 0.0002$; Figure 2). There was significantly more biomass on the edge versus interior ($F_{1, 61} = 55.9$, $P< 0.0001$), and the edge effect was greater for PS ($t_{61} = 8.2$, $P < 0.0001$) than PW ($t_{61} = 2.1$, $P = 0.039$; Figure 2). Contributing to this community-level effect were significant spatial position $\times$ treatment interaction observed in *H. maximiliani* ($F_{1, 61} = 18.4$, $P = 0.0001$; Figure 2) and *E. canadensis*, ($F_{1, 61} = 4.054$, $P = 0.0485$; Figure 2).

Floral composition: In 2012, there were significant spatial position $\times$ date (t_{234} = 10.12, *P* <0.0001), treatment $\times$ date (t_{234} = 3.23, *P* = 0.0014) and treatment $\times$ spatial position (t_{38} = 6.38, *P* <0.0001) effects on total flowers. Two dominant species (*H. maximiliani* and *R. hirta*) constituted 99.6% of all flowers. There was significantly more *R. hirta* early in season for PW versus PS (t_{93} = -4.21, *P* = 0.0001) and marginally more *H. maximilliani* in PS versus PW (t_3 = 2.53, *P* = 0.09).

In 2013, there was a significant treatment $\times$ position $\times$ date interaction (t_{378} = 2.99, *P* = 0.0029) on flowers. Four dominant species (*H. maximiliani*, *A. novae angliae*, *E. canadensis*, and *M. fistulosa*) composed 86.4% of all flowers. There were significantly more *A. novae angliae* (t_3 = -4.91, *P* = 0.016), marginally more *M. fistulosa* (t_3 = -3.14, *P* = 0.052), and no difference in *H. maximiliani* (t_3 = -0.16, *P* = 0.88) or *E. canadensis* (t_3 = -0.35, *P* = 0.75) in the interior of PW versus PS plots. No significant differences were detected for *E. canadensis*.

In 2014, when we expanded our sample size of edge and interior plots, we detected more flowers in the PW versus PS interior (t_3 = 3.29, *P* = 0.046; Figure 3) and there were significant treatment $\times$ spatial position $\times$ date interactions (*P* = 0.0012) and date^2 (*P* = 0.0011) on total flowers. We analyzed flower abundance for the four most common species (*H. maximiliani*, *A. novae angliae*, *E. canadensis*, and *M. fistulosa*) constituting 82.8% of all flowers. Contributing most to the spatial position $\times$ treatment interaction on total flowers were *H. maximilliani* (t_{54} = 7.80, *P* < 0.0001) and *E. canadensis* (t_{54} = 2.33, *P* = 0.0233) which were most abundant on the edge of PS (Figure

3). Contrasting this pattern, there were more *A. novae angliae* ($t_3 = 5.16$, $P = 0.0141$) and *M. fistulosa* ($t_3 = 4.00$, $P = 0.0279$) flowers in PW versus PS flowers (Figure 3).

Cover- Leaf Area Index

There was greater cover (leaf area index) in the WS versus PS and the effect varied with date in 2013 (date × treatment: $F_{1, 234} = 32.92$, $P < 0.0001$) and 2014 ($F_{1, 236} = 42.58$, $P < 0.0001$; Figure 4). There was no detectable difference in cover between the PW versus PS in 2013 but significantly more cover in PW versus PS late in the 2014 season (treatment × date: $F_{1, 116} = 13.31$, $P = 0.0004$; Figure 4). *Aster novae angliae* flowering peaked late in the season (Figure 3) and there was significantly more *A. novae angliae* biomass in PW versus PS suggesting this species likely contributed to the greater cover in PW versus PS late in the 2014 season.

Biomass and theoretical ethanol yields

For SRC willow, there was a significant treatment × position interaction ($F_{1, 18} = 7.75$, $P = 0.0123$) where the edge of the WS yielded more than interior ($t_{18} = 2.84$, $P = 0.0109$) but yield was similar among spatial positions in the WPS ($t_{18} = 0.922$, $P = 0.37$). For senesced polyculture biomass we observed a significant effect of edge in 2012 and a significant spatial position × treatment interaction in 2013 ($F_{1, 18} = 16.8$, $P = 0.0007$) where biomass increased much more from the interior to the edge of the PS than the PW. In 2014, we observed a significant position by treatment effect with no effect of treatment within the interior.

We focused on theoretical ethanol yield because polyculture biomass yield accounted for 96% (R^2) of the variation in theoretical ethanol yield (L EtOH m^{-2}) and we assumed constant EtOH L per g biomass of SRC willow. Furthermore, there was little variation in theoretical ethanol concentration (L g^{-1}) of among herbaceous polyculture species. Over three years, SRC willow produced more theoretical ethanol than polyculture and the magnitude of the difference was greater in WS than WPS treatment (treatment × crop: $F_{1, 6} = 7.38$, $P = 0.0348$; Figure 5). The treatment effects also differed with spatial position (treatment × spatial position: $F_{1, 15} = 8.15$, $P = 0.012$). PS ($t_{14} = 2.59$, $P = 0.022$) and WS ($t_{14} = 3.38$, $P = 0.0045$) both showed more biomass on the edge versus interior while there were no differences in biomass among spatial positions in PW or WPS.

Natural Enemies of pest insects

There were more natural enemies in the PS than WS and the effect was significant in 2014 ($t_3 = -6.31$, $P = 0.0081$; Figure 6) but not 2012 ($t_3 = 2.65$, $P = 0.0771$) or 2013 ($t_3 = 2.32$, $P = 0.10$). In PS versus WS, we captured more Syrphidae in 2013 ($t_3 = 4.22$, $P = 0.0243$) and 2014 ($t_3 = -5.29$, $P = 0.013$; Figure 6) as well as more spiders in 2013 ($t_3 = 4.08$, $P = 0.0267$) and 2014 ($t_3 = 6.39$, $P = 0.0078$; Figure 6). Coccinellidae, however, were more abundant in WS versus PS late in the 2012 season ($P = 0.0013$).

There were more natural enemies in PW versus PS late in the 2013 season (treatment × $date^2$: $t_{77} = 2.13$, $P = 0.036$; Figure 7) but not in 2012 or 2014. In 2013 we captured more *Orius insidiosus* (treatment × date: $t_{37} = 2.62$, $P = 0.0127$) and spiders (treatment × date: $t_{77} = 2.49$, $P = 0.015$) in PW versus PS late in the season (Figure 7).

Discussion:

This study evaluated integrated perennial cropping systems for potential synergies and tradeoffs in floral resources, biofuels, and natural enemies. We observed tradeoffs between biofuel production and natural enemy abundance. SRC willow produced substantially more biofuels than polyculture. Consistent with other experiments (Jungers et al., 2013), variation in biomass drove patterns in biofuel production, not variation in cell wall carbohydrates. For example, there was low variation in theoretical ethanol yield per dry gram polyculture, depending on the year $6 - 11\%$ variation coefficient. The magnitude of the difference was greater in willow integrated with soybeans than willow with polyculture. Willow was less productive when sharing an edge with polyculture, an effect that minimized the difference in yield between crops at this position. SRC willow growing in monoculture or adjacent to soybeans was much more productive than polyculture. Our data suggest that the large variation and differences in yield observed among woody and herbaceous from cropping structure is substantial and comparable to variation observed across landscapes position (Thelemann et al., 2010). A strategy focused primarily on biofuels with the secondary goal of supporting beneficial insects could integrate SRC willow into soybeans.

Nitrogen fertilization of willow but not polyculture may have contributed to the difference in production between crops. Application of 100 lb N/ acre for nitrogen fertility management of willow is a common practice (Abrahamson et al., 2010), and we applied less than the typical rate (~74 lb N/acre). However, fertilization of willow typically shows no effect especially during the first rotation (Fillion et al., 2009; Quaye et

al., 2011; Quaye and Volk, 2013). Studies reporting fertilizer responses have involved multiple rotations and applied different forms of N (e.g. slow release) and/or multiple macro- and micro- nutrients in a sludge/slurry leading to variable gains in willow yield, 7 – 45% more biomass (Adegbidi et al., 2003; Cavanagh et al., 2011; Nissim et al., 2013). The lack of response to fertilization largely owes to the efficient transfer of nutrients from decomposing leaf and fine roots to mitigate potential nutrient deficiencies in the first rotation (Hangs et al., 2014). In comparison, though short-term fertilization of established grasslands enhances productivity (Jungers et al., 2015), fertilization of polycultures of the kind used in our study can degrade productivity of native plants, especially during polyculture establishment, by encouraging non-native weed populations (Fornara et al., 2012). It is unlikely but possible that fertilization contributed significantly to the differences we observed between cropping systems.

The benefit of polyculture over the willow was in supporting greater abundance of natural enemies of pest insects, mostly in the third year of the study. This was primarily driven by greater abundances of spiders (Order Araneae) and hoverflies (family Syrphidae) in polyculture compared to SRC willow. Hoverfly adults show strong affinities for floral resources as they feed exclusively on pollen and nectar (Almohamad et al., 2009). Our results add to mounting evidence showing that enhancement of these resources via floral strips increases hoverfly abundance (Cowgill et al., 1993; Bowie et al., 1999; Frank, 1999; MacLeod, 1999; van Rijn et al., 2006; Pontin et al., 2006; Haenke et al., 2009). Multiple factors in polyculture may contribute greater spider abundance. The presence of grasses and forbs can support greater spider populations than open environments by supporting greater prey populations and enhancing habitat complexity to

minimize intraguild predation (Riechert and Bishop, 1990; Sunderland and Samu, 2000; Langellotto and Denno, 2006). The thicker thatch and leaf layer of polyculture compared to the decaying leaf layer in willow likely contributed to these differences. Spiders are also known to consume pollen and nectar as an alternative food resource which was enhanced in the polyculture (Jackson et al., 2001; Peterson et al., 2010). In contrast to spiders and hoverflies, lady beetles were more abundant in SRC willow. Fertilization of willow is known to enhance Aphididae and may have contributed to greater willow aphid and lady beetle populations (Wiesenborn, 2011). These results suggest that a cropping strategy focused on supporting natural enemies would emphasize polyculture.

Greater abundance of natural enemies in polyculture may enhance biological control of the soybean aphid. There is significant information suggesting that enhanced hoverfly population in floral strips also increase in adjoining crops (White et al., 1995; Hickman and Wratten, 1996; van Rijn et al., 2006; Walton and Isaacs, 2011). However, hoverflies are generally sparse in soybeans potentially due to intra-guild predation and soybean leaf pubescence (Eckberg et al., 2015). Furthermore the presence of greater populations of natural enemies in polyculture may not lead to spillover and enhancement of natural enemies in soybeans if, as other studies suggest, favorable habitat function as a sink for natural enemies, diminishing potential biological control (Corbett 1998). The current study thus provides an assessment of how perennial biomass cropping systems mediates natural enemy abundance in an agriculture landscape and future work should evaluate how these effects interact with predator – aphid dynamics and biological control in surrounding conventional agricultural systems.

Our data suggest there are tradeoffs between producing biofuels and supporting natural enemy populations yet there is potential to design systems that temper such tradeoffs. An approach seeking to optimize perennial biofuels and natural enemy populations could integrate alley strips into soybeans. Such systems would not only benefit from including perennial crops that excel at different functions, our data suggests there is some compatibility among systems. Despite the prevalence of competitive interactions resulting from light limitation of herbaceous crops in alley configurations with woody crops (Jose et al., 2004), integrating polyculture with willow led to no depression in yield of polyculture. Instead, some herbaceous species were promoted in alley configurations including species that flowered early (i.e. wild bergamot) and late in the season (i.e. new england aster). Species vary in their shade tolerance (Lin et al., 1999), therefore, establishing diverse polyculture can lead to herbaceous plant communities with similar productivity in partial shade or full sun as we observed. Furthermore, polyculture integrated with willow showed significantly greater cover late in the season owing primarily to a dominance of new England aster. Enhanced structural diversity from integrating polyculture with willow may support more natural enemies in polyculture, though the current study showed limited support for this interaction, alley configuration enhanced spider abundance in polyculture late in the 2013 season. Habitat structure can affect late season dispersal (ballooning) in spiders (Blandenier et al., 2013); willow may have limited dispersal of spiders in the current study enhancing their abundance in polyculture.

Integrated cropping structure showed drawbacks, primarily for willow which showed significant variation in yield depending on the cropping structure. Willow

adjacent to soybeans showed a ~40% increase in yield whereas this same yield increase was not observed along the shared edge with polyculture. There is apparently strong belowground competition among perennial crops that limits willow yield. While the indirect effects of willow on polyculture appear positive or at least neutral, the direct competitive of these crops appear negative especially for willow. The presence of strong positive perennial-soybean edge effects on willow and polyculture yield suggest that integrating perennial crops into soybeans in a way that maximizes the amount of perennial crop-soybean edge enhances perennial biofuel yields and could support greater spillover of natural enemies.

In conclusion, there were strong tradeoffs between the production of biofuels and abundance of natural enemies across multiple perennial cropping systems. Cropping design only partly ameliorated such tradeoffs suggesting that the value placed on biofuels versus natural enemies will drive the selection and integration of perennial crops in conventional agriculture. Future work is needed to characterize the extent to which natural enemies from perennial crops spillover to conventional agricultural crops and provide biological control of pests.

Figure Legends

Figure 1- Species Composition in polyculture surrounded by willow (closed circles) versus polyculture surrounded by soybeans (open triangles). All slopes are back-transformed from log10. Significantly more *Monarda fistulosa* ($F_{1,11}$ = 14.4, P = 0.0030) and *Aster novae angliae* ($F_{1,11}$ = 21.8, P=0.0007) in alley versus non-alley and significantly more *Sorghastrum nutans* in non-alley versus alley ($F_{1,11}$ = 10.5, P = 0.0079). There are no other significant differences.

Figure 2: 2014 Treatment (alley = polyculture surrounded by willow, polyculture = polyculture surrounded by soybeans) and spatial position (1= interior, 2= edge) effects on **total polyculture biomass** MS = Maximilian Sunflower (H. maximiliani), CWR = Canada Wild Rye (Elymus Canadensis) (g m-2).

Figure 3- 2014 Floral composition. Species level response to treatment and position. Total flowers is reported for the interior of PW (polyculture surrounded by willow) and PS (polyculture surrounded by soybeans). Interior = closed symbol, Edge = open symbol. PW (polyculture willow) = circle, PS (polyculture soybean) = triangle.

Figure 4- 2014 Leaf Area Index. Left Panel: Willow Monoculture (triangles) versus Polyculture (circles). Right Panel: Polyculture surrounded by willow, PW (triangles) versus Polyculture surrounded by soybeans, PS (circles). Presented are means and statistically significant model terms (back-transformed from square-root).

Figure 5- Biofuel production from 3 years of polyculture and willow production. Shown is the yield of polyculture integrated with SRC willow in an alley configuration (polyculture-willow, PW) or in soybeans (polyculture-soybeans, PS) and SRC willow

integrated with polyculture in an alley configuration (willow-polyculture, WP) or in soybeans (willow-soybeans, WS) for the interior versus edge of plots. The primary findings indicate that SRC willow produced more ethanol than polyculture when these crops are integrated with soybeans versus each other. And the edge effects were much stronger for polyculture and willow integrated with soybeans versus each other.

Figure 6- 2014 polyculture (PS, circles) versus SRC willow (WS, triangles). Syrphidae, spiders and total natural enemies presented.

Figure 7- 2013 Polyculture Willow (PW, filled triangles) versus polyculture soybeans (PS, open circles)- Significantly more predators in the alley prairie versus prairie monoculture late in the season driven by spiders and Orius.
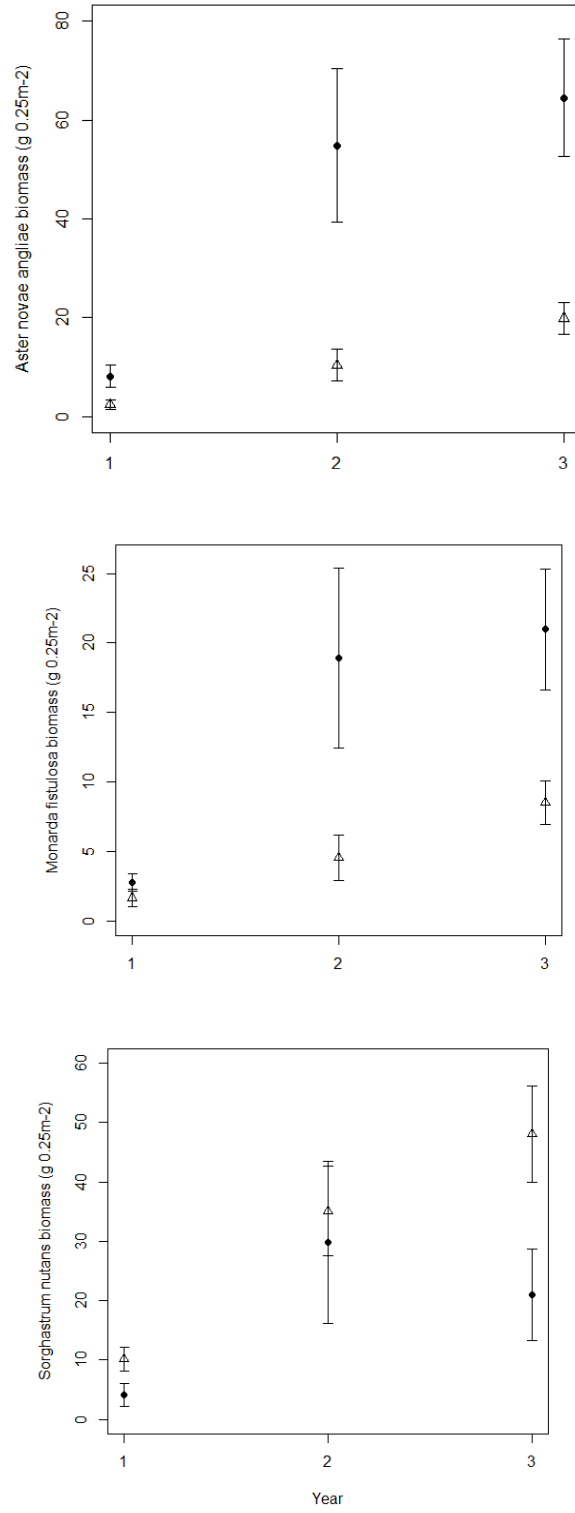
Figure 1

Figure 2
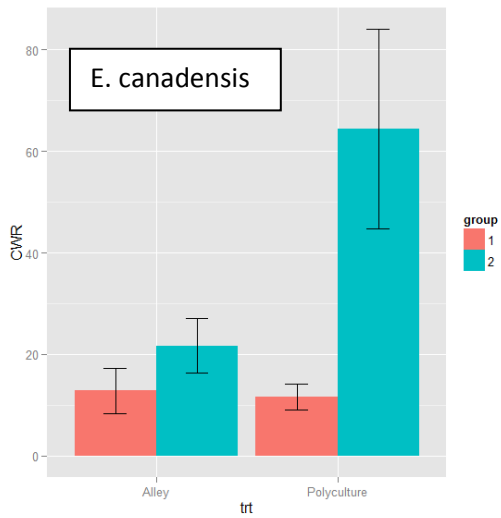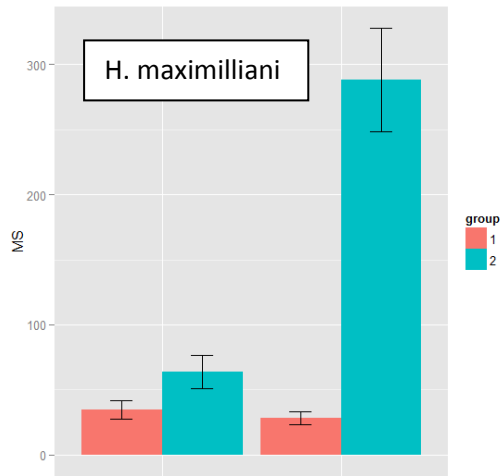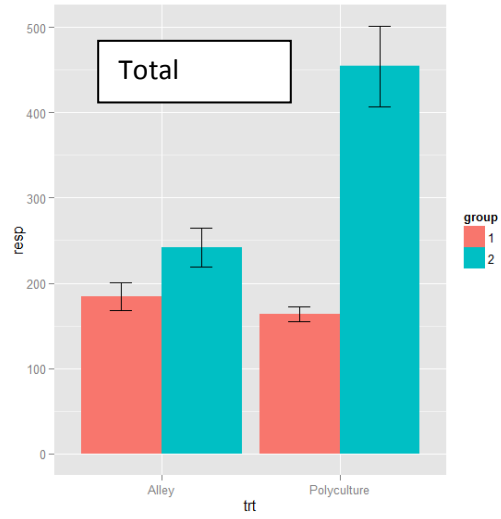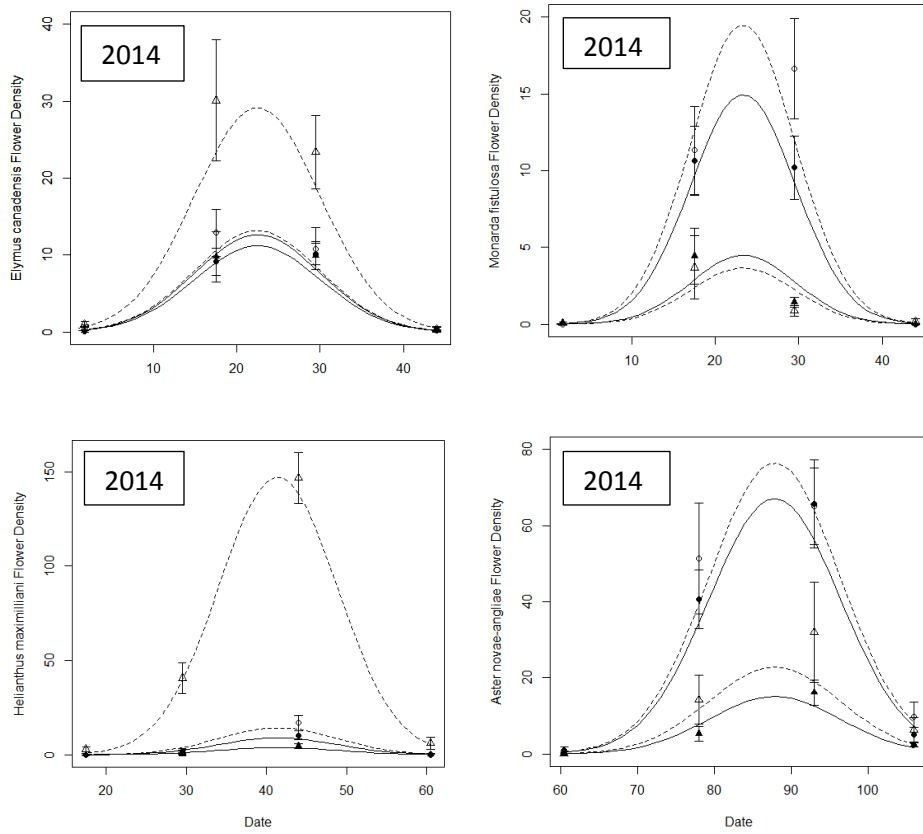
Figure 3
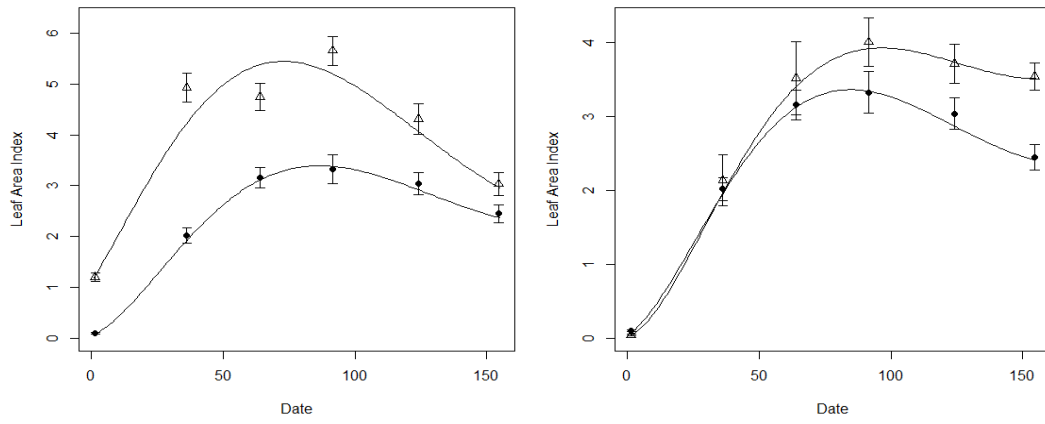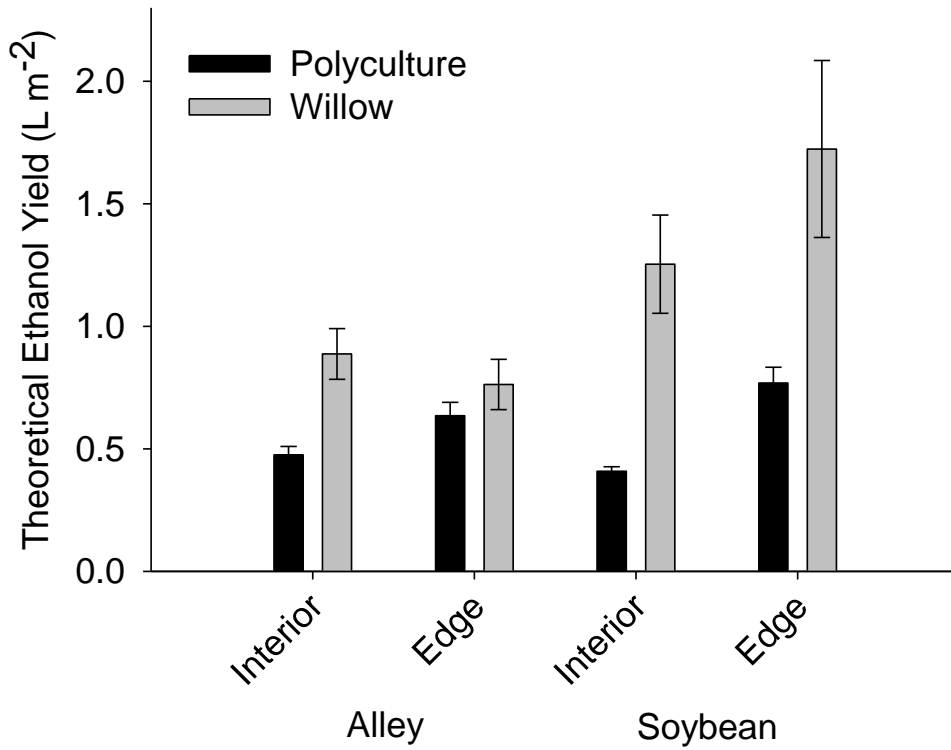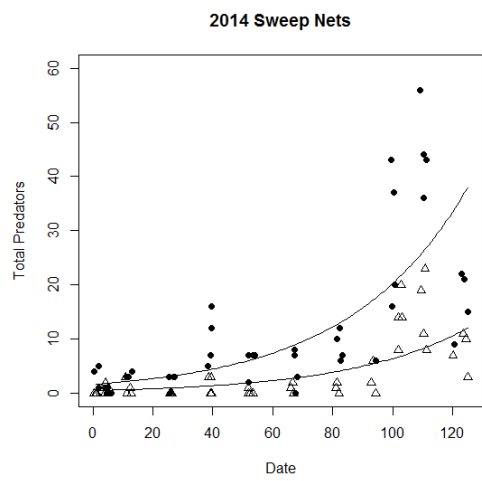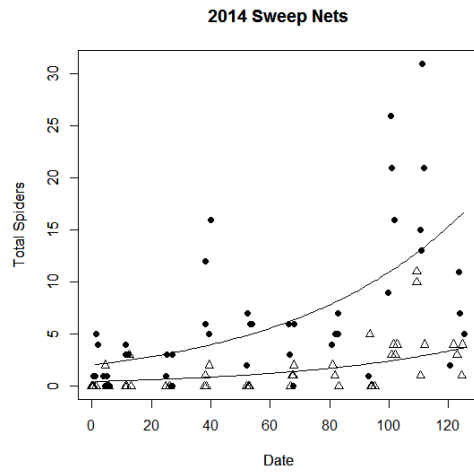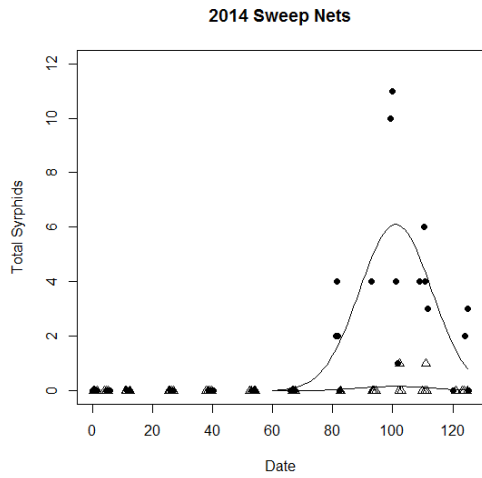
Figure 4

Figure 5

Figure 6

2013 Sweep Nets- Prairie Alley versus Prairie Monoculture
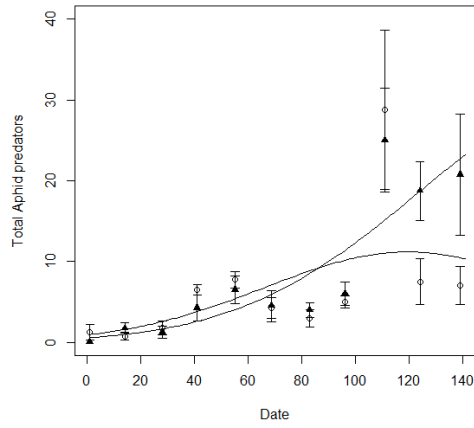
2013 Sweep Nets- alley prairie versus prairie monoculture
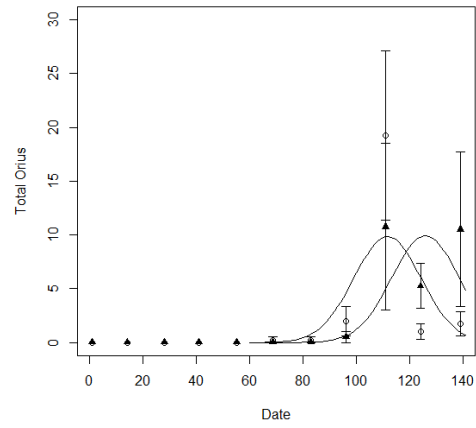
2013 Sweep Nets- Prairie Alley versus Prairie Monoculture
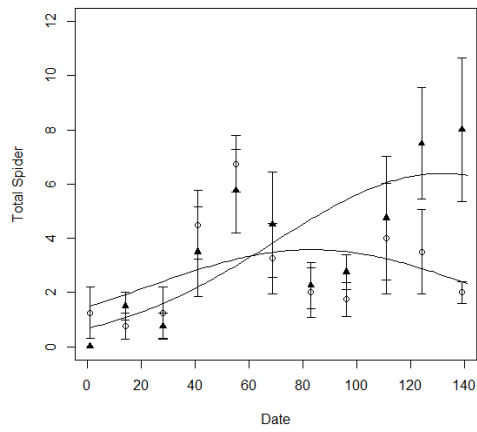
Figure 7

CHAPTER 4

Competitive interactions of cultivar and wild switchgrass with native grasses

Introduction:

There is growing interest in the United States to develop switchgrass (*Panicum virgatum* L.) as a biomass feedstock for bioenergy and biomaterial applications. Switchgrass is being selected and bred for rapid establishment and greater biomass yield to increase efficiency and economic viability of bioenergy production systems (Boe and Ross 1998, Casler 2012, Perrin et al. 2008). Breeding and cultivation of native switchgrass for bioenergy could enhance its weediness yet little attention has been paid to switchgrass in the context of invasiveness (Barney and DiTomaso 2008, Simberloff 2008). Using plant species as biomass crops in their native distribution also increases risk of introgression of genes into wild populations (Kwit and Stewart 2012). The long-term sustainability of switchgrass biomass production systems would benefit from a better understanding of the interactions between switchgrass phenotype and potential weediness.

Switchgrass breeding has focused on improving several traits for bioenergy production including increased seed size for greater emergence and seedling vigor (Boe and Ross 1998), reduced dormancy (Burson et al. 2009), extended growing season (Casler 2012), increased resistance to insect herbivory, and direct selection for high yield of vegetative biomass (Casler 2010, 2012, Missaoui et al. 2005, Rose et al. 2007). Selection of upland cultivars for seed size, emergence and early seedling vigor increased these traits by 39 – 140% compared to other cultivars (Boe and Ross 1998). In a

phylogenetically controlled study encompassing many plant species, increased seedling vigor was associated with invasiveness (Grotkopp and Rejmanek 2007) suggesting that breeding switchgrass for large seeds and enhanced seedling vigor (Boe and Ross 1998) may also increase their weediness.

Selection for greater biomass can also elevate the invasion potential of switchgrass. In a meta-analysis of 117 studies, greater growth rate and biomass were associated with 125 invasive plant species compared to non-invasive congeners (van Kleunen et al. 2010). To date breeding and selection have increased biomass yield of switchgrass by 20-30% (Casler 2012). Hybridization of lowland-upland cultivars has led to further increases in yield of 25-40% compared to the best adapted upland cultivar depending on the location (Vogel et al. 2014) with some hybrids being up to 70% more productive than their parent cultivars (Casler and Vogel 2014). Hybridization and increased genetic variation have been implicated in some plant invasions (Ellstrand and Schierenbeck 2000), most notably the highly invasive reed canary grass (Lavergne and Molofsky 2007). Despite potential for weediness of high-yielding switchgrass hybrids, such cultivars may have limited potential for seed production given their extended growing seasons and delayed flowering (Casler and Vogel 2014). Reducing seed set in biomass production fields could enhance biosafety of switchgrass by limiting outcrossing with wild relatives and population spread via seeds (Kwit and Stewart 2012).

The potential for even minor gains in seed size, seedling vigor, or biomass yield to contribute to invasiveness in switchgrass is largely unknown. Ecological studies involving advanced switchgrass cultivars and wild relatives are critical to identifying less invasive phenotypes and providing guidance for selection and breeding to reduce

75

invasive potential in switchgrass (Anderson et al. 2006). Moreover, the emergence of revolutionary high-throughput genomic technologies has fueled optimism that rapid feedback between breeding and ecological studies can result in development of productive biomass crops with less risk of invasion (Runck et al. 2014).

A first step to understanding invasion potential in switchgrass is to evaluate how variation in seed size, seedling vigor, and biomass among advanced cultivars and wild populations mediate competition of switchgrass with native plants and affect native plant performance. Further, the growth and impacts of invasive plants often vary with plant invader density suggesting competition studies using an experimental range of plant density can provide more insight on the context of invader impacts (Cappuccino 2004). We performed a factorial experiment in the greenhouse testing the effect of switchgrass seed source ('Trailblazer' and 'EG-2101' selectively bred for biomass and five wild populations with no history of selection) and switchgrass density (0, 3, 9, or 18 plants per pot) on the performance of two widely distributed grasses (*Elymus canadensis* L. and *Bouteloua curtipendula* (Michx.) Torr.). This experiment utilized a diverse group of selected and wild switchgrass populations, a wide range of switchgrass density, and functionally distinct focal species (e.g. C3 and C4 pathways) to provide a broad evaluation of the potential for switchgrass selection and breeding to impact native grass performance.

Materials and Methods

Seeds. We collected wild populations from five tallgrass prairies in southern and east-central Minnesota from 12–25 September 2011. We selected these wild populations because they represented five separate and uniquely managed tallgrass prairies and thereby provide some independence among replicate populations as well as increased representation of native switchgrass. To ensure that the seed represents locally native populations, collections were made from remnant or locally re-seeded (seed collected within 40 km) prairie plant communities in the Scientific and Natural Areas (SNAs) program (Minnesota Department of Natural Resources and the Minnesota Chapter of the Nature Conservancy). Collections were made away from 1) prairie reconstructions likely to have used non-local seed, and 2) borders of the SNA (all collections were at least 40 m away from the SNA border). Within each SNA, seed was collected from four to eight collection areas (N = 29 collections) with collection radii no greater than 30 m. We recorded the geographic position (UTM) of the center of each collection area to verify locations within SNA and distance to SNA property edge using a Geographic Information System. This protocol helped ensure each collection was likely representative of locally native switchgrass.

We evaluated two cultivars that have resulted from selection for increased biomass production, Trailblazer and EG-2101. Trailblazer originated from several seed accessions that were collected in Nebraska and Kansas, intercrossed to form a regional gene pool, and selected for improved agronomic performance (including biomass yield) and increased forage digestibility (Casler 2012, Vogel et al. 1991). Trailblazer typically produces 7.7 Mg ha^{-1}, approximately 8% more biomass than other upland switchgrass

cultivars (Berdahl et al. 2005, Casler and Boe 2003, Lemus et al. 2002). EG-2101

represents an advanced selection of 'Cave-in-rock' (an upland cultivar) for enhanced

biomass production and has shown yields in excess of 13 Mg ha^{-1} (Blade Energy Crops

2009, Christensen 2010). Seeds for Canada wild rye (*Elymus canadensis*) and sideoats

grama (*Bouteloua curtipendula*) originated from Benton and Pope Counties, respectively,

in central Minnesota and were produced in Benton County.

Experimental Design. We evaluated switchgrass competition on focal plants with a

factorial combination of treatments: three switchgrass population treatments (EG-2101,

Trailblazer and wild), four switchgrass plant densities (0, 3, 9, and 18 plants pot^{-1}) and

two tallgrass prairie focal species (Canada wild rye and sideoats grama). We established

the switchgrass density gradient for each combination of the five wild populations and

focal plants (Wild populations pots: 4 densities × 5 replicate wild populations × 2 focal

species = 40 pots). Two replicates of the switchgrass density gradient × focal plant

combination were established for each cultivar (Cultivar pots: 4 densities × 2 cultivars ×

2 focal species × 2 replicates = 32 pots; N = 72 pots). Treatments and pots were randomly

arranged in a staggered 12 × 12 pot grid at an initial spacing of 13 cm and 24 days later

increased to 20 cm to minimize shading effects among pots. Pots were re-randomized

weekly.

The experimental unit, a pot, consisted of one of the two focal species planted in

the center of a 9.3 L cylinder pot (24 cm diameter by 20 cm height) containing sphagnum

peat moss medium (Sunshine$^{®}$ Mix #8/ LC8, Sun gro, Agawam, MA, USA) fertilized at a

concentration of 7.7 g 14-14-14 osomocote (Everris, Dublin, OH, USA) per L medium.

One of three switchgrass densities was planted within 8.9 cm (3 switchgrass pot^{-1}), 5.1 cm (9 switchgrass pot^{-1}) or 3.0 cm (18 switchgrass pot^{-1}) of the focal plant. For each density, switchgrass was arranged equidistantly around the central focal plant and each other at 180º angles (Figure 1). A 2.5 cm buffer between the outermost switchgrass and the pot edge was maintained for each density (Figure 1).

Planting. Wild switchgrass populations, Canada wild rye and sideoats grama seeds were cold-moist stratified to improve germination but cultivars were not stratified given their high germination (Eckberg et al. 2015). Seeds were planted into trays from 31 January – 7 February and transplanted to experimental pots on 26 – 27 February. For each species, we selected and transplanted similarly sized seedlings that were approximately 6 – 9 cm height to obviate initial size differences related to time of emergence and better test differences in post-emergence growth rate.

Experimental Conditions and Maintenance. Pot medium was monitored daily and watered to maintain conditions that minimize potential for water stress or waterlogging. Day length increased from 12 – 14 hours over the duration of the experiment (28 February – 24 April, 2014). From 28 February – 25 April, we monitored air temperature with four hobo data loggers positioned north-south and east-west amongst the pot grid. Air temperature was generally consistent, average temperature varied by 27.2 – 28.6º C among the data loggers. Average minimum temperatures were 20.9 –21.9 ºC and maximum temperatures were 36.6 – 38.5 ºC. Thrips (order Thysanoptera) infested plants and caused variable damage among switchgrass and focal plants which we scored

visually. Focal plant damage was generally low (1 – 10% leaf area damaged) with 89% of Canada wild rye and 50% of sideoats grama affected. Switchgrass damage was slightly greater (>10% leaf area damaged) on 72% of EG-2101 plants, 17% of Trailblazer plants, and 22% of wild plants at the observed thrip peak (26 March). Elevated thrips damage may make this a conservative test of the hypothesis that EG-2101 is more productive than other populations. To control thrips bifenthrin (Talstar, Philadelphia, PA, USA) was applied at a 0.024% concentration on 14 March and 7 April and by the final harvest thrip damage was >10% on less than 4% of switchgrass plants.

Measurements. We measured size of the focal plant and the three surrounding switchgrass plants (Figure 1) every two weeks starting 28 February until 25 April (five measurements). Size measurements included number of leaves (>50% unfurled), number of stems, length of the longest leaf (ligule to leaf tip) and number of panicles. Senesced leaves were counted and found to constitute a small percentage of the total leaves (6 – 8%). We analyzed switchgrass total leaf number versus biomass and focal plant green leaves versus biomass because there was more support for these variables (AIC_c). Stem counts included only those stems with a length greater than 1 cm. Plant growth was observed as change in plant measurements over time. Panicles were first observed on 21 March and counted on subsequent two week intervals. Above-ground dry biomass was measured for all individual focal plants and switchgrass at the end of the experiment (23 – 25 April). To determine dry plant mass, biomass was dried for 72 hours at 60º C.

Leaf area index and intercepted PAR (photosynthetically active radiation) for above- ground biomass were measured in each pot at the end of the experiment (23 –

April) using the AccuPAR LP-80 ceptometer (Decagon Devices Inc., Pullman, WA, USA). We collected two perpendicular measurements centered on each pot. To control ambient light conditions, measurements were taken directly under the same greenhouse light using an external sensor mounted 95 cm above the pot.

Statistical analysis. All data were analyzed using the software environment R (R Development Core Team, version 3.1.3, Vienna, Austria). Effects of switchgrass population, density, focal plant identity and their two and three-way interactions on focal plant biomass and panicle number were analyzed using analysis of covariance (ANCOVA). We focused the analysis on the treatments containing both switchgrass and the focal plant (switchgrass densities 3 – 18 plants pot^{-1}); including the zero switchgrass density in the analysis had little effect on the results. Effects of switchgrass population, density, and their interaction on switchgrass above-ground biomass, leaf area index, panicle number, and light interception were tested using ANCOVA. Switchgrass biomass and panicle number was observed on the three individual switchgrass plants surrounding each focal plant so pots were treated as a random effect. We used multiple regression to test the effects of switchgrass biomass, leaf area index, and light interception on focal plant biomass. To evaluate switchgrass and focal plant growth we first performed AIC (Akaike's Information Criterion)- based model selection to determine the best candidate size measurement (leaf number, stem number or longest leaf) accounting for variation in final harvested biomass. We compared models using AIC_c (AIC corrected for sample size) Akaike weights (w_i), which represent the proportion of evidence supporting each candidate model (Bolker 2008, Hurvich and Tsai 1991). Then we used those

measurements to evaluate growth in relation to treatments using a linear mixed effects model that accounted for non-independence among multiple observations from the same pot and plant over time. We fit linear and quadratic terms for day as well as their interactions with treatment factors, and we assumed an autoregressive moving average autocorrelation structure among observation over time. Residual errors of all models were evaluated, and response variables were log transformed as needed to improve homogeneity of variance. Switchgrass panicle number was highly heteroscedastic, even with log+1 transformation, so we used a generalized linear mixed model (glmer) with an underlying poisson error distribution, and to reduce overdispersion we included a random effect for each observation (Harrison 2014). Significance tests of treatments were based on the F-statistic (ANCOVA or ANCOVA mixed effects model) or χ^2 statistic (generalized linear mixed model) of the full model. Differences among specific treatments were evaluated using unadjusted t- or z-statistics of models including only significant terms.

Results

Initial Size. Despite our effort to select and establish plants of similar initial size, there were several statistically significant differences in plant size measurements among treatments. Initial leaf number differed among switchgrass population treatments ($F_{2, 42} = 13.84$, $P < 0.0001$) by as much as 13%. Initial focal plant leaf number varied up to 28%

with switchgrass density and focal plant identity ($F_{1, 42} = 4.60$, $P = 0.038$). Focal plant longest leaf differed up to 18% among switchgrass population and density ($F_{2, 42} = 4.66$, $P = 0.015$). No covariates significantly influenced final plant biomass at the end of the experiment or improved the model fit (reduced AIC_c), so they were not included in the analysis of biomass.

Growth Rate. For switchgrass we focused on growth of longest leaf because it best represented variation in final switchgrass biomass (AIC_c $w_i = 1$). There were significant differences in growth of longest leaf over time among switchgrass populations ($F_{2, 468} = 22.3$, $P < 0.0001$; Figure 2). Wild switchgrass populations grew slower than EG-2101 ($t = 8.2$, $P < 0.0001$; Figure 2) and Trailblazer ($t = 9.7$, $P < 0.0001$; Figure 2). Switchgrass growth rate significantly decreased by the end of the experiment (day^2: $F_{1, 468} = 307.4$, $P<0.0001$) suggesting final measurements approximately reflected full plant size for the experimental conditions.

We analyzed growth rate based on focal plant stem number because it was the most strongly associated with variation in biomass of sideoats grama (AIC_c $w_i = 0.60$) and Canada wild rye (AIC_c $w_i = 0.99$). Growth rate of sideoats grama stem number differed significantly among population treatments ($F_{2, 63} = 4.9$, $P = 0.010$; Figure 3). Sideoats grama surrounded by wild switchgrass showed significantly greater growth than those surrounded by EG-2101 ($t = 2.6$, $P = 0.012$; Figure 3) or Trailblazer ($t = 2.5$, $P = 0.015$; Figure 3). Sideoats grama growth rate significantly decreased toward the end of the experiment (day^2: $F_{1, 63} = 47.0$, $P<0.0001$) suggesting final measurements were approximately representative of full plant size for the experimental conditions. Canada

wild rye growth rate also differed among switchgrass population ($F_{2, 63}$ = 8.4, P = 0.0006). Canada wild rye surrounded by Trailblazer compared to wild switchgrass populations showed a statistically lower growth rate (day: t = 3.6, P = 0.0006) yet this led to only minor reductions in final stem number (up to 7% less) given other interacting model terms (i.e. intercept, day^2).

Panicle Production. There was significant variation in total number of panicles among switchgrass population (χ^2 = 12.3, P = 0.0021); wild populations produced more panicles than EG-2101 (z = 2.3, P = 0.020) and Trailblazer (z = 3.4, P = 0.00077). Panicle production differed between sideoats grama and Canada wild rye ($F_{1, 42}$ = 7.5, P = 0.009) but there were no detectable effects of switchgrass population or density on their panicle number.

Focal Plant Biomass. Focal grass biomass was reduced by increasing switchgrass plant density ($F_{1, 42}$ = 47.0, P < 0.0001). The effects of switchgrass on focal plants was also mediated by an interaction between switchgrass population and focal species (population × focal species $F_{2, 42}$ = 3.4, P = 0.044). Specifically, EG-2101 (t = 4.1, P = 0.00017) and Trailblazer (t = 3.7, P = 0.00050) reduced sideoats grama biomass more so than wild switchgrass populations (Figure 4a). In contrasts, there were no significant differences among switchgrass populations in their effects on Canada wild rye biomass.

Factors Associated with Focal Plant Biomass. Switchgrass populations differed significantly in individual biomass ($F_{2, 48}$ = 21.2, P <0.0001). Individual EG-2101 plants

produced approximately 132% more biomass than wild populations ($t = 5.5$, $P < 0.0001$) and Trailblazer was 119% more productive than individuals from wild populations ($t = 5.1$, $P < 0.0001$). There were also significant differences among switchgrass populations in their total biomass per pot ($F_{2, 45} = 78.3$, $P < 0.0001$; Figure 4b). Greater switchgrass density resulted in more switchgrass biomass per pot ($F_{1, 45} = 109.5$, $P < 0.0001$, Figure 4b). Depending on the density of switchgrass, EG-2101 was $76 - 120\%$ more productive than wild populations ($t = 11.4$, $P < 0.0001$) on a per pot basis and Trailblazer was $49 - 113\%$ more productive ($t = 8.4$, $P < 0.0001$; Figure 4b). The more advanced cultivar, EG-2101, was $3 - 19\%$ more productive than Trailblazer ($t = 2.5$, $P = 0.014$; Figure 4b). Differences in switchgrass biomass among treatments contributed to effects on focal species biomass: switchgrass biomass and identity of the focal plant explained 62% of variation in focal plant biomass ($R^2 = 0.62$).

Leaf area index of pots containing cultivars was moderately greater than those with wild populations ($F_{2, 45} = 3.6$, $P = 0.036$). Depending on switchgrass density, Trailblazer leaf area index was $10 - 28\%$ more than wild populations ($t = 2.5$, $P = 0.016$) and EG-2101 was $5 - 20\%$ more than wild populations, a marginally significant difference ($t = 1.9$, $P = 0.065$). Leaf area index and identity of the focal plant explained 63% of the variation in focal plant biomass ($R^2 = 0.63$). Associated with LAI, there was also more light interception by cultivars versus wild populations ($F_{2, 45} = 4.3$, $P = 0.019$), but the difference was slight ($2 - 14\%$).

Switchgrass biomass, leaf area index and light interception all appeared to influence focal plant biomass, and so we evaluated the effects of these factors using multiple regression and model selection (Akaike's Information Criteria). Stepwise

multiple regression revealed that focal plant biomass was significantly influenced by entire pot biomass of switchgrass ($t = 2.6$, $P = 0.014$) and leaf area index ($t = 2.9$, $P = 0.0062$) but not light interception ($t = 0.7$, $P = 0.51$). There was more support for the model containing leaf area index and biomass of switchgrass (AIC_c $w_i = 0.64$) than the full model (AIC_c $w_i = 0.23$), or models with only biomass of switchgrass (AIC_c $w_i = 0.04$), leaf area index (AIC_c $w_i = 0.08$), or light interception (AIC_c $w_i = 0.02$). Thus, both leaf area and biomass of switchgrass contributed to variation in focal plant biomass.

Switchgrass seed size influences germination and early seedling vigor (Eckberg et al. 2015, Smart and Moser 1999) which may contribute to variation in switchgrass biomass and impacts on focal plants in the current study. To test this hypothesis we evaluated the effect of mean seed size per switchgrass populations (two cultivars and five wild populations, N = 7 populations), measured for each seed lot in a previous study (Eckberg et al. 2015), on switchgrass biomass observed in the current study. Larger seeded switchgrass populations produced more biomass ($F_{1, 5} = 46.3$, $P = 0.0010$; Figure 5) and the same effect was significant when only wild populations were compared ($F_{1, 3} = 51.2$, $P = 0.0056$). These data suggest that population variation in seed size contributes significantly to switchgrass biomass, at least in the short-term context of this experiment.

Switchgrass population seed size, along with focal plant species, weakly mediated the effects on focal plant biomass (Seed size × focal plant: $F_{1, 37} = 5.3$, $P = 0.028$). In particular there was a marginally significant negative effect of seed size on sideoats grama ($t = 2.5$, $P = 0.057$) and no detectable effect on Canada wild rye ($t = 0.25$, $P = 0.81$). Thus, seed size may contribute to variation in the impacts of switchgrass on focal plant biomass.

Discussion

There is concern over the invasion potential of grasses developed for bioenergy production (Raghu et al. 2006). We evaluated how competitive interactions between switchgrass and two native tallgrass prairie grasses varied across a density gradient of selectively bred cultivars versus wild populations. Depending on the specific cultivar and its density, selectively bred switchgrass yielded 47 – 162% more biomass and produced 9 – 21% more leaf area than wild populations. Multiple regression revealed that switchgrass biomass and leaf area both affected native grass performance, primarily sideoats grama. Sideoats grama was 25 – 59% less productive when surrounded by selectively bred cultivars compared to wild populations. Competitive effects on Canada wild rye were less clear, potentially owing to very warm conditions which can limit C3 growth and may have minimized differences in competitive performance against C4 switchgrass populations (Kemp and Williams 1980). These data suggest selection and breeding to improve switchgrass biomass production can significantly reduce performance of some native prairie grass species, with competition potentially being more intense between functionally similar grasses.

Cultivars selectively bred for biomass production were more productive than wild populations. The difference in biomass (47 – 162% more biomass) was much greater than that observed for cultivars and wild populations in field studies, 14% more biomass (Casler 2005). Potential differences in plant density in the field and greenhouse could contribute to differences in yield among cultivars and wild populations, cultivars were more productive at the lowest density in the greenhouse. Field to greenhouse differences in yield also reflect the inclusion of several cultivars not bred for biomass in the field

comparison (Casler 2005) further bolstering the significance of breeding for improving production of switchgrass biomass (Casler 2012).

Seed size of switchgrass populations, as measured in a previous study (Eckberg et al. 2015), was associated with productivity and potentially competitive interactions. Switchgrass populations with larger seed size were significantly more productive with marginally greater impacts on sideoats grama performance. In contrast, Smart and Moser (1999) showed small, short-term effects of seed size on switchgrass seedling vigor that diminished with time. That study, however, examined a smaller range in seed size (1.4 versus 2.0 mg seed^{-1}) than ours encompassed (0.9 – 1.9 mg seed^{-1}). The wider population level variation allowed for a broader test of the role of seed size yet potentially confounds population-level genetic and environmental variance with the direct effects of seed size on productivity (Platenkamp and Shaw 1993, Vange et al. 2004). Cultivars with larger seed also have a history of selection for biomass and originate from lower latitudes with longer growing seasons than wild populations (Blade Energy Crops 2009, Vogel et al. 1991). To minimize the potential for systematic genetic-environmental covariance with seed size we included a large sample of switchgrass populations and accounted for non-independence of observations within population in our analysis. Furthermore, we confirmed the significant relationship between population seed size and productivity when analyzing only wild populations with similar latitudinal origins and no history of selection. Estimates of seed size effects on switchgrass productivity may be conservative because we controlled switchgrass density and minimized variation in initial seedling size yet seed size has been shown to contribute to early emergence and growth (Smart and

Moser 1999). Thus, our study suggests that differences in seed size among populations contributed to post-emergence growth and biomass of switchgrass.

Switchgrass productivity increases at lower latitudes in part because plants flower later under an extended growing season (Casler 2012, Casler et al. 2007). Consistent with previous studies we observed less flowering (fewer number of panicles) and more biomass after 8 weeks of growth in the greenhouse for two cultivars originating from latitudes south of the wild populations (Nebraska, Kansas and Illinois versus Minnesota). Less flowering by cultivars may reflect a delay in flowering relative to the wild populations. This implies that later flowering is positively associated with production of vegetative biomass (Casler 2012), yet the current study does not allow us to separate the effects of reduced flowering versus direct selection on biomass in cultivars. Instead, these data suggest flowering related to latitudinal origin, seed size, and selection history jointly influence biomass production.

Our study suggests that greater biomass production from delayed flowering (Casler 2012), large seed size, and/or selection history can also increase competition with some native grasses. These data imply important tradeoffs in the factors potentially contributing to invasiveness, a result that warrants a nuanced approach to evaluating the role of seed and vegetative productivity in potential invasiveness. Current breeding approaches to increase biomass yield are focused on delayed flowering which could reduce seed production from biomass fields in latitudes >40ºN (Casler 2012). For example, the new cultivar Liberty, a hybrid derivative of upland (early) × lowland (late) crosses, is 10-14 days later in flowering than locally adapted populations (Vogel et al. 2014). Ultimately, breeding goals are to develop cultivars that would be 35-40 days later

in flowering than locally adapted germplasm by combining the late-flowering trait of southern populations with cold tolerance of northern populations (Casler 2012). This approach would enhance biosafety of cultivars through reduced outcrossing potential (Kwit and Stewart 2012). Late flowering switchgrass varieties with reduced seed production may have less potential to spread from production fields and impact plant communities. Reducing seed production is especially important because seed size is often enhanced in cultivar populations (Eckberg et al. 2015). Large seeded populations showed greater seedling vigor/ biomass and potentially stronger competitive effects and seedling vigor is associated with invasiveness in other plants (Grotkopp and Rejmanek 2007). Yet the reduction or elimination of seed production in switchgrass would not guarantee a benign biomass crop. Highly rhizomatous plants such as switchgrass have become invasive primarily through vegetative propagation (Saltonstall 2002). The extended growing season of late flowering switchgrass could also increase weediness as suggested for other grasses (Mangla et al. 2011). The contribution of seed versus vegetative propagation to invasive potential of switchgrass merits further investigation given the significance of both pathways for grass invasion (Matlaga and Davis 2013, West et al. 2014) and the need for these data to inform breeding for less invasive biomass crops.

Results from this greenhouse experiment underscore the need for field experiments to test the ecological interactions of switchgrass cultivars with native plant communities. Differences in switchgrass performance and competitive pressure may be specific to environments like a greenhouse with ample resource availability; such interactions can differ between greenhouse and field experiments (Stanton 1984). Furthermore, large environmental variation in the expression of improved cultivar vigor

(Vogel et al. 2014) implies that the effects of selective breeding on invasiveness could vary across environments (Abay and Bjørnstad 2009, Simmonds 1991). Future studies are needed on switchgrass phenotype expression and impacts on native plant communities across a range of habitat characteristics. Such studies can yield important insight by considering switchgrass in a broader invasion context involving phenotype interaction with native plant communities at the propagule dispersal, establishment, and impact stages of an invasion.

Figure 1. Planting Arrangement. The focal plant (squares) was planted in the center of each pot and surrounded by 0 (not shown), 3, 9, or 18 switchgrass (circles). Switchgrass were planted equidistantly from each other and the focal plant and a constant buffer was maintained for the outermost switchgrass and the pot. The diagram is not to scale.

Figure 2. Switchgrass growth of longest leaf in relation to switchgrass density (3, 9, 18 plants per pot) and population treatment (EG-2101, 'Trailblazer', and Wild). Longest leaf and leaf and stem number were measured on the three closest switchgrass surrounding each focal plant (2 focal plants $\times$ 27 pots per focal plant $\times$ 3 switchgrass per pot; $N = 162$). Growth of longest leaf is presented because it best represented variation in final harvested biomass and the differences in biomass among switchgrass population treatment and density. Patterns in data among population treatments were difficult to view within each switchgrass density so we added random noise to each density (x-axis) using the jitter function in R.

Figure 3. Sideoats grama growth of stem number in relation to switchgrass density (3, 9, 18 plants per pot) and population treatment (EG-2101, 'Trailblazer', and Wild). Longest leaf and leaf and stem number were measured on sideoats grama ($N = 27$). Stem number best represented variation in final sideoats biomass and the effects of switchgrass population treatment and density on sideoats grama biomass. Patterns in data among population treatments were difficult to view within each switchgrass density so we added random noise to each density (x-axis) using the jitter function in R.

Figure 4. a) Sideoats grama biomass (N = 27) at the end of the experiment in relation to switchgrass density (3, 9, 18 plants per pot) and population treatment (EG-2101, 'Trailblazer', and Wild). The switchgrass cultivars (EG-2101, 'Trailblazer') have been selected for improved agronomic traits and wild populations represent no selection history. b) Total switchgrass biomass per pot versus switchgrass density and population treatment. Shown are switchgrass biomass for all pots (2 focal plants × 27 pots per focal plant; N = 54). Patterns in data among population treatments were difficult to view within each switchgrass density so we added random noise to each density (x-axis) using the jitter function in R.

Figure 5: A) Switchgrass mean seed size per population and plant density (3, 9, 18 plants per pot) versus total switchgrass biomass per pot.

3 Switchgrass

2.5 cm

8.9 cm

9 Switchgrass
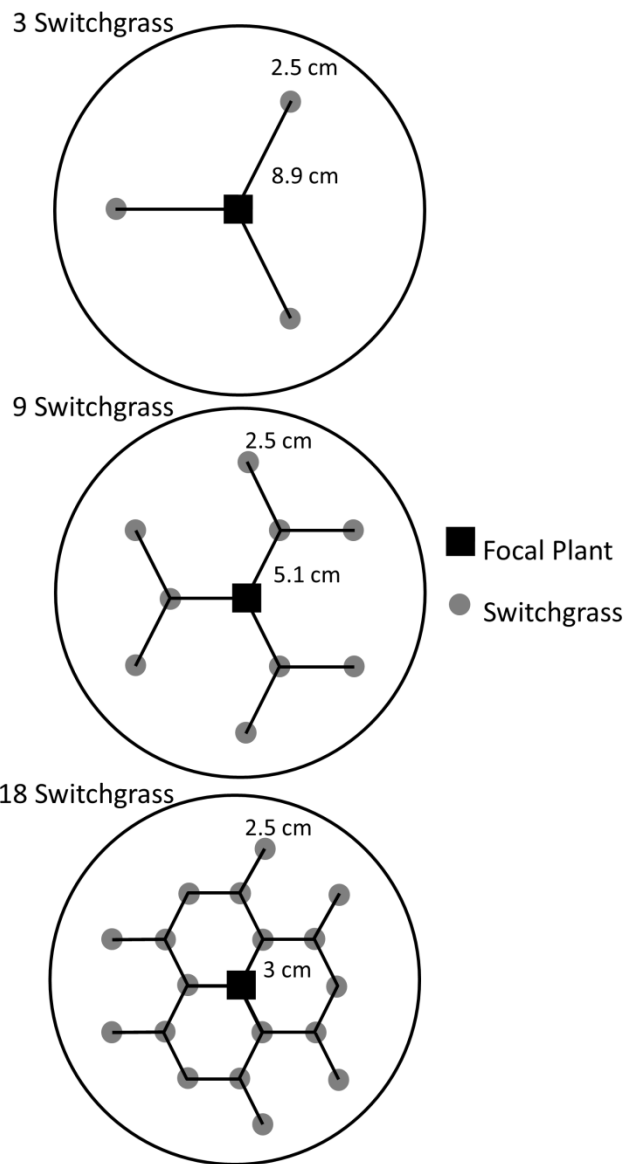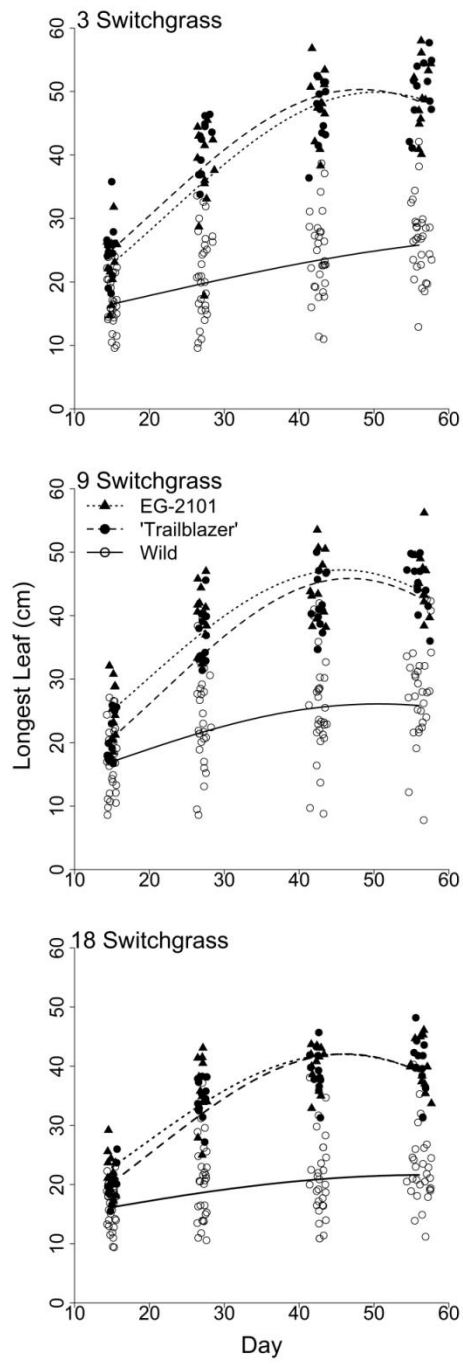
2.5 cm

5.1 cm

■ Focal Plant

● Switchgrass

18 Switchgrass
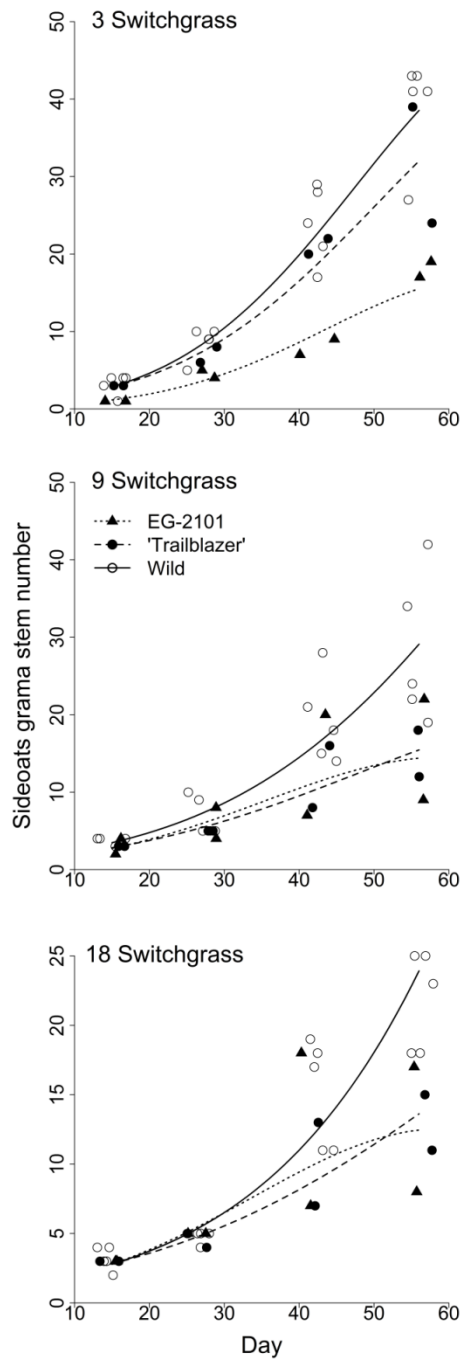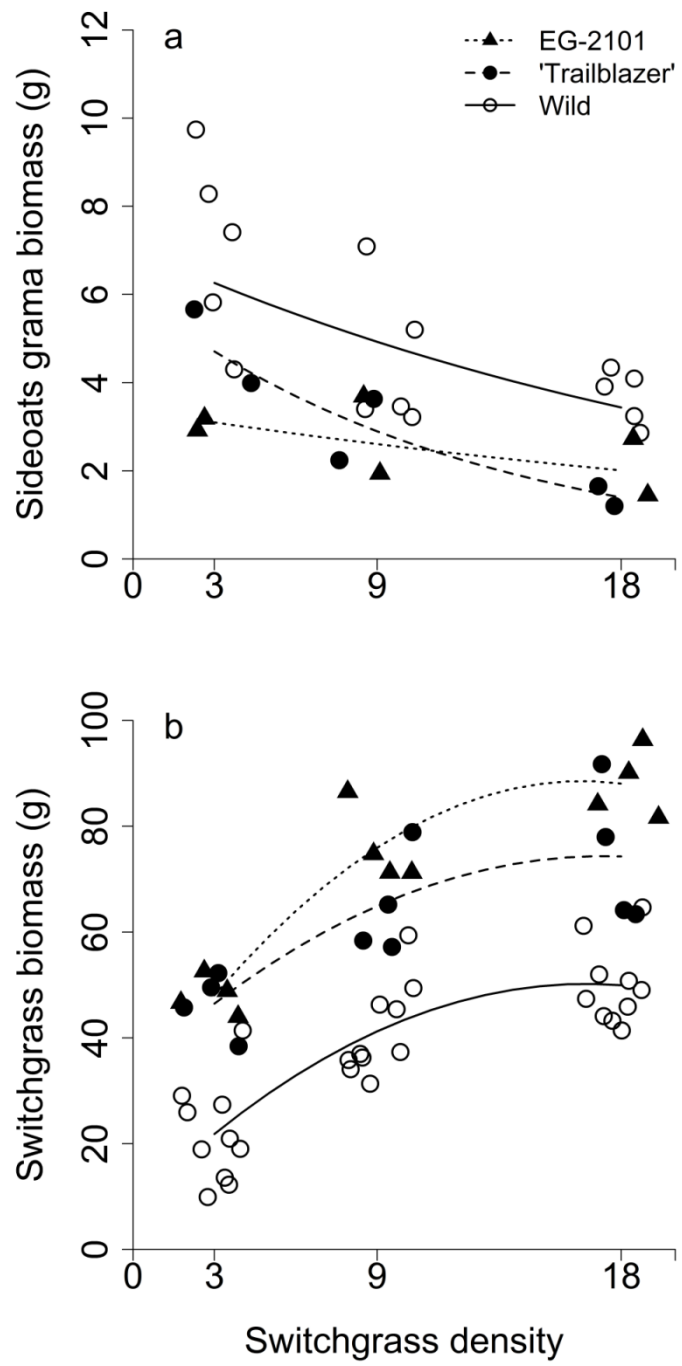
2.5 cm

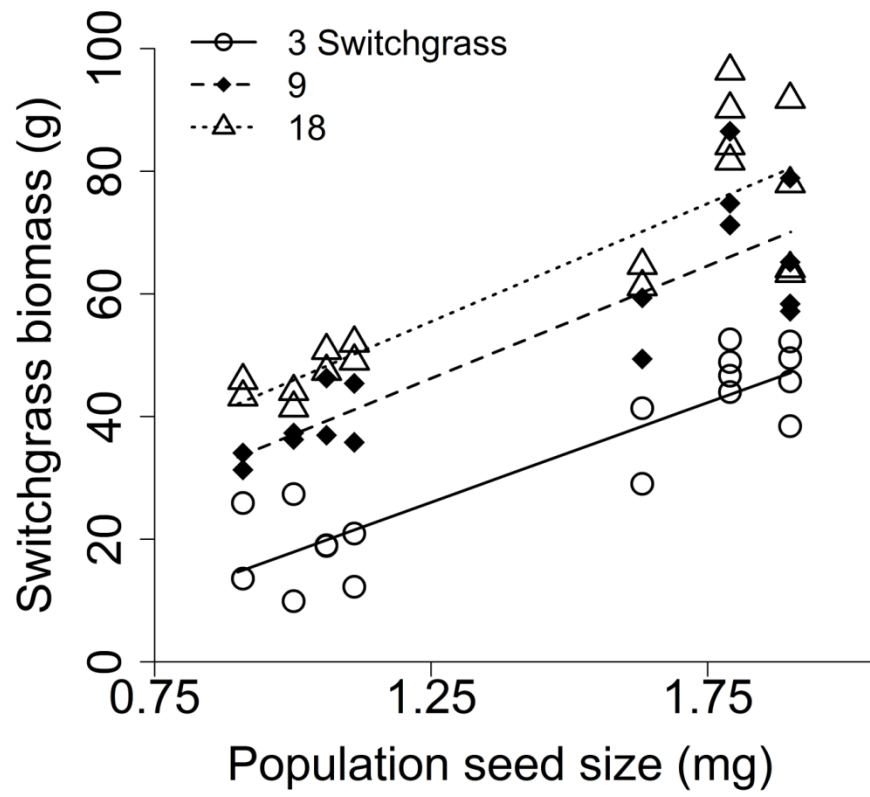3 cm

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

CHAPTER 5

Switchgrass population and cold-moist stratification mediate germination

Introduction

The development of perennial grasses for bioenergy and biomaterial production is a current challenge for agronomists and geneticists. For nearly 70 years the native warm season switchgrass (*Panicum virgatum* L.) has been the focus of breeding and selection, first for forage and conservation applications and more recently for bioenergy production (Casler, 2012). Because of the large net energy yields and potential for future improvement (Schmer et al., 2008), switchgrass is poised to support the Renewable Fuel Standards and Energy Independence and Security Act of 2007 (EPA, 2010). Despite major gains in switchgrass agronomics and breeding (McLaughlin and Kszos, 2005; Casler, 2012), several limitations must be addressed to improve the efficiency of switchgrass-based biomass production systems. Switchgrass seed dormancy can be high, sometimes over 80% (Blake, 1935; Sanderson et al., 1996; Shen et al., 2001), and this can limit the establishment and long-term productivity of the crop (Mitchell and Vogel, 2012). While variation in other agronomic traits (e.g. biomass yield, survival, and cell wall carbohydrates) has been characterized (Casler, 2005), similar studies are lacking for germination and dormancy.

Breeding and selection programs have presumably reduced dormancy in switchgrass cultivars because dormant seeds are eliminated by successive cycles of selection (Casler, 2012). For example, four cycles of direct selection against dormancy increased germination of the cultivar 'Alamo' by 16-71% depending on the seed lot and

germination conditions (Burson et al., 2009). Germination is also greater for heavier seed (Aiken and Springer, 1995; Smart and Moser, 1999) and selection for large seed size has led to the release of cultivars with enhanced germination and seedling emergence (Boe and Ross, 1998). The relative effects of seed size per se versus selection history on germination have not been directly quantified. Studies on germination in switchgrass have been limited to cultivars only (Zarnstorff et al., 1994; Aiken and Springer, 1995; Smart and Moser, 1999; Shen et al., 2001), all of which likely underwent some selection for less dormancy (Casler, 2012). More insight on the factors affecting germination could be realized by a broader comparison of cultivars versus wild populations, which have little to no artificial selection for less dormancy. Such an evaluation could inform breeding strategies by providing insight on the extent to which selection and large seed size are associated with reduced dormancy in cultivars and wild populations.

The application and potential benefits of seed treatments on germination could be better understood in the broader context of population-level variation in switchgrass germination. Many seed treatments have been investigated as a means to alleviate dormancy in switchgrass including cold-moist stratification, scarification (mechanical and chemical), variable storage durations and conditions, and growth hormones (Zarnstorff et al., 1994; Haynes et al., 1997; Madakadze et al., 2000). Chemical treatments, while effective at increasing germination (Haynes et al., 1997; Madakadze et al., 2000), can be costly or impractical when applied to commercial-scale biomass production. Cold-moist stratification is both practical and effective at enhancing germination (Zarnstorff et al., 1994; Shen et al., 2001). However, little is known about

how the effect of cold-moist stratification differs among wild and cultivated populations that vary in dormancy.

In this study, we compare the proportion of germinating versus dormant seeds of four cultivars and eight wild switchgrass populations and evaluate how variation in seed size among switchgrass populations influences germination (experiment 1). The four cultivars have a known history of selection for agronomic traits or have undergone seed multiplication; dormancy is one of the first wild traits to be reduced in breeding programs (Casler, 2012). All eight wild populations were collected from Scientific and Natural Areas in Minnesota and have little to no artificial selection. We then evaluate the interactive effect of population and cold-moist stratification on germination and dormancy (experiment 2).

Materials and Methods

Switchgrass Seeds: Wild Populations

Both experiments used switchgrass seed collected from wild populations and cultivars. From 11 to 24 September 2011, switchgrass seed was collected from eight prairies, each considered a separate wild population, in central and southern of Minnesota. One population (Black Dog) represented seed collected in 2011 and 8 September 2012. We minimized the potential for genetic contamination by cultivar seed or cross-pollination by collecting populations 1) from prairie in the Scientific & Natural Areas (SNAs) program (Minnesota Department of Natural Resources and Minnesota Chapter - the Nature Conservancy) that represent remnant plant populations or re-seeded populations collected from remnant prairie within 40 km; 2) avoiding SNAs that were adjacent to DNR

Wildlife Management Areas with large-scale prairie reconstructions; and 3) making every practical effort to collect seed close to the interior of the SNA. Within each SNA switchgrass seed was collected from four to eight locations (30 meter maximum search radius). Based on GIS analysis, only one of 46 collection points was located outside the SNA, 68 m away in a plant community similar to the sampled SNA. All other collection areas were located 40 to 413 m away from the edge of each SNA. Finally, we reviewed MN-DNR management notes and 1991 aerial photos for evidence of field cultivation and re-seeding as such populations may have reduced dormancy (Casler, 2012). Two of the eight populations were re-seeded using remnant prairie seed sources within 40 km while the others are likely remnant populations.

Switchgrass Seeds: Cultivars

We studied three upland switchgrass cultivars: 'Summer' (Kaste Seed Inc.), 'Trailblazer' (Stock Seed Farms), and EG-2101 (CERES Blade), as well as a lowland cultivar selected for biomass production, WS12L-IL (Casler, 2012). Cultivars have undergone selection for agronomic traits or seed multiplication which both entail elimination of dormant seeds with potential for reducing cultivar dormancy (Casler, 2012). 'Trailblazer' represents several seed accessions from Nebraska and Kansas that have potential intra-specific hybridization (Vogel et al., 1991). EG-2101 resulted from selection of 'Cave-in-Rock' for increased biomass production (Blade Energy Crops, 2009; Christensen, 2010). Summer is the only cultivar in this study which has not been selectively bred but has undergone numerous generations of seed multiplication since its release as a cultivar (Alderson and Sharp, 1994). WS12L-IL consists of a broad genetic

base of lowland germplasm selected for winter hardiness in Madison, Wisconsin and represents a significant increase in yield compared to many other cultivars associated with its late flowering time (Casler, 2012).

Experiment 1: Test of Switchgrass Populations on Germinability-Dormancy

We tested the proportion germinable and dormant seeds of the cultivars 'Summer', EG-2101, WS12L-IL, 'Trailblazer', and eight remnant populations (N = 12 populations) in a growth chamber between 28 Feb. and 15 Mar. 2013. Prior to the germination test, seeds were dry pre-chilled at 1 to 4°C for 10 months and then surface sterilized with a 5% v/v bleach solution and triple rinsed with deionized water. For each population we established six replicate petri dishes (100 x 15 mm) with moistened 0.24" Versa-Pak germination paper (Seedburo, Des Plaines, IL) each containing 50 fully formed, randomly selected seeds (six replicate petri dishes × 50 seeds/ replication = 300 seeds/ population; N = 72 petri dishes). We recorded dry mass (g) of each 50-seed-replicate. Petri dishes were randomly distributed in the growth chamber maintained at 30/15°C – 8/16 hour – day/night, respectively, for 14 d (Association of Official Seed Analysts [AOSA], 2010a). We recorded and removed germinated (radicle length ≥ 2 mm) seeds daily. After 14 days, we tested viability of non-germinated seed using a 0.1% tetrazolium solution (AOSA, 2010b). Germination proportion is calculated as the number germinated (**g**) divided by germinated (**g**) plus viable, non-germinated seeds (**d**):

$$\frac{g}{g + d} \times 100$$

We removed seeds that were either confirmed dead by the TZ test or covered by 90% mold leaving 3,307 seeds (91.9% of the original seeds) to estimate germination proportion.

Experiment 2: Test of Cold-Moist Stratification and Populations on Germinability-Dormancy

We selected three cultivars and five wild populations representative of the variation in germination observed in experiment 1 and tested the effect of cold-moist stratification on germination from 9 Dec. 2013 to 24 Jan. 2014. Seeds were dry pre-chilled at 1 to 4°C for 19 months before the start of experiment 2, nine months after experiment 1. We established five replicate petri dishes each with 50 seeds per treatment (cold-moist and control) and population (five replicate petri dishes/ treatment $\times$ 50 seeds/ replication = 250 seeds/ population/ treatment; N = 80 petri dishes). We recorded dry mass (g) of each 50 seed-replicate. To impose the cold-moist stratification treatment we randomly arranged all petri dishes in a dark storage container at 4°C for 28 days. Cold-moist stratification seeds were surface sterilized with a 5% v/v bleach solution, triple rinsed with deionized water, and then placed on moistened 0.24" Versa-Pak germination paper. Control petri dish seeds remained dry inside a coin envelope to prevent seed loss. After 28 days petri dishes were transferred to the growth chamber, control seeds were surface sterilized and moistened, and all petri dishes were randomly arranged for the 14 day germination test using the same conditions as in experiment 1.

Daily monitoring, data collection, tetrazolium tests and germination calculations followed the protocols established in experiment 1. We removed seeds that were either

confirmed dead (negative TZ test), covered by more than 90% mold or showing damaged root growth which left 3,396 seeds (84.9 % of the original seeds) to estimate germination proportion. Despite a longer saturation period for cold-moist stratified seeds, their mortality was only 2.3% greater than seeds in the control and this difference was not statistically significant (P=0.35).

Statistical Analysis

All data were analyzed in R version 3.1.1 (R Development Core Team, 2014). Petri dish was the experimental unit. We used a generalized linear mixed model (glmer) to conduct likelihood ratio tests (chi-square distribution, χ^2) of the effect of switchgrass population (individual population, cultivar versus wild), cold-moist stratification, and their interaction on germination in experiments 1 and 2. The response of each seed followed a Bernoulli distribution (germinated, dormant) and petri dish was treated as a random effect to account for non-independence among seeds in the same petri dish. Seed mass based on 50 seed samples was analyzed using ANOVA and model residuals were normally distributed and homogeneous among treatments. In experiment 1 we evaluated all pairwise comparisons of germination and seed mass among each population using unadjusted p-values at the 0.05 level to provide a preliminary test of mean differences among populations. To address the potential for inflated experiment-wise type I error rate, we used the results from Experiment 1 to test a subset of eight populations of interest (Saville, 2013). The effect of cold-moist stratification on each of the eight populations was evaluated using unadjusted p-values in experiment 2.

Population-level analysis was performed with ANCOVA on the mean seed mass and germination for each population in both experiments. In experiment 1, we evaluated the effect of seed origin (cultivar versus wild) and mean seed mass on germination at the population level. In experiment 2, the population-level response to cold-moist stratification (Germination with stratification minus germination without stratification) was evaluated in relation to mean seed mass, germination without stratification, and origin (cultivar versus wild).

Results and Discussion

Wild and cultivar populations of switchgrass were tested to evaluate differences in germination proportion and, from this, progress in breeding efforts for improved germination. We also tested the response of switchgrass populations to cold-moist stratification to determine the benefit of stratification treatments for further improving germination. Germination of the four cultivar populations significantly exceeded that of eight wild populations (57.1 vs. 18.5%; $\chi^2 = 43.1$, $P < 0.001$; Figure 1a). There were also significant differences in germination within each group, especially among wild populations ($\chi^2 = 150.6$, $P < 0.001$; Figure 1a). We then used the same procedure to re-test germination in a subset of populations representative of the variation in germination. Consistent with experiment 1, there were significant differences in germination among switchgrass populations ($\chi^2 = 117.3$, $P < 0.001$). The relative differences and mean statistical comparison results were generally consistent between experiments (Figure 1a and control treatments of Figure 2), further supporting the finding of large variation in germination among wild populations and generally higher germination of cultivars.

Higher seed dormancy of wild versus cultivar switchgrass populations likely reflects different selection history. Dormancy may be reduced for cultivars by elimination of non-germinating seeds during successive cycles of selection for other agronomic traits or seed multiplication as mechanisms that unconsciously select against dormancy (Casler, 2012). The significant response of switchgrass dormancy to selection suggests there is a strong genetic basis and high heritability for dormancy (Burson et al., 2009) as shown in other plants (Goggin et al., 2010). In contrast to cultivars, wild populations persist in environments with elevated spatio-temporal heterogeneity in growing conditions. Seed dormancy is adaptive in such environments, allowing for the formation of seedbanks that persist through times of unfavorable conditions (Pake and Venable, 1996; Rees et al., 2006). While longer lived plants often show less seed dormancy than shorter lived plants under variable environments (Rees, 1993; Rees et al., 2006), there is still considerable dormancy in perennial plants including switchgrass (Shen et al., 2001) as our results confirm. While most wild populations were highly dormant, several showed low dormancy. Re-seeding wild populations may selectively reduce dormancy if recently disturbed restoration environments promote rapidly germinating seedlings over dormant seeds. There is some evidence in support of this hypothesis as the only re-seeded populations, Joseph Tauer and Lost Valley, showed low dormancy (Figure 1a). Nevertheless, these results demonstrate genetic variation for seed dormancy levels in native switchgrass, establishing the basis for genetic improvements in germination of commercial cultivars.

Seeds of switchgrass cultivars were 26% heavier than wild switchgrass seed (1.56 versus 1.23 mg seed^{-1}; $F_{1, 70} = 16.4$, $P < 0.001$) but there was substantial variation in seed

mass among wild and cultivar populations ($F_{11, 60} = 100.76$, $P <0.001$; Figure 1b). Seed mass, origin (cultivar, wild), and their interaction explained most of the population-level variation in germination ($R^2 = 92.1\%$, $P<0.001$, Figure 3). There was a strong positive relationship between seed size and germination for wild populations ($P<0.001$) but not cultivars ($P=0.16$, Figure 3). This pattern was confirmed with the subset of eight cultivar and wild populations re-tested in the second experiment. Selection for low dormancy itself and selection for larger seed mass may jointly influence seed germination as the current study and others indicate (Vange et al., 2004). Greater seed size can provision resources for seedlings to endure variable establishment conditions as experienced by wild populations in natural environments (Rees, 1993; Leishman et al., 2000); but seed size may be less important to cultivars where it has been shown to only have an ephemeral effect on seedling performance in more homogeneous and predictable agricultural environments (Smart and Moser, 1999). Alternatively, because cultivar seed was generally much larger than that of wild populations and limited in its range of sizes, the potential to detect a relationship between seed size and germination in cultivars may be lacking. Aiken and Springer (1995) showed a positive correlation between seed size and germination in cultivars up to approximately 1.0-1.2 mg seed^{-1} at which point further increases in seed size did not promote germination. The majority of our cultivars were larger than 1.2 mg seed^{-1}. Regardless of the underlying mechanism, these data suggest both seed size and selection history jointly influence germination.

Seed of all populations were grown in different environments and some populations were subjected to different storage conditions and duration. While these environmental differences were to some extent unavoidable (i.e. wild populations must

be collected from tallgrass prairies to be considered a wild population), they raise the potential for genotype × environment covariance to confound our inference on the role of selection history in germination. To minimize the potential for systematic environmental effects to confound our inference of genetically based differences among populations we collected and compared numerous wild populations to cultivars with a known history of selection. Our results are consistent with other studies suggesting a strong genetic basis for dormancy (Vange et al., 2004; Burson et al., 2009; Goggin et al., 2010). Further, in a study that directly compared both effects, dormancy rankings among maternal genotypes were conserved across environment suggesting no genotype × environment interaction (Platenkamp and Shaw, 1993). Therefore while growing environment may have influenced dormancy in our study, its effect are likely non-systematic as to not alter the general patterns in germination among cultivar and wild populations. Further, our estimates of dormancy are relevant to growers and breeders seeking to incorporate wild populations and current cultivars into research and breeding projects.

Dormancy is also mediated by seed age and storage conditions (Zarnstorff et al., 1994). All seed was stored at 1 to 5°C for 10 months before the first experiment began, but beforehand two cultivars (EG-2101 and 'Trailblazer') were stored for 12 months longer and at warmer temperatures than all other populations. Warm storage temperatures (23 to 30°C) for three to 12 months can substantially enhance germination (Zarnstorff et al., 1994; Shen et al., 2001). However, our data suggest differences in storage conditions did not significantly contribute to population-level variation in germination in the first experiment. For example, the two cultivars with extended storage times and exposure to warmer temperatures showed slightly lower germination than the other cultivars. From

the first to second experiment we observed a 4 to 45% increase in germination when seeds were stored at 1 to 5°C for one more year; highly dormant populations remained mostly dormant (Figure 1a versus 2). While we urge caution in interpretation of the effects of aging on germination because we did not experimentally test this effect (e.g. by controlling for observer effects of seed selection in each trial), these observations are consistent with the hypothesis that some wild populations of switchgrass are persistently dormant even after an extensive after-ripening period.

Cold-moist stratification treatment increased germination overall ($\chi^2 = 100.1$, $P<0.001$) and for all but one population, EG-2101, which showed a consistent but non-significant trend ($P = 0.13$; Figure 2). The magnitude of the treatment effect varied greatly among populations ($\chi^2 = 23.3$, $P = 0.001$; Figure 2). This is consistent with other experiments that showed strong positive effects of cold-moist stratification on seed germination and emergence with large variation in the response among different cultivars and seed lots (Zarnstorff et al., 1994; Shen et al., 2001). We further evaluated variation in population-level response to stratification (Population germination under cold-moist stratification minus germination of control) and tested variables associated with the response. There were no significant effects of seed mass ($t = -0.35$, $P = 0.74$) or origin ($t = 1.2$, $P = 0.31$) on response to cold-moist stratification. Instead, populations with a higher dormancy showed a greater response to cold-moist stratification ($t = -12.7$, $P <0.001$) explaining 95.8% of the variation (R^2) in response (Figure 4). For every 10% increase in dormancy among populations, there was a 6.5% increase in the response to cold-moist stratification suggesting an important offsetting effect of this seed treatment. Other studies have similarly shown that cold-moist stratification increased the

germination of highly dormant populations much more so than less dormant populations (Zarnstorff et al., 1994; Shen et al., 2001).

In conclusion, seed origin and mass jointly influenced population-level variation in switchgrass germination. This information can be used in breeding programs to improve switchgrass germination and establishment success as well as provide an initial roadmap for screening and integration of wild populations into current breeding programs. This study has also shown that cold-moist stratification increased germination of nearly all populations suggesting that such treatments could be widely applied to enhance germination in the field. Future studies could use seed grown and stored in similar environments to improve the estimation of population genetic variation in dormancy.

Figure Legends

Figure 1. Mean ($\pm$ SE) a) germination and b) seed mass (based on samples of 50 seeds) of eight wild and four cultivar populations of switchgrass. Letters indicate significant differences among populations ($P<0.05$). The names of wild populations represent their collection sites.

Figure 2. Mean ($\pm$ SE) germination of switchgrass populations in relation to cold-moist stratification. Letters indicate significant differences ($P<0.05$) among switchgrass populations without stratification. Asterisks indicate statistical significance of the effect of cold-moist stratification (** $P<0.001$, *$P<0.05$).

Figure 3. Mean seed mass and origin (cultivar, wild) versus germination for eight wild and four cultivar populations of switchgrass.

Figure 4. Relationship between mean germination of each switchgrass population and the increase in germination after cold-moist stratification (Germination with stratification minus germination without stratification for each population).
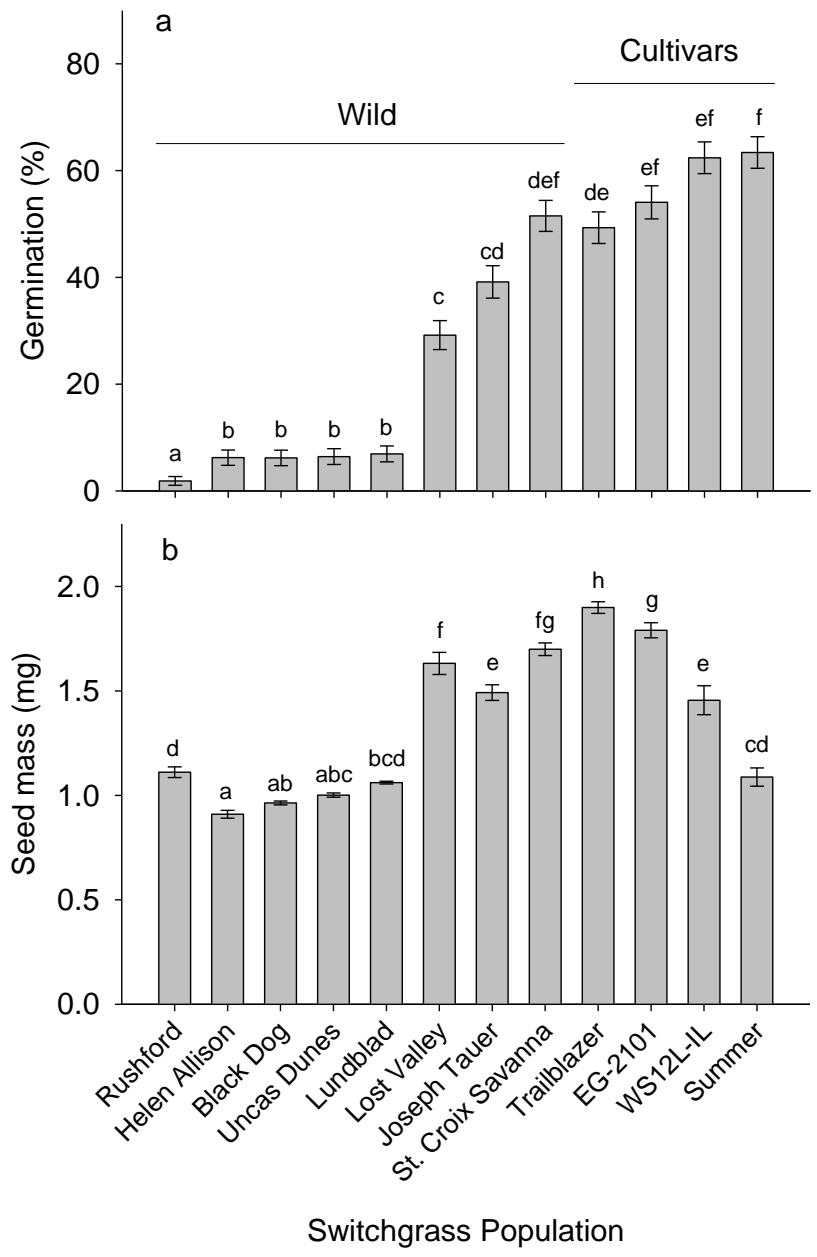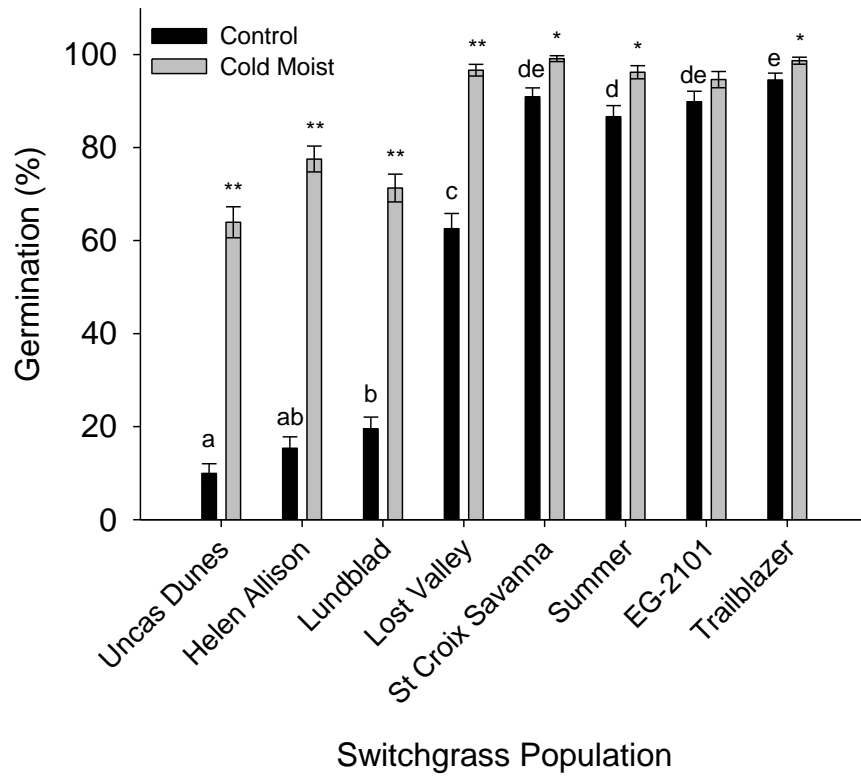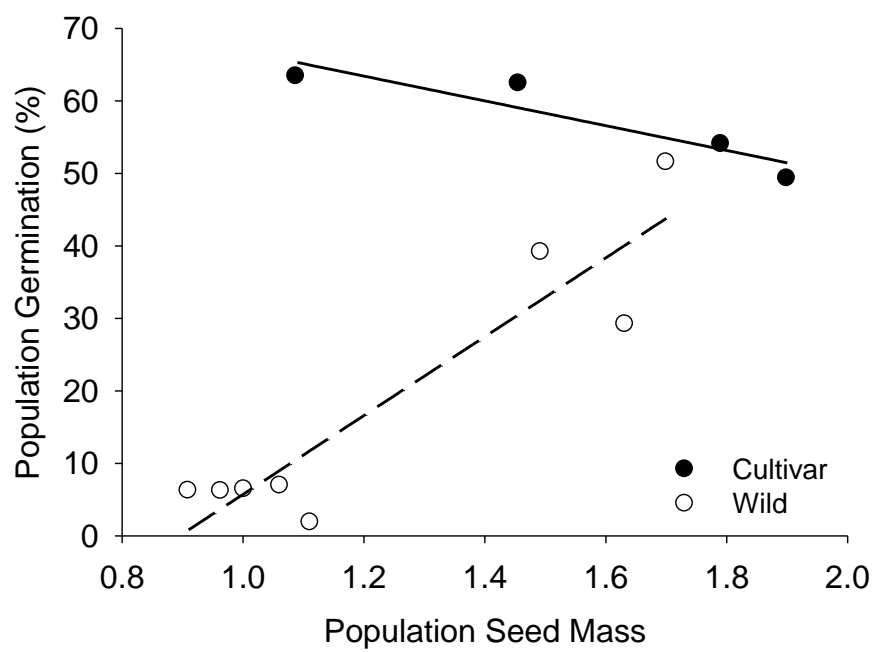
Figure 1

113

Figure 2

Figure 3

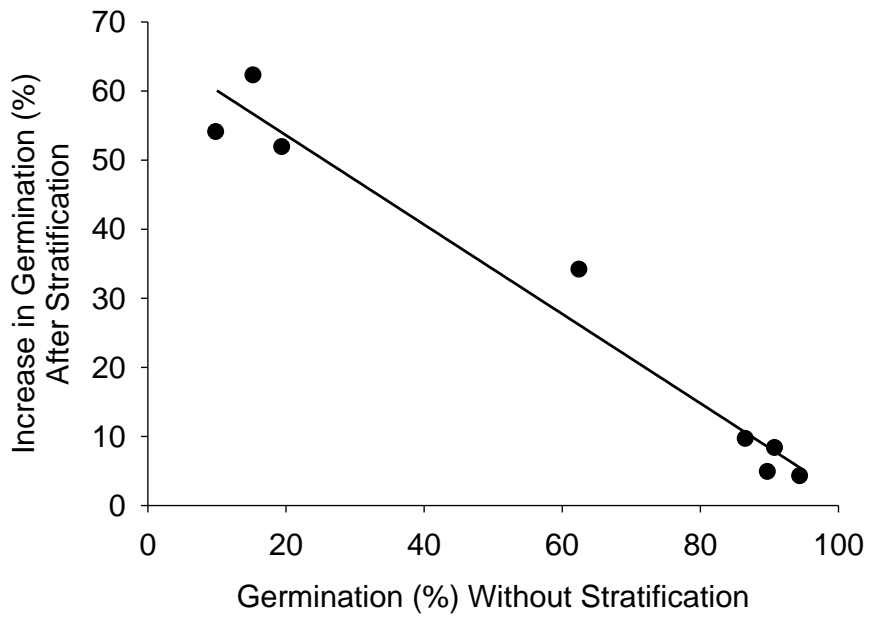Figure 4

Bibliography

Abay, F, and A Bjørnstad. 2009. Specific adaptation of barley varieties in different locations in Ethiopia. Euphytica. 167:181–195

Abrahamson, L.P., T.A. Volk, L.B. Smart, and K.D. Cameron. 2010. Shrub Willow Biomass Producer 's Handbook. State University of New York, Syracuse, NY.

Adegbidi, H.G., R.D. Briggs, T. A. Volk, E.H. White, and L.P. Abrahamson. 2003. Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. Biomass and Bioenergy 25: 389–398.

Aiken, G.E., and L. Springer. 1995. Seed size distribution , germination, and emergence of 6 switchgrass cultivars. Journal of Range Management. 48: 455–458.

Akaike H. 1974. A New Look at the Statistical Model Identification. IEEE Transactions on Automatic Control. 19: 716–723.

Alderson, J., and W. Sharp. 1994. Grass varieties in the United States (USDA Agriculture Handbook, Ed.). No. 170. U.S. Government Printing Office, Washington, DC.

Alhmedi, A., E. Haubruge, and F. Francis. 2010. Intraguild interactions and aphid predators: biological efficiency of *Harmonia axyridis* and *Episyrphus balteatus*. Journal of Applied Entomology. 134: 34–44.

Almohamad, R., F.J. Verheggen, and É. Haubruge. 2009. Searching and oviposition

behavior of aphidophagous hoverflies (Diptera : Syrphidae): a review.

Biotechnologie Agronomie Societe Et Environement. 13: 467–481.

Andersen, C., and S.M. Louda. 2008. Abundance of and floral herbivory on exotic bull

thistle versus native tall thistle in western tallgrass prairie. Proceedings of the North

American Prairie Conference. 20: 33–50.

Anderson, N.O., N. Gomez, and S.M. Galatowitsch. 2006. A non-invasive crop ideotype

to reduce invasive potential . Euphytica 148:185–202

Anderson-Teixeira, K.J., S.C. Davis, M.D. Masters, and E.H. DeLucia. 2009. Changes in

soil organic carbon under biofuel crops. Global Change Biology- Bioenergy 1: 75–

96.

Andow, D. 1991. Vegetational diversity and arthropod population response. Annual

Review of Entomology. 36: 561–586.

Association of Official Seed Analysts. 2010a. AOSA rules for testing seeds. AOSA,

Ithaca, NY.

Association of Official Seed Analysts. 2010b. Tetrazolium testing handbook. AOSA,

Ithaca, NY.

AOSA. 2012. Association of Official Seed Analysts. Ithaca, New York, USA.

Bach, C. 2001. Long-term effects of insect herbivory on responses by *Salix cordata* to

sand accretion. *Ecology*. 82: 397–409.

Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. Annual Review of Ecology, Evolution, and Systematics. 40: 1–20.

Barney, J.N., and J.M. DiTomaso. 2008. Nonnative species and bioenergy: Are we cultivating the next invader? Bioscience. 58:64–70.

Bell A.C., Clawson S., and Watson S. 2006. The long-term effect of partial defoliation on the yield of short-rotation coppice willow. Annals of Applied Biology. 148: 97–103.

Berdahl, JD, A.B. Frank, J.M. Krupinsky, P.M. Carr, J.D. Hanson, and H.A. Johnson. 2005. Biomass yield, phenology, and survival of diverse switchgrass cultivars and experimental strains in western North Dakota. Agronomy Journal. 97:549–555

Björkman C., Hoglund S., Eklund K., Larsson S. 2000. Effects of leaf beetle damage on stem wood production in coppicing willow. Agricultural and Forest Entomology. 2: 131–139.

Blade Energy Crops. 2009. New varieties 2010 - Other varieties. http://www.progressiveforage.com/forage-types/other-forage/new-varieties-2010-other-varieties. Accessed July 21, 2015

Blake, A.K. 1935. Viability and germination of seeds and early life history of prairie plants. Ecological Monographs. 5: 405–460.

Blanco-Canqui, H., C.J. Gantzer, S.H. Anderson, E.E. Alberts, and A. L. Thompson. 2004. Grass barrier and vegetative filter strip effectiveness in reducing runoff,

sediment, nitrogen, and phosphorus loss. Soil Science Society of America Journal. 68: 1670–1678.

Blandenier, G., O.T. Bruggisser, R.P. Rohr, and L.F. Bersier. 2013. Are phenological patterns of ballooning spiders linked to habitat characteristics? Journal Arachnology. 41: 126–132.

Boe, A., and J. Ross. 1998. Registration of "Sunburst" switchgrass. Crop Science. 38: 540.

Bolker, B.M. 2008. Ecological Models and Data in R. Princeton, New Jersey: Princeton University Press

Bowie, M.H., G.M. Gurr, Z. Hossain, L.R. Baggen, and F.C. M. 1999. Effects of distance from field edge on aphidophagous insects in a wheat crop and observations on trap design and placement. International Journal of Pest Management. 45: 69–73.

Brewer, M. J., and T. Noma. 2010. Habitat affinity of resident natural enemies of the invasive *Aphis glycines* (Hemiptera: Aphididae), on soybean, with comments on biological control. Journal of Economic Entomology. 103: 583–596.

Broberg C.L., Borden J.H., Humble L.M. (2001) Host range, attack dynamics, and impact of Cryptorhynchus lapathi (Coleoptera: Curculionidae) on Salix (Salicaceae) spp. Canadian Entomologist. 133: 119–130.

Burson, B.L., C.R. Tischler, and W.R. Ocumpaugh. 2009. Breeding for reduced post-harvest seed dormancy in switchgrass: Registration of TEM-LoDorm switchgrass germplasm. Journal of Plant Registrations. 3: 99–103.

Cappuccino, N. 2004. Allee effect in an invasive alien plant, pale swallow-wort *Vincetoxicum rossicum* (Asclepiadaceae). Oikos. 106: 3–8.

Casler, M.D. 2005. Ecotypic variation among switchgrass populations from the northern USA. Crop Science. 45: 388–398.

Casler, MD. 2010. Changes in mean and genetic variance during two cycles of within-family selection in switchgrass. BioEnergy Research. 3:47–54

Casler, M.D. 2012. Switchgrass breeding, genetics, and genomics. p. 29–53. *In* Monti, A. (ed.), Switchgrass: A valuable biomass crop for energy. Springer-Verlag.

Casler, M.D., A.R. Boe. 2003. Cultivar x environment interactions in switchgrass. Crop Science. 43: 2226–2233.

Casler, M.D., and K.P. Vogel. 2014. Selection for biomass yield in upland, lowland, and hybrid switchgrass. Crop Science. 54: 626–636.

Casler, M.D., K.P. Vogel, C.M. Taliaferro, N.J. Ehlke, J.D. Berdahl, E.C. Brummer, R.L. Kallenbach, C.P. West, and R.B. Mitchell. 2007. Latitudinal and longitudinal adaptation of switchgrass populations. Crop Science. 47: 2249–2260.

Cavanagh, A., M.O. Gasser, and M. Labrecque. 2011. Pig slurry as fertilizer on willow plantation. Biomass and Bioenergy. 35: 4165–4173.

Chacon, J., and G. Heimpel. 2010. Density-dependent intraguild predation of an aphid parasitoid. Oecologia. 164: 213–220.

Chambers, R. J., and T. H. L. Adams. 1986. Quantification of the impact of hoverflies (Diptera : Syrphidae) on Cereal Aphids in Winter Wheat: an analysis of field populations. Journal of Applied Ecology. 23: 895–904.

Christensen, C. 2010. Development of dedicated energy crops to supply the biofuel and biopower industries. *In* 7th Annual Bioenergy Feedstocks Symposium.

Collins C.M., R.G. Rosado, and S.R. Leather. 2001. The impact of the aphids *Tuberolachnus salignus* and *Pterocomma salicis* on willow trees. Annals of Applied Biology. 138: 133–140.

Costamagna, A. C., and D. A. Landis. 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecological Applications. 16: 1619–28.

Costamagna, A. C., and D. A. Landis. 2007. Quantifying predation on soybean aphid through direct field observations. Biological Control. 42: 16–24.

Costamagna, A. C., D. A. Landis, and M. J. Brewer. 2008. The role of natural enemy guilds in *Aphis glycines* suppression. Biological Control. 45: 368–379.

Costamagna, A. C., D. A. Landis, and C. D. Difonzo. 2007. Suppression of soybean

aphid by generalist predators results in a trophic cascade in soybeans. Ecological

Applications. 17: 441–51.

Cowgill, S.E., S.D. Wratten, and N.W. Sotherton. 1993. The effect of weeds on the

numbers of hoverfly (Diptera : Syrphidae) adults and the distribution and

composition of their eggs in winter wheat. Annals of Applied Biology. 123: 499–

515.

Coyle D.R., T.E. Nebeker, E.R. Hart, and W.J. Mattson. 2005. Biology and management

of insect pests in North American intensively managed hardwood forest systems.

Annual Review of Entomology. 50: 1–29.

Dalin, P., T. Demoly, M.F. Kabir, and C. Björkman. 2011. Global land-use change and

the importance of zoophytophagous bugs in biological control: Coppicing willows

as a timely example. Biological Control. 59: 6–12.

Dalin P., O. Kindvall, and C. Björkman. 2009. Reduced population control of an insect

pest in managed willow monocultures. PloS one. 4: e5487.

Denison, R.F. 2014. Increasing cooperation among plants, symbionts, and farmers is key

to past and future progress in agriculture. J of Bioeconomics: 223–238.

Desneux, N., R. Barta, K. Hoelmer, K. Hopper, and G. Heimpel. 2009. Multifaceted

determinants of host specificity in an aphid parasitoid. Oecologia. 160: 387–398.

Desneux, N., R. J. O'Neil, and H. J. S. Yoo. 2006. Suppression of population growth of

    the soybean aphid, *Aphis glycines* Matsumura, by predators: the identification of a

    key predator and the effects of prey dispersion, predator abundance, and

    temperature. Enviromental Entomology. 35: 1342–1349.

Desneux, N., R. J. O'Neil, and H.J.S. Yoo. 2006. Suppression of population growth of

    the soybean aphid, *Aphis glycines* Matsumura, by predators: the identification of a

    key predator and the effects of prey dispersion, predator abundance, and

    temperature. Enviromental Entomology. 35: 1342–1349.

Doane, C.C. and D.E. Leonard. 1975. Orientation and dispersal of late-stage larvae of

    Porthetria dispar (Lepidoptera: Lymantriidae). The Canadian Entomologist. 107:

    1333–1338.

Donaldson, J., S. Myers, and C. Gratton. 2007. Density-dependent responses of soybean

    aphid (*Aphis glycines* Matsumura) populations to generalist predators in mid to late

    season soybean fields. Biological Control. 43: 111–118.

Eckberg, J.O., M.D. Casler, G.A. Johnson, L.L. Seefeldt, K.E. Blaedow, and R.G. Shaw.

    2015. Switchgrass population and cold-moist stratification mediate germination.

    Crop Science. 55: 2746-2752.

Eckberg, J.O., J. A. Peterson, C. P. Borsh, J. M. Kaser, G. A. Johnson, J. C. Luhman,

    D.L. Wyse, and G.E. Heimpel. 2015. Field Abundance and Performance of

    Hoverflies (Diptera: Syrphidae) on Soybean Aphid. Annals of Entomological

    Society of America. 108: 26–34.

Ellstrand, N.C. and K.A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Science. 97: 7043–7050.

EPA. 2010. Environmental Protection Agency. Regulation of fuels and fuel additives: Changes to renewable fuel standard program.

Fargione, J.E., T.R. Cooper, D.J. Flaspohler, J. Hill, C. Lehman, T. McCoy, S. McLeod, E.J. Nelson, K.S. Oberhauser, and D. Tilman. 2009. Bioenergy and wildlife: Threats and opportunities for grassland conservation. Bioscience 59: 767–777.

Fiedler, A.A.K., and D.A. Landis. 2007a. Attractiveness of Michigan Native Plants to Arthropod Natural Enemies and Herbivores. Environmental Entomology. 36: 751–765.

Fiedler, A.A.K., and D.A. Landis. 2007b. Plant characteristics associated with natural enemy abundance at Michigan native plants. Environmental Entomology 36: 878–886.

Fillion, M., J. Brisson, T.I. Teodorescu, S. Sauvé, and M. Labrecque. 2009. Performance of Salix viminalis and Populus nigra × Populus maximowiczii in short rotation intensive culture under high irrigation. Biomass and Bioenergy 33: 1271–1277.

Finke, D., and R. Denno. 2002. Intraguild Predation Diminished in Complex-Structured Vegetation: Implications for Prey Suppression. Ecology 83(3): 643–652.

Fitzgerald, T.D. 1995. The tent caterpillars. Ithaca, New York, USA: Cornell University Press.

Fitzgerald T.D., T. Casey, and B. Joos. 1988. Daily foraging schedule of field colonies of the eastern tent caterpillar *Malacosoma americanum*. Oecologia. 76: 574–578.

Fitzgerald T.D., and J.S. Edgerly. 1979. Exploration and recruitment in field colonies of the eastern tent caterpillar. Journal of the Georgia Entomological Society. 14: 312–314.

Fitzgerald T.D., Peterson S.C. 1983. Elective recruitment by the eastern tent caterpillar (*Malacosoma americanum*). Animal Behaviour. 31: 417–423.

Fornara, D. A., D. Tilman, and B.Z. Houlton. 2012. Soil carbon sequestration in prairie grasslands increased by chronic nitrogen addition. Ecology 93: 2030–2036.

Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. Difonzo. 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environmental. Entomology. 33: 608–618.

Frank, T. 1999. Density of adult hoverflies (Dipt ., Syrphidae) in sown weed strips and adjacent fields. Journal of Applied Entomology. 123: 351–355.

Gagnon, A.E., G. E. Heimpel, and J. Brodeur. 2011. The ubiquity of intraguild predation among predatory arthropods. PLoS One. 6: 1–7.

Gamble, J.D., G. Johnson, C.C. Sheaffer, D. a. Current, and D.L. Wyse. 2014. Establishment and early productivity of perennial biomass alley cropping systems in Minnesota, USA. Agroforestry. Systems. 88: 75–85.

Gardiner, M. M., D. A. Landis, C. Gratton, C. D. DiFonzo, M. O'Neal, J. M. Chacon, M. T. Wayo, N. P. Schmidt, E. E. Mueller, and G. E. Heimpel. 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecological Applications. 19: 143–154.

Gardiner, M. M., and D. A. Landis. 2007. Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies. Biological Control. 40: 386–395.

Gardiner, M.A., J.K. Tuell, R. Isaacs, J. Gibbs, J. Ascher, and D. Landis. 2010. Implications of three biofuel crops for beneficial arthropods in agricultural landscapes. Bioenergy Research. 3: 6–19.

Gill, K., R. Cox, and M. O'Neal. 2014. Quality Over Quantity : Buffer Strips can be Improved with Select Native Plant Species. Environmental. Entomology. 43: 298–311.

Gladbach D.J., Holzschuh A., Scherber C., Thies C., Dormann C.F., Tscharntke T. 2011. Crop-noncrop spillover: arable fields affect trophic interactions on wild plants in surrounding habitats. Oecologia. 166: 433–41.

Goggin, D.E., R.J.N. Emery, S.B. Powles, and K.J. Steadman. 2010. Initial characterisation of low and high seed dormancy populations of *Lolium rigidum* produced by repeated selection. Journal of Plant Physiology. 167: 1282–1288.

Gontijo, L. M., S. D. Cockfield, and E. H. Beers. 2012. Natural enemies of Woolly Apple Aphid (Hemiptera : Aphididae) in Washington State. Environmental Entomology 41: 1364–1371.

Grotkopp, E, and M. Rejmanek. 2007. High seedling relative growth rate and specific leaf area are traits of invasive species: Phylogenetically independent contrasts of woody angiosperms. American Journal of Botany. 94:526–532.

Haenke, S., B. Scheid, M. Schaefer, T. Tscharntke, and C. Thies. 2009. Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscapes. Journal of Applied Ecology. 46: 1106–1114.

Hangs, R.D., J.J. Schoenau, K.C.J. Van Rees, N. Bélanger, T. Volk, and T. Jensen. 2014. First Rotation Biomass Production and Nutrient Cycling within Short-Rotation Coppice Willow Plantations in Saskatchewan, Canada. BioEnergy Research: 1091–1111.

Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. PeerJ 2:1–19

Haynes, J.G., W.G. Pill, and T.A. Evans. 1997. Seed treatments improve the germination and seedling emergence of switchgrass (*Panicum virgatum* L.). HortScience 32: 1222–1226.

Heaton, E.A., L.A. Schulte, M. Berti, H. Langeveld, W. Zegada-Lizarazu, D. Parrish, and A. Monti. 2013. Managing a second-generation crop portfolio through sustainable intensification: Examples from the USA and the EU. Biofuels, Bioproducts, and Biorefining. 7: 702–714.

Heimpel, G. E., Y. Yang, J. D. Hill, and D. W. Ragsdale. 2013. Environmental consequences of invasive species: greenhouse gas emissions of insecticide use and the role of biological control in reducing emissions. PLoS One. 8: e72293.

Hickman, J., and S. Wratten. 1996. Use of Phacelia tanacetifolia strips to enhance biological control of aphids by hoverfly larvae in cereal fields. Journal of Economic Entomology. 89: 832–840.

Hilborn R., Mangel M. 1997. The Ecological Detective: Confronting Models with Data. Princeton, New Jersey: Princeton University Press.

Hindayana, D., R. Meyhofer, D. Scholz, and H. M. Poehling. 2001. Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera: Syrphidae) and other aphidophagous predators. Biological Control. 20: 236–246.

Hjältén J., and P.W. Price. 1997. Can plants gain protection from herbivory by association with unpalatable neighbours?: A field experiment in a willow-sawfly system. Oikos. 78: 317–322.

Hochwender C.G., and R.S.Fritz. 2004. Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. Oecologia. 138: 547–57.

Holzmueller E.J., and S. Jose. 2012. Biomass production for biofuels using agroforestry: potential for the North Central Region of the United States. Agroforestry Systems. 85: 305–314.

Hopper, J. V., E. H. Nelson, K. M. Daane, and N. J. Mills. 2011. Growth, development and consumption by four syrphid species associated with the lettuce aphid, *Nasonovia ribisnigri*, in California. Biological Control. 58: 271–276.

Hopper, K. R., S. M. Prager, and G. E. Heimpel. 2013. Is parasitoid acceptance of different host species dynamic? Functional Ecology. 27: 1201–1211.

Hurvich, CM, and C.L. Tsai. 1991. Bias of the corrected AIC criterion for underfitted regression and time series models. Biometrika 78:499–509.

Ingels, B., and P. De Clercq. 2011. Effect of size, extraguild prey and habitat complexity on intraguild interactions: a case study with the invasive ladybird *Harmonia axyridis* and the hoverfly *Episyrphus balteatus*. BioControl. 56: 871–882.

Isaacs, R., J. Tuell, A. Fiedler, M. Gardiner, and D. Landis. 2009. Maximizing arthropod-mediated ecosystem services in agricultural landscapes: the role of native plants. Frontiers in Ecology and the Environment. 7: 196–203.

Jackson, R.R., S.D. Pollard, X.J. Nelson, G.B. Edwards, and A.T. Barrion. 2001. Jumping spiders (Aranae: Salticidae) that feed on nectar. Journal of Zoology. 255: 25–29.

Johnson, G.A., D.L. Wyse, and C.C. Sheaffer. 2013. Yield of perennial herbaceous and woody biomass crops over time across three locations. Biomass and Bioenergy 58: 267–274.

Jordan N., Boody G., Broussard W., Glover J.D., Keeney D., Mccown B.H., McIsaac G., Muller M., Murray H., Neal J., Pansing C., Turner R.E., Warner K., Wyse D. 2007. Sustainable development of the agricultural bio-economy. Science. 316: 1570–1571.

Jose, S., A. R. Gillespie, and S.G. Pallardy. 2004. Interspecific interactions in temperate agroforestry. Agroforestry Systems. 61-62: 237–255.

Jungers, J.M., C.C. Sheaffer, and J. a. Lamb. 2015. The Effect of Nitrogen, Phosphorus, and Potassium Fertilizers on Prairie Biomass Yield, Ethanol Yield, and Nutrient Harvest. BioEnergy Research. 8: 279–291

Jungers, J.M., J.E. Fargione, C.C. Sheaffer, D.L. Wyse, and C. Lehman. 2013. Energy Potential of Biomass from Conservation Grasslands in Minnesota, USA. PLoS One 8: e61209

Kaiser, M. E., T. Noma, M. J. Brewer, K. S. Pike, R. Vockeroth, and S. D. Gaimari. 2007. Hymenopteran parasitoids and Dipteran predators found using soybean aphid after its midwestern United States invasion. Annals of the Entomological Society of Americ. 100: 196–205.

Kemp, P.R., and G.J. Williams. 1980. A physiological basis for niche separation between *Agropyron smithii* (C3) and *Bouteloua gracilis* (C4). Ecology. 61: 846–858

Kendall D. A. and C.W.Wiltshire. 1998. Life-cycles and ecology of willow beetles on *Salix viminalis* in England. Forest Pathology. 28: 281–288.

Kendall D.A., Hunter T., Arnold G.M., Liggitt J., Morris T., Wiltshire C.W. 1996. Susceptibility of willow clones (Salix spp.) to herbivory by *Phyllodecta vulgatissima* (L.) and *Galerucella lineola* (Fab.) (Coleoptera, Chrysomelidae). Annals of Applied Biology. 129, 379–390.

Keoleian G., and T. Volk 2005. Renewable Energy from Willow Biomass Crops: Life Cycle Energy, Environmental and Economic Performance. Critical Reviews in Plant Sciences. 24: 385–406.

Van Kleunen, M, E Weber, and M Fischer. 2010. A meta-analysis of trait differences between invasive and non-invasive plant species. Ecology Letters. 13: 235–245.

Knapp R., and T.M. Casey. 1986. Thermal ecology, behavior, and growth of gypsy moth and eastern tent caterpillars. Ecology. 67: 598–608.

Koh, I., H. I. Rowe, and J. D. Holland. 2013. Graph and circuit theory connectivity models of conservation biological control agents. Ecological Applications. 23: 1554–73.

Kosmala, M. C. 2013. Effects of human actions on four ecological systems, with a focus on trophic relationships. Ph.D. Dissertation. University of Minnesota, Saint Paul.

Kwit, C, and C.N. Stewart. 2012. Gene flow matters in switchgrass (*Panicum virgatum* L.), a potential widespread biofuel feedstock. Ecological Applications. 22: 3–7.

Landis, D., M. Gardiner, W. van der Werf, and S. Swinton. 2008. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. Proceedings of the National Academy of Science. 105: 20552–20557.

Landis, D., M. Gardiner, W. van der Werf, and S. Swinton. 2008. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. Proceedings of the National Academy of Science. 105: 20552–20557.

Landis, D. A., F.D. Menalled, A.C. Costamagna, and T.K. Wilkinson. 2005. Manipulating plant resources to enhance beneficial arthropods in agricultural landscapes. Weed Science. 53: 902–908.

Landis, D., and B. Werling. 2010. Arthropods and biofuel production systems in North America. Insect Science. 17: 220–236.

Landis, D. A, S.D. Wratten, and G.M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology. 45: 175–201.

Langellotto, G. A., and R.F. Denno. 2006. Refuge from cannibalism in complex-structured habitats: implications for the accumulation of invertebrate predators. Ecological Entomology. 31: 575–581.

Lavergne, S, and J. Molofsky. 2007. Increase genetic variation and evolutionary potential drive the success of an invasive grass. Proceedings of the National Academy of Science. 104: 3883–3888.

Leishman, M.R., I.J. Wright, A.T. Moles, and M. Westoby. 2000. The evolutionary ecology of seed size. p. 31–57. *In* Seeds: The ecology of regeneration in plant communities.

Lemus, R, E.C. Brummer, K.J. Moore, N.E. Molstad, C.L. Burras, and M.F. Barker. 2002. Biomass yield and quality of 20 switchgrass populations in southern Iowa, USA. Biomass and Bioenergy. 23: 433–442.

Letourneau, D.K., I. Armbrecht, B.S. Rivera, J.M. Lerma, E.J. Carmona, M.C. Daza, S. Escobar, V. Galindo, C. Gutiérrez, S.D. López, J.L. Mejía, A.M.A. Rangel, J.H. Rangel, L. Rivera, C.A. Saavedra, A.M. Torres, and A.R. Trujillo. 2011. Does plant diversity benefit agroecosystems? A synthetic review. Ecol. Appl. 21: 9–21.

Lin, C., R. McGraw, M. George, and H. Garrett. 1999. Shade effects on forage crops with potential in temperate agroforestry practices. Agrofor. Syst. 44: 109–119.

MacLeod, A. 1999. Attraction and retention of Episyrphus balteatus DeGeer (Diptera: Syrphidae) at an arable field margin with rich and poor floral resources. Agriculture Ecosystems and Environment. 73: 237–244.

Madakadze, I.C., B. Prithiviraj, R.M. Madakadze, K. Stewart, P. Peterson, B.E. Coulman, and D.L. Smith. 2000. Effect of preplant seed conditioning treatment on the germination of switchgrass (*Panicum virgatum* L.). Seed Sci. Technol. 28: 403–411.

Mangan, M.E., C. Sheaffer, D.L. Wyse, N.J. Ehlke, and P.B. Reich. 2011. Native Perennial Grassland Species for Bioenergy: Establishment and Biomass Productivity. Agronomy Journal. 103: 509–519.

Mangla, S, R.L. Sheley, and J.J. James. 2011. Field growth comparisons of invasive alien annual and native perennial grasses in monocultures. Journal of Arid Environments 75: 206–210.

Martel, J., and S. Malcolm. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. Journal of Chemical Ecology 30: 545–561.

Matlaga, D.P. and A.S. Davis. 2013. Minimizing invasive potential of *Miscanthus × giganteus* grown for bioenergy: Identifying demographic thresholds for population growth and spread. Journal of Applied Ecology. 50:479–487.

McLaughlin S.B. and L.A. Kszos. 2005. Development of switchgrass (*Panicum virgatum*) as a bioenergy feedstock in the United States. Biomass and Bioenergy 28: 515–535.

Mignault, M.P., M. Roy, and J. Brodeur. 2006. Soybean aphid predators in Québec and the suitability of *Aphis glycines* as prey for three Coccinellidae. BioControl. 51: 89–106.

Missaoui, A.M., V.A. Fasoula, and J.H. Bouton. 2005. The effect of low plant density on response to selection for biomass production in switchgrass. Euphytica 142: 1–12.

Mitchell, R.B., and K.P. Vogel. 2012. Germination and emergence tests for predicting switchgrass field establishment. Agronomy Journal. 104: 458–465.

Moser, S. E., Y. Kajita, J. D. Harwood, and J. J. Obrycki. 2011. Evidence for utilization of Diptera in the diet of field-collected coccinellid larvae from an antibody-based detection system. Biological Control. 58: 248–254.

Nakamura M., Miyamoto Y., Ohgushi T. 2003. Gall initiation enhances the availability of food resources for herbivorous insects. Functional Ecology. 17: 851–857.

Neal J.W., Chittams J.L., and J.A. Bentz. 1997. Spring emergence by larvae of the eastern tent caterpillar (Lepidoptera: Lasiocampidae): a hedge against high-risk conditions. Annals of the Entomological Society of America. 90: 596–603.

Nieto, D. J., C. Shennan, W. H. Settle, R. O'Malley, S. Bros, and J. Y. Honda. 2006. How natural enemies and Cabbage Aphid (*Brevicoryne brassicae* L .) population dynamics affect organic broccoli harvest. Environmental Entomology. 35: 94–101.

Nissim, W.G., F.E. Pitre, T.I. Teodorescu, and M. Labrecque. 2013. Long-term biomass productivity of willow bioenergy plantations maintained in southern Quebec, Canada. Biomass and Bioenergy. 56: 361–369.

Noma, T., and M. J. Brewer. 2008. Seasonal abundance of resident parasitoids and predatory flies and corresponding soybean aphid densities, with comments on classical biological control of soybean aphid in the Midwest. Journal of Economic Entomology. 101: 278–287.

Noma, T., C. Gratton, M. Colunga-Garcia, M. J. Brewer, E. E. Mueller, K. a G. Wyckhuys, G. E. Heimpel, and M. E. O'Neal. 2010. Relationship of soybean aphid (Hemiptera: Aphididae) to soybean plant nutrients, landscape structure, and natural enemies. Environmental Entomology. 39: 31–41.

Nordman E., Robison D., Abrahamson L., and T. Volk. 2005. Relative resistance of willow and poplar biomass production clones across a continuum of herbivorous insect specialization: Univariate and multivariate approaches. Forest Ecology and Management. 217: 307–318.

Pake, C.E., and D.L. Venable. 1996. Seed banks in desert annuals : Implications for persistence and coexistence in variable environments. Ecology. 77: 1427–1435.

Peacock L., and S. Herrick. 2000. Responses of the willow beetle *Phratora vulgatissima* to genetically and spatially diverse *Salix* spp. plantations. Journal of Applied Ecology. 37: 821–831.

Peng, R.K., L.D. Incoll, S.L. Sutton, C. Wright, and A. Chadwick. 1993. Diversity of airborne arthropods in a silvoarable agroforestry system. Journal of Applied Ecology. 30: 551–562.

Perrin, R, K. Vogel, M. Schmer, and R. Mitchell. 2008. Farm-Scale Production Cost of Switchgrass for Biomass. BioEnergy Research. 1: 91–97.

Peterson, J. A., S. A. Romero, and J.D. Harwood. 2010. Pollen interception by linyphiid spiders in a corn agroecosystem: Implications for dietary diversification and risk-assessment. Arthropod-Plant Interactions. 4: 207–217.

Pimentel, D., C. Harvey, P. Resosudarmo, K. Sinclair, D. Kurz, M. McNair, S. Crist, L. Shpritz, L. Fitton, R. Saffouri, and R. Blair. 1995. Environmental and economic costs of soil erosion and conservation benefits. Science. 267: 1117–1123.

Pineda, A., and M. Á. Marcos-García. 2008. Seasonal abundance of aphidophagous hoverflies (Diptera: Syrphidae) and their population levels in and outside Mediterranean sweet pepper greenhouses. Annals of the Entomological Society of America. 101: 384–391.

Platenkamp, G.A.J., and R.G. Shaw. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. Evolution. 47: 540–555.

Pontin, D.R., M.R. Wade, P. Kehrli, and S.D. Wratten. 2006. Attractiveness of single and multiple species flower patches to beneficial insects in agroecosystems. Annals of Applied Biology. 148: 39–47.

Quaye, A.K., and T. A. Volk. 2013. Biomass production and soil nutrients in organic and inorganic fertilized willow biomass production systems. Biomass and Bioenergy. 57: 113–125.

Quaye, A.K., T. A. Volk, S. Hafner, D.J. Leopold, and C. Schirmer. 2011. Impacts of paper sludge and manure on soil and biomass production of willow. Biomass and Bioenergy 35(7): 2796–2806.

R Development Core Team. 2005. R: A language and environment for statistical computing.

R Development Core Team. 2014. R: A language and environment for statistical computing.

Raghu, S, R.C. Anderson, C.C. Daehler, A.S. Davis, R.N. Wiedenmann, D. Simberloff, and R.N. Mack. 2006. Adding biofuels to the invasive species fire? Science 313: 1742.

Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annual Reviews of Entomology. 56: 375–99.

Ragsdale, D. W., B. P. McCornack, R. C. Venette, B. D. Potter, I. V Macrae, E. W. Hodgson, M. E. O'Neal, K. D. Johnson, R. J. O'Neil, C. D. DiFonzo, T. E. Hunt, P. A. Glogoza, and E. M. Cullen. 2007. Economic threshold for soybean aphid (Hemiptera : Aphididae). Journal of Economic Entomology. 100: 1258–1267.

Rand T.A., Tylianakis J.M., and T. Tscharntke. 2006. Spillover edge effects: the dispersal of agriculturally subsidized insect natural enemies into adjacent natural habitats. *Ecology letters*, 9, 603–14.

Rees, M. 1993. Trade-offs among dispersal strategies in British plants. Nature. 366: 150–152.

Rees, M., D.Z. Childs, J.C. Metcalf, K.E. Rose, A.W. Sheppard, and P.J. Grubb. 2006. Seed dormancy and delayed flowering in monocarpic plants: Selective interactions in a stochastic environment. American Naturalist. 168: E53–E71.

Riechert, S.E., and L. Bishop. 1990. Prey control by an assemblage of generalist predators: spiders in garden test systems. Ecology 71: 1441–1450.

Rieske L.K., and L.H. Townsend (2005) Orientation and dispersal patterns of the eastern tent caterpillar, *Malacosoma americanum* F. (Lepidoptera: Lasiocampidae). Journal of Insect Behavior. 18: 193–207.

Robertson, B. A., C. Porter, D. A. Landis, and D.W. Schemske. 2012. Agroenergy Crops Influence the Diversity, Biomass, and Guild Structure of Terrestrial Arthropod Communities. BioEnergy Research. 5: 179–188.

Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: The Fauna of collards (*Brassica oleracea*). Ecological Monographs. 43: 95–124.

Rose, L.W., M.K. Das, R.G. Fuentes, and C.M. Taliaferro. 2007. Effects of high- vs. low-yield environments on selection for increased biomass yield in switchgrass. Euphytica. 156: 407–415.

Runck, B.C., M.B. Kantar, N.R. Jordan, J. a. Anderson, D.L. Wyse, J.O. Eckberg, R.J. Barnes, C.L. Lehman, L.R. DeHaan, R.M. Stupar, C.C. Sheaffer, and P.M. Porter. 2014. The Reflective Plant Breeding Paradigm: A Robust System of Germplasm Development to Support Strategic Diversification of Agroecosystems. Crop Science.54: 1939–1948.

Rutledge, C. E., R. J. O'Neil, T. B. Fox, and D. A. Landis. 2004. Soybean aphid predators and their use in integrated pest management. Annals of the Entomological Society of America. 97: 240–248.

Ruzicka, Z. 1975. The effects of various aphids as larval prey on the development of *Metasyrphus corollae* [Dipt.: Syrphidae]. Entomophaga. 20: 393–402.

Sage R.B. and K. Tucker. 1997. Invertebrates in the canopy of willow and poplar short rotation coppices. Aspects of Applied Biology. 49: 105–111.

Saltonstall, K. 2002. Cryptic invasion by a non-native genotype of the common reed, Phragmites australis, into North America. Proceedings of the National Academy of Science. 99: 2445–2449

Sanderson, M.A., R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B. V Conger, D.J. Parrish, D.D. Wolf, C. Taliaferro, A.A. Hopkins, W.R. Ocumpaugh, M.A. Hussey, J.C. Read, and C.R. Tischler. 1996. Switchgrass as a sustainable bioenergy crop. Bioresource Technology. 56: 83–93.

Saville, D.J. 2013. Multiple comparison procedures—cutting the Gordian Knot. Agronomy Journal 105: 1–6.

Schmer, M.R., K.P. Vogel, R.B. Mitchell, and R.K. Perrin. 2008. Net energy of cellulosic ethanol from switchgrass. Proceedings of the National Academy of Science. 105: 464–469.

Schmer, M.R., K.P. Vogel, R.B. Mitchell, B.S. Dien, H.G. Jung, and M.D. Casler. 2012. Temporal and Spatial Variation in Switchgrass Biomass Composition and Theoretical Ethanol Yield. Agronomy Journal. 104: 54-64.

Schmidt, N. P., M. E. O'Neal, and P. M. Dixon. 2008. Aphidophagous predators in Iowa soybean: a community comparison across multiple years and sampling methods. Annals of the Entomological Society America. 101: 341–350.

Shen, Z., D.J. Parrish, D.D. Wolf, and G.E. Welbaum. 2001. Stratification in switchgrass seeds is reversed and hastened by drying. Crop Science 41: 1546–1551.

Simberloff, D. 2008. Invasion biologists and the biofuels boom: Cassandras or
colleagues? Weed Science 56:867–872

Simmonds, N.W. 1991. Selection for local adaptation in a plant breeding programme.
Theoretical and Applied Genetics. 82: 363–367.

Sluiter, A., B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker.
2010. Determination of Structural Carbohydrates and Lignin in Biomass. U. S.
Depatment of Energy - National Renewable Energy Laboratory, Golden, Colorado.

Smart, A.J., and L.E. Moser. 1999. Switchgrass seedling development as affected by seed
size. Agronomy Journal. 91: 335–338.

Smith, H. A., and W. E. Chaney. 2007. A survey of syrphid predators of *Nasonovia
ribisnigri* in organic Lettuce on the Central Coast of California. Journal of Economic
Entomology. 100: 39–48.

Smith, H. A., W. E. Chaney, and T. A. Bensen. 2008. Role of syrphid larvae and other
predators in suppressing aphid infestations in organic Lettuce on California's
Central Coast. Journal of Economic Entomology. 101: 1526–1532.

Soleyman-Nezhadiyan, E., and R. Laughlin. 1998. Voracity of larvae, rate of
development in eggs, larvae and pupae, and flight seasons of adults of the hoverflies
Melangyna viridiceps Macquart and *Symosyrphus grandicornis* Macquart (Diptera :
Syrphidae). Australian Journal of Entomology. 37: 243–248.

Solomon J.D., and W.K. Randall. 1978. Biology and damage of the willow shoot sawfly
in willow and cottonwood. Annals of the Entomological Society of America. 71:
654–657.

Stamps, W.T., R.L. McGraw, L. Godsey, and T.L. Woods. 2009. The ecology and
economics of insect pest management in nut tree alley cropping systems in the
Midwestern United States. Agriculture Ecosystems and Environment. 131: 4–8.

Stanton, M.L. 1984. Seed variation in wild radish: Effect of seed size on components of
seedling and adult fitness. Ecology. 65:1105–1112.

Strong, D.R., E.D. McCoy, and J.R. Rey. 1977. Time and the number of herbivore
species: the pests of sugarcane. Ecology. 58: 167–175.

Sunderland, K., and F. Samu. 2000. Effects of agricultural diversification on the
abundance, distribution, and pest control potential of spiders: a review. Entomologia
Experimentalis et Applicata. 95: 1–13

Tenhumberg, B. 1995. Estimating predatory efficiency of *Episyrphus balteatus* (Diptera:
Syrphidae) in Cereal fields. Environmental Entomology. 24: 687–691.

Tenhumberg, B., and H. M. Poehling. 1995. Syrphids as natural enemies of Cereal
Aphids in Germany: aspects of their biology and efficacy in different years and
regions. Agriculture Ecosystems and Environment. 52: 39–43.

Thelemann, R., G. Johnson, C. Sheaffer, S. Banerjee, H. Cai, and D. Wyse. 2010. The Effect of Landscape Position on Biomass Crop Yield. Agronomy Journal. 102: 513-522.

Thevathasan, N.V., and a. M. Gordon. 2004. Ecology of tree intercropping systems in the North temperate region: Experiences from southern Ontario, Canada. Agroforestry Systems. 61-62: 257–268.

Tilman, D., P.B. Reich, J. Knops, D. Wedin, T. Mielke, and C. Lehman. 2001. Diversity and productivity in a long-term grassland experiment. Science. 294: 843–845.

Van Rijn, P.C.J., J. Kooijman, F.L. Wäckers, and P.C.J. van Rijn. 2006. The impact of floral resources on syrphid performance and cabbage aphid biological control. International Organisation for Biological and Integrated Control of Noxious Animals and Plants/ West Paleartic Bull. 29: 149–152.

Vange, V., I. Heuch, and V. Vandvik. 2004. Do seed mass and family affect germination and juvenile performance in Knautia arvensis? A study using failure-time methods. Acta Oecologica 25: 169–178.

Vanhaelen, N., C. Gaspar, and F. Francis. 2002. Influence of prey host plant on a generalist aphidophagous predator: *Episyrphus balteatus* (Diptera: Syrphidae). European Journal of Entomology. 99: 561–564.

Verdinelli M., Sanna-Passino G. 2003. Development and feeding efficiency of *Malacosoma neustrium* larvae reared with *Quercus* spp. leaves. Annals of Applied Biology. 143: 161–167.

Verheggen, F. J., Q. Capella, E. G. Schwartzberg, D. Voigt, and E. Haubruge. 2009. Tomato-aphid-hoverfly: a tritrophic interaction incompatible for pest management. Arthropod-Plant Interactions. 3: 141–149.

Vockeroth, J. R. 1992. The flower flies of the subfamily Syrphinae of Canada, Alaska, and Greenland, Insects Arachn. Canada, Part 18. Canada Communication Group-Publishing, Ottawa, Ontario.

Vogel, K.P., F.A. Haskins, H.J. Gorz, B.A. Anderson, and J.K. Ward. 1991. Registration of "Trailblazer" switchgrass. Crop Sci. 31: 1388.

Vogel, KP, RB Mitchell, MD Casler, and G Sarath. 2014. Registration of "Liberty" Switchgrass. Journal of Plant Registration: 242–247.

Wackers, F. 2004. Assessing the suitability of flowering herbs as parasitoid food sources: flower attractiveness and nectar accessibility. Biological Control 29: 307–314.

Wade M.J., and F. Breden. 1986. Life History of natural populations of the imported willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). Annals of the Entomological Society of America. 79: 73–79.

Walton, N.J., and R. Isaacs. 2011. Influence of Native Flowering Plant Strips on Natural Enemies and Herbivores in Adjacent Blueberry Fields. Environomental Entomology 40: 697–705.

Weih M., U.M.E. Didon, A.C. Rönnberg-Wästljung, and C. Björkman. 2008. Integrated agricultural research and crop breeding: Allelopathic weed control in cereals and long-term productivity in perennial biomass crops. Agricultural Systems. 97: 99–107.

West, NM, D.P. Matlaga, and A.S. Davis. 2014. Managing Spread from Rhizome Fragments is Key to Reducing Invasiveness in   Miscanthus × giganteus. Invasive Plant Science and Management. 7: 517–525.

Wheeler, A. G. 1977. Studies on the arthropod fauna of Alfalfa VII. Predaceous insects. Canadian Entomologist. 109: 423–427.

White, A.J., S.D. Wratten, N.A. Berry, U. Weigmann, and U. Weigmannl. 1995. Habitat Manipulation to Enhance Biological Control of Brassica Pests by Hover Flies (Diptera : Syrphidae). Journal of Economic Entomology. 88: 1171–1176.

Wiesenborn, W.D. 2011. Biomasses of arthropod taxa differentially increase on nitrogen-fertilized willows and cottonwoods. Restoration Ecology. 19: 323–332.

Zarnstorff, M.E., R.D. Keys, and D.S. Chamblee. 1994. Growth regulator and seed storage effects on switchgrass germination. Agronomy Journal. 86: 667–672.